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VERSITA

Redescriptions and comments on the validity of *Acuaria subula* and *A. skrjabini* (Nematoda, Spirurida, Acuariidae), parasites of passerine birds

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Abstract

Acuaria subula (Dujardin, 1845) is redescribed by light microcopy (LM) and scanning electron microscopy (SEM) on the basis of specimens from its type host, *Erithacus rubecula* (Passeriformes, Muscicapidae), from Curonian Spit (Kaliningradskaya Oblast', Russia) and Bulgaria. *Acuaria skrjabini* (Ozerskaya, 1926) is redescribed by LM and SEM on the basis of specimens from *Passer domesticus* (type host) and *P. hispaniolensis* (Passeriformes, Passeridae) from Bulgaria. Contrary to previous opinions recognizing *A. skrjabini* as a junior synonym of *A. subula*, the present study confirms that they are distinct species. They can be distinguished on the basis of the ratio between the length of cordons and the body length, the ratio between the length of muscular oesophagus and glandular oesophagus, and the ratio between the total length of oesophagus and the body length. In addition, the plates forming the cordons in these two species exhibit different morphological characters. Another difference between these two species is associated with the particular irregular mosaic ornamentation of the cuticle on the ventral and lateral sides of body around the region of vulva of *A. subula* and its absence in *A. skrjabini*. Data on their host and geographical ranges are surveyed. The type series of *Acuaria buttnerae* Chabaud et Petter, 1961, described as a parasite of *Calandrella brachydactyla* (Passeriformes, Alaudidae) in France, is re-examined; the latter species is recognized as a junior synonym of *A. skrjabini* (new synonymy).

Keywords

Nematoda, Acuariidae, *Acuaria subula*, *Acuaria skrjabini*, *Acuaria buttnerae*, *Erithacus rubecula*, *Passer domesticus*, *Passer hispaniolensis*, *Calandrella brachydactyla*

Introduction

The nematodes of the genus *Acuaria* Bremser, 1811 are parasites occurring under the lining of the gizzard, mainly in passerine birds. Among European species, *A. subula* (Dujardin, 1845) and *A. skrjabini* (Ozerskaya, 1926) are frequently recorded. *A. subula* has been described from the European Robin, *Erithacus rubecula* (L.) (Muscicapidae) in France and subsequently reported from diverse European and Asian birds of the Passeriformes (Alaudidae, Bombycillidae, Corvidae, Emberizidae, Fringillidae, Hirundinidae, Laniidae, Motacillidae, Muscicapidae, Passeridae, Sittidae, Sturnidae, Sylviidae and Turdidae) as well as from the Columbiformes (Columbidae), Piciformes (Picidae), Apodiformes (Apodidae) and Coraciiformes (Coraciidae) (see below). *Acuaria skrjabini* was originally described as a parasite of *Passer domesticus* (L.) in Russia and recorded from a variety of passerines (Estrildidae, Passeridae, Prunellidae, Sturnidae and Thraupidae), with distribution in Europe, Asia, Australia and New Zealand (see below). The overlapping host ranges of the two species and the poor knowledge of their morphological variations (Sobolev in Kurashvili 1957, Sobolev in Skrjabin *et al.* 1965, Baron 1967) indicate the possible presence of erroneous identifications in the published records. Furthermore, Skrjabin *et al.* (1965) discussed the validity of *A. skrjabini* as doubtful and considered it as a possible synonym of *A. subula*; they accepted conditionally its validity, "pending more detailed studies of its variation based on more abundant materials". Sonin *et al.* (1983) considered *A. subula* and *A. skrjabini* as synonymous. Smogorzhevskaya (1990) emphasized the necessity of further comparative studies of specimens identified as *A. subula* and *A. skrjabini* collected from various passerine birds in or-

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der to confirm them as distinct species and to specify their host ranges. These contradictions concerning the recognition of *A. subula* and *A. skrjabini* as distinct species provoked us to examine their morphology in comparative aspect.

During a survey of acuariid nematodes from Bulgaria and Curonian Spit (Kalingradskaya Oblast', Russia), we had the opportunity to examine new specimens of *A. subula* and *A. skrjabini*, including individuals collected from the type hosts of both species. The aim of the present article is to provide detailed morphological descriptions of the two species based on light microscopy and scanning electron microscopy in order to develop the basis for their identification. We also re-evaluate previous records in view of the new morphological data.

Materials and Methods

Specimens from the helminthological collections of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER-BAS), Institute of Ecology, Nature Research Centre, Vilnius, and Muséum National d'Histoire Naturelle, Paris (MNHN), were studied. Details of the collection data and number of specimens are given in the text for each species.

The nematodes were preserved in 70% ethanol or 4% formaldehyde solution. For light-microscopy, specimens were cleared and examined as temporary mounts in glycerol. Specimens used for SEM observations were transferred from 70% ethanol to 40% ethanol (10 min), rinsed in 0.1 M cacodylate buffer, post-fixed in 1% OsO_4 for 2 h and dehydrated in an ethanol series. They were coated with gold in fine coater JEOL JFS 1200 and examined using a JEOL JSM 5510 microscope at 10 kV.

Metrical data are given as the range, with the mean and the number of measurements taken (n) in parentheses. Measurements are in micrometres except where otherwise stated.

The following indices were used: cordon length/body length = $I_{CL/BL}$; length of muscular oesophagus/length of glandular oesophagus = $I_{mOE/gOE}$; length of oesophagus/body length = $I_{OE/BL}$; length of caudal alae/body length = $I_{CA/BL}$; length of left spicule/length of right spicule = $I_{LSP/RSP}$; distance from vulva to posterior end/body length = $I_{V/BL}$.

Results

***Acuaria subula* (Dujardin, 1845) Railliet, Henry et Sisoff, 1912** (Figs 1–3)

Material studied

From *Erithacus rubecula* (6 birds), collected at Fringilla Point, 12 km south to Rybachiy (Curonian Spit), Kaliningradskaya Oblast', Russia, 27 April-24 May 2004, collector J. Komisarovas (3 male and 15 female nematodes): 08.05.2004, IBER-BAS N001.051 (1 female); 08.05.2004, IBER-BAS N001.080 (1 female and 1 male), N001.081 (1 female and 1 male) and MNHN 1YT (2 females); 08.05.2004, IBER-BAS N001.081 (1 female); SEM stub; 27.04.2004, IBER-BAS N001.071 (2 females); 24.05.2004, IBER-BAS N001.052 (2 females); 04.05.2004, IBER-BAS N001.073 (5 females and 1 male); from *Erithacus rubecula*, Kavarna, Bulgaria, 9 May 2009, IBER-BAS N001.072 (1 male).

Description (based on individuals from Curonian Spit): Medium-sized acuariid nematodes. Anterior end with 2 triangular pseudolabia, each bearing single amphid and a pair of papillae (Fig. 2A, B). Cordons four, arise dorsally and ventrally between pseudolabia, extending laterally in longitudinal direction to posterior end of muscular oesophagus (Fig. 1A). Each cordon consisting of two longitudinal rows of cuticular plates delimiting longitudinal canal situated between them (Fig. 2C). Cuticular plates with rather uniform shape; posterior rim of each plate overlapping anterior part of adjacent posterior plate (Fig. 2D). Deirids rounded (Fig. 2E), small, *c*. 1 µm in diameter, situated anteriorly to nerve ring. Excretory pore behind nerve ring (Fig. 1A). Buccal cavity long. Phasmids subterminal (Fig. 2H).

Male (n = 2; see Table I for other measurements): Maximum width about mid-body. Body width at level of cloaca 63–81. Cordons 9–10 wide. Deirids and excretory pore at 122–168 and 170–215, respectively, from anterior end. Left and right postdeirids (Fig. 2F) at 1,206 and 1,518 from posterior extremity, respectively (in a specimen 3.7 mm long). Buccal cavity 8–9 wide. Muscular oesophagus 29–36 wide. Glandular oesophagus 63–72 wide. Nerve ring at 133–143 from anterior end. Cuticle 6–7 thick; distance between cuticular striations about 4–5 µm. Caudal alae 394–408 long. Caudal papillae 10 pairs (4 precloacal and 6 postcloacal); single ventral precloacal papilla (Figs 1B, 2G, H). Left spicule longer, 11–12 wide; right spicule 14–16 wide (Fig. 1C).

Female (n = 8 except otherwise stated; see Table I for other measurements): Maximum body width in region between posterior end of glandular oesophagus and vulva; body width at anus 63–107. Tail conical, bent dorsally (Fig. 1H), with small terminal protuberance (Fig. 3E). Cordons 11–14 wide. Deirids and excretory pore at 127–206 (162) and 209–295 (267), respectively, from anterior end. Buccal cavity 11–13 wide. Muscular oesophagus 36–50 (43) wide. Glandular oesophagus 93–120 (100) wide. Nerve ring at 152–200 (178, n = 7) from anterior body end. Cuticle 9–10 thick; distance between cuticular striation 7–8 µm. Cuticle mostly with transverse striations, undulated along lateral sides of body (Figs 2C, 3A, B), except region of vulva characterized by irregular mosaic ornamentations on ventral and lateral sides of body (Fig. 3C, D). Distinct circular folds surround vulva (Fig. 3D). Reproductive system didelphic. Vagina simple, posteriorly directed, with circular musculature, passing into long ovejector with thin muscular walls, filled with spermatozoa or/and eggs (Fig. 1D, E). Uteri two; their terminal parts lined with epithelium consisting of tall, narrow cells. Spermatheca oval, between oviduct and uterus

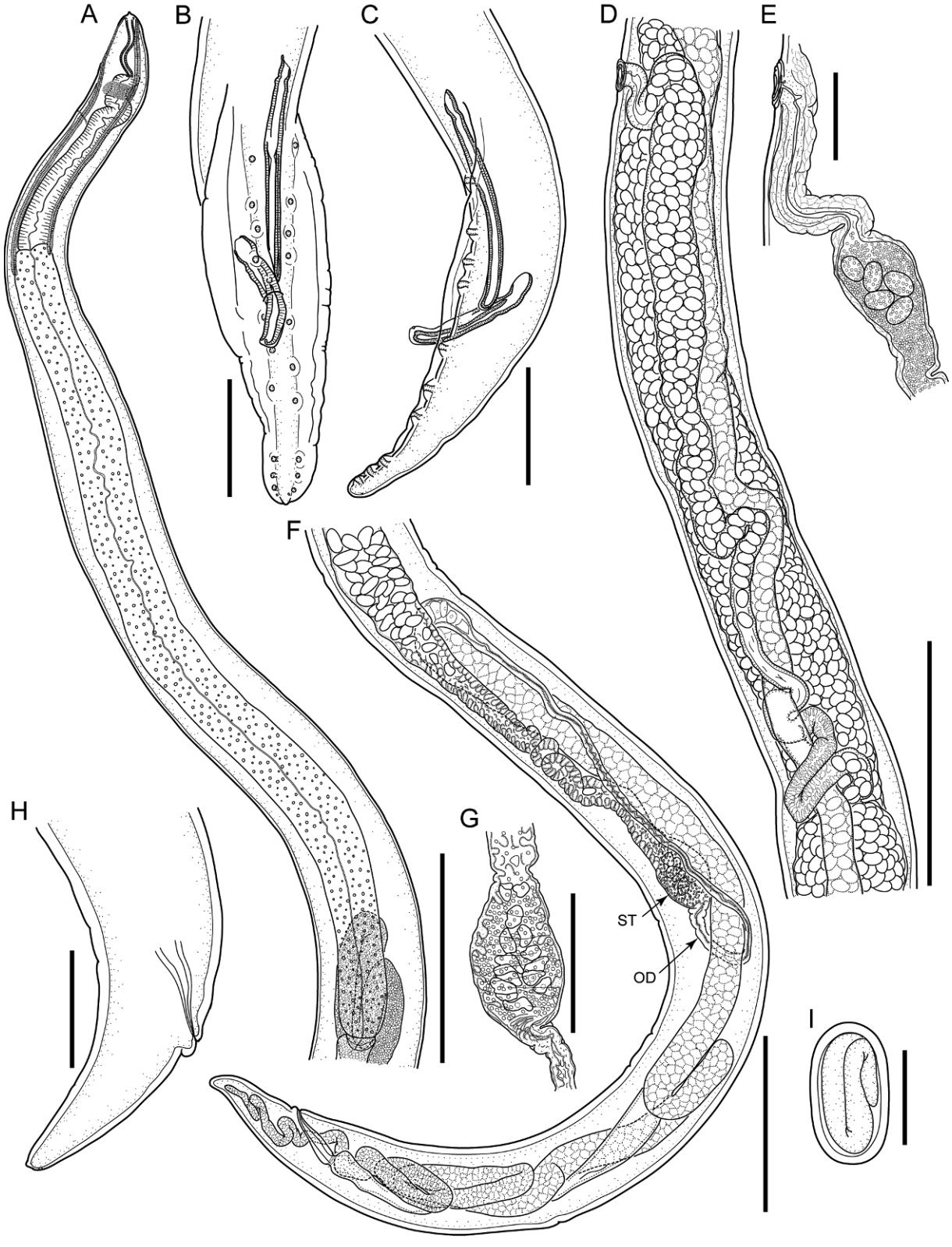

Fig. 1. A-I. *Acuaria subula.* **A.** Anterior end, lateral view, female. **B.** Posterior end, ventral view, male. **C.** Posterior end, lateral view, male. **D.** Terminal part of female genital system, lateral view. **E.** Vulva, lateral view. **F.** Posterior end, lateral view, female; note spermatheca (ST) and oviduct (OD). **G.** Spermatheca. **H.** Tail, lateral view, female. **I.** Egg. Scale bars = 500 μm (A, D); 250 μm (F); 100 μm (B, C, E, G, H); 25 μm (I)

(Fig. 1F, G). Eggs oval, containing first stage larva (Fig. 1I). $I_{V/BL}$ 0.435–0.561 (0.474).

Host range and geographical distribution

Records: [1] Dujardin (1845); [2] Gendre (1913); [3] Kurashvili (1953); [4] Kurashvili (1957); [5] Ryšavý (1957); [6] Dubinina and Kulakova (1960); [7] Bashkirova (1960) – ?, see Remarks; [8] Chabaud and Petter (1961); [9] Joszt (1962); [10] Oshmarin and Parukhin (1963); [11] Oshmarin (1963); [12] Gundłach (1965); [13] Baron (1967) – ?, see Remarks; [14] Kontrimavichus (1969); [15] Jögis (1971); [16] Ryzhikov *et al.* (1973); [17] Sonin and Larchenko (1974); [18] Okulewicz (1982); [19] Okulewicz (1984); [20] Smogorzhevskaya (1990); [21] Okulewicz and Okulewicz (2008); [22] present study.

Descriptions: 1, 2, 7 (?, see Remarks), 8, 13 (?, see Remarks), 15, 18, 20, 22; see also Kurashvili (1957) and Skrjabin *et al.* (1965) for the description from the unpublished thesis by Sobolev (1947).

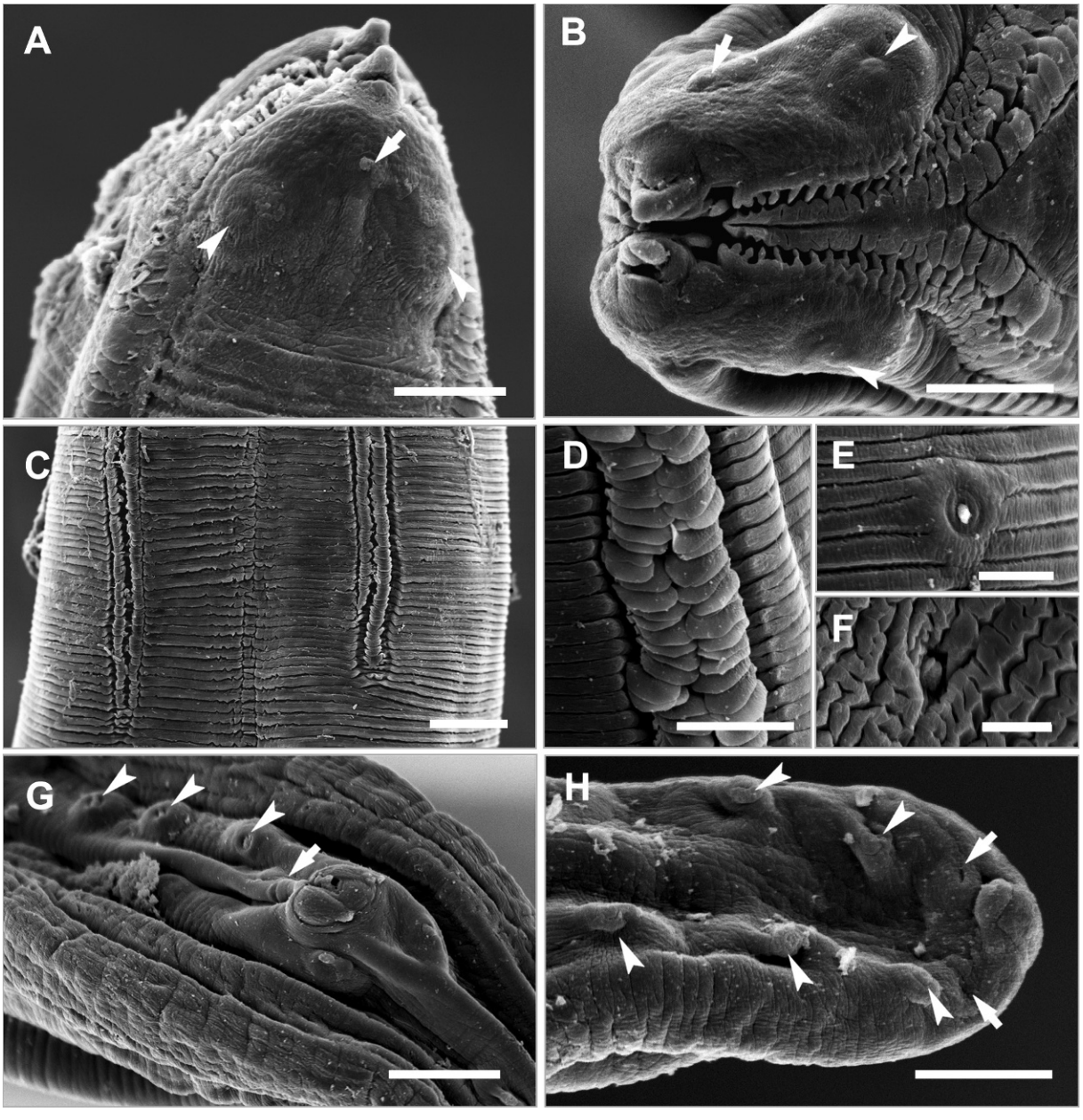

Fig. 2. A-H. *Acuaria subula*, SEM. **A.** Anterior end, lateral view; note cephalic papillae (arrowheads) and amphid (arrow). **B.** Anterior end, ventral view; note cephalic papillae (arrowheads) and amphid (arrow). **C.** Posterior ends of cordons. **D.** Detail of cordon structure. **E.** Deirid. **F.** Postdeirid. **G.** Cloacal region; note precloacal papillae (arrowheads) and medial papilla (arrow). **H.** Posterior end, male; note postcloacal papillae (arrowheads) and phasmids (arrows). Scale bars = 10 μm (A, B, D, H); 20 μm (C, G); 5 μm (E, F)

Table I. Metrical data of *A. subula* from various hosts and localities

Source	Dujardin (1845)	Gendre (1913)	Chabaud and Petter (1961)	Jögis (1971)	Okulewicz (1982)		Smogorzhevskaya (1990)	Present study	
Host	*Erithacus rubecula*	*E. rubecula*	*E. rubecula*	*E. rubecula, Motacilla alba*	*Hippolais icterina*	*Ficedula hypoleuca*	*E. rubecula*	*E. rubecula*	
Locality	France	France	France	Russia	Poland	Poland	Ukraine	Russia	Bulgaria
Male	**n = 1**	**n = 1**	**n = 1**	**n = 2**	**n = 2**	–	–	**n = 2**	**n = 1**
Body length (mm)	7.0	4.9	4.2	3.8–4.0	4.2	–	–	3.7–5.6	6.0
Maximum body width	167	120	110	120–150	86	–	–	140–160	156
Tail, length	–	–	175	–	159	–	–	161–189	165
Buccal cavity, length	136	150	120	100–110	114	–	–	108–116	134
Muscular oesophagus, length	510	430	400	280–330	420	–	–	302–402	447
Glandular oesophagus, length	–	1,580	1,600	440–1,100	1,550	–	–	875–1,592	1,575
Cordons, length	–	380	205	170–230	198	–	–	267–380	456
Left spicule, length	227	190	190	180–210	192	–	–	192–206	208
Right spicule, length	134	120	110	110–120	121	–	–	120–122	117
$I_{CL/BL}$	–	0.078	0.049	0.051	0.047	–	–	0.068–0.072	0.075
$I_{mES/gES}$	–	0.272	0.250	–	0.271	–	–	0.253–0.345	0.284
$I_{ES/BL}$	–	0.410	0.476	–	0.469	–	–	0.319–0.359	0.334
$I_{Lsp/Rsp}$	1.694	1.583	1.727	–	–	–	–	1.600–1.689	1.778
Pairs of postcloacal papillae	–	6	6	7	–	–	–	6	6
Female	**n = 1**	**–**	**–**	**n = 1**	**–**	**n = 1**	**n = 2**	**n = 8**	**–**
Body length (mm)	18.0	–	–	10.0	–	9.5	14.0–18.0	8.6–15.2	–
Maximum body width	290	–	–	330	–	210	232–266	201–335	–
Tail, length	170	–	–		–	159	184	159–291	–
Buccal cavity, length	170	–	–	130	–	140	140	120–157	–
Muscular oesophagus, length	840	–	–	440	–	470	480	418–670	–
Glandular oesophagus, length	–	–	–	1,700	–	1,510	1,577	1,233–1,923	–
Cordons, length	–	–	–	500	–	206	786–860	389–771	–
Vulva from posterior end, distance (mm)	8.0	–	–	5.0	–	5.1	6.0	4.1–8.6	–
Eggs	37–39	–	–	36 × 24–28	–	40–28 × 19–23	38–40 × 24	35–37 × 21–24	–
$I_{CL/BL}$	–	–	–	0.050	–	0.022	0.051*	0.027–0.085	–
$I_{mES/gES}$	–	–	–	0.259	–	0.311	0.340	0.263–0.339**	–
$I_{ES/BL}$	–	–	–		–	0.280	0.129*	0.148–0.191**	–

*Presented as mean; **n = 6

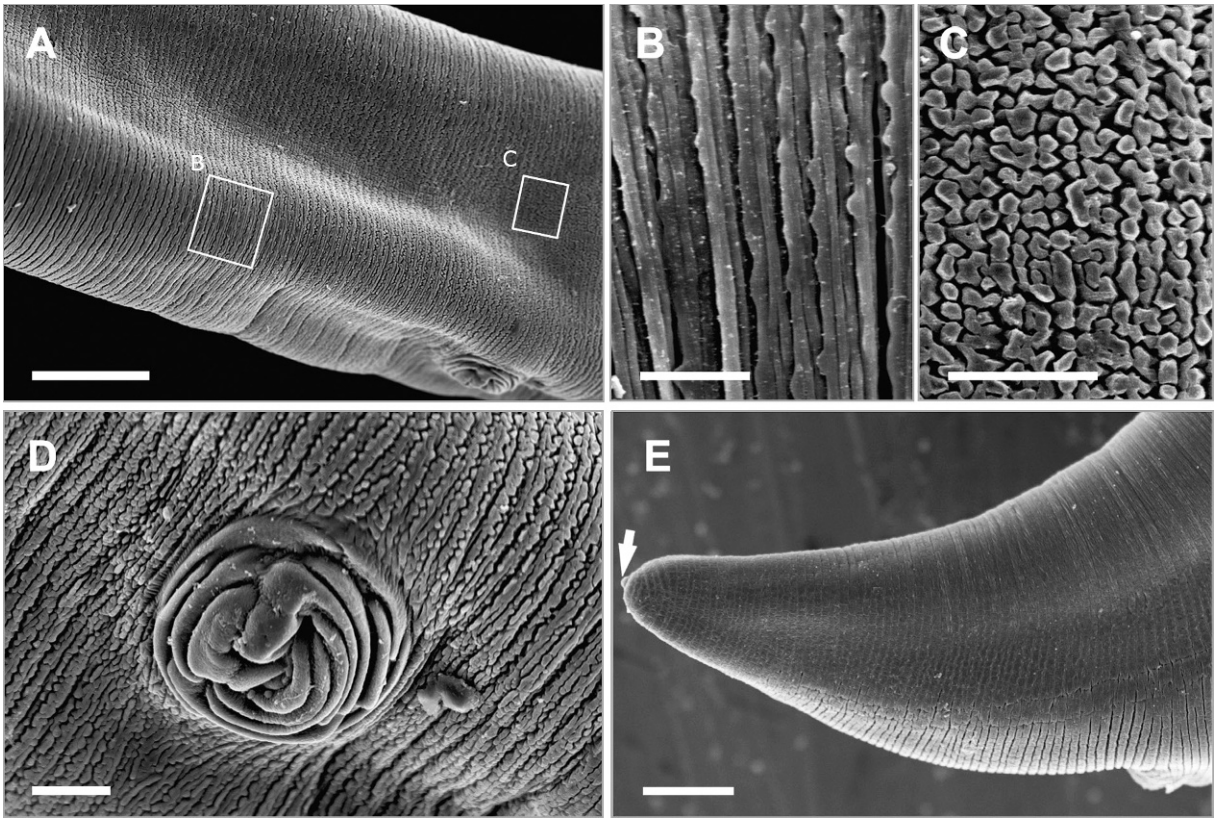

Fig. 3. A-E. *Acuaria subula*, female, SEM. **A.** Body at the level of cloaca, lateral view; white rectangles indicate regions shown in 3B and 3C. **B, C.** Cuticle, details, see 3A. **D.** Vulva. **E.** Tail, lateral view; note small terminal protrusion (arrow). Scale bars = 50 μm (A); 10 μm (B-D); 20 μm (E)

Type-host: *Erithacus rubecula* (L.) [1, 2, 4, 8, 15, 19, 20, 22] (Passeriformes, Muscicapidae).

Other hosts: Passeriformes, Alaudidae: *Calandrella cinerea* (Gmelin) [17], *Eremophila alpestris* (L.) [17]; Bombycillidae: *Bombycilla garrulus* (L.) [16]; Corvidae: *Garrulus glandarius* L. [17], *Nucifraga caryocatactes* (L.) [10], *Pica pica* L. [3, 4]; Emberizidae: *Emberiza hortulana* L. [17], *E. leucocephalos* Gmelin [15, 16]; Fringillidae: *Coccothraustes coccothraustes* (L.) [11]; Hirundinidae: *Riparia riparia* (L.) [16]; Laniidae: *Lanius cristatus* L. [17], *L. excubitor* L. [4], *L. minor* Gmelin [4]; Motacillidae: *Motacilla alba* L. [14, 15, 16, 17], *M. citreola* Pallas [4, 11, 16]; Muscicapidae: *Cyanoptila cyanomelana* (Temminck) [11], *Ficedula hypoleuca* (Pallas) [18], *Luscinia calliope* (Pallas) [11], *Muscicapa striata* (Pallas) [17], *Oenanthe hispanica* (L.) [17], *O. isabellina* (Temminck) [17], *O. oenanthe* (L.) [4], *O. picata* (Blyth) [4]; Passeridae: *Passer domesticus* (L.) [4, 9, 13 – ?, see Remarks, 17], *P. montanus* (L.) [4, 6, 7 – ?, see Remarks, 11]; Sittidae: *Sitta europaea* L. [5, 10]; Sturnidae: *Sturnus cineraceus* Temminck [10], *S. vulgaris* L. [12]; Sylviidae: *Acrocephalus arundinaceus* (L.) [6], *A. dumetorum* Blyth [6], *A. scirpaceus* (Hermann) [21], *Hippolais icterina* (Vieillot) [18], *Phylloscopus collybita* (Vieillot) [16]; *P. trochiloides* (Sundevall) [21]; Turdidae: *Zoothera dauma* (Latham) [11]; Coraciiformes, Coraciidae: *Coracias garrulus* L. [6]; Columbiformes, Columbidae: *Streptopelia orientalis* (Latham) [7 – ?, see Remarks]; Apodiformes, Apodidae: *Hirundapus caudacutus* (Latham) [11]; Piciformes, Picidae: *Dendrocopos leucotos* (Bechstein) [7 – ?, see Remarks], *Dryocopus martius* [11], *Picus canus* Gmelin [7 – ? see Remarks, 11].

Distribution: France (type locality) [1, 2, 8], England [13 – ?, see Remarks], Slovakia [5], Poland [9, 12, 18, 19, 21], Lithuania [14], Bulgaria [22], Ukraine [20], Georgia [3, 4]; Russia: Kaliningradskaya Oblast' [15, 22], Astrakhanskaya Oblast' [6], Yakutia [14, 16], Tuva [17], Buryatia [16], Primorsky Kray [7, 10, 11], Chukotka [16].

Remarks

Several studies redescribed the morphology of *A. subula* from its type host (Gendre 1913, Chabaud and Petter 1961, Jögis 1971, Smogorzhevskaya 1990). They reported males with smaller body dimensions and shorter spicules than those in the original description (Table I). However, they all resemble the original description by spicule length ratio. We believe that metrical differences are within the variation of the species and consider these identifications as reliable.

Okulewicz (1982) reported *A. subula* from *Hippolais icterina* and *Ficedula hypoleuca* in Poland. These samples are

Table II. Metrical data of *A. skrjabini* from various hosts and localities

Source	Ozerskaya (1926)	Singh (1948)	Baron (1967)*	Mawson (1972)	Smogorzhevskaya (1990)	Sato *et al.* (2005)		Present study	Present study
Host	***Passer domesticus***	***P. domesticus***	***P. domesticus***	***Tiaris canorus, Lonchura malacca, Estrilda melpoda***	***P. domesticus***	***P. montanus***	***Sturnus cineraceus***	***Calandrella brachydactyla***	***Passer hispaniolensis, P. domesticus***
Locality	**Russia**	**India**	**England**	**Australia**	**Ukraine**	**Japan**	**Japan**	**France****	**Bulgaria**
Male	**n = 3**	**n = 1**	–	–	–	**n = 13**	**n = 3**	**n = 3**	**n = 2**
Body length (mm)	8.8–9.2	5.5	7.0–9.8	7.1–9.0	5.0–7.0	3.6–6.7	4.2–4.6	5.3–5.8	7.0–7.6
Maximum body width	150–190	154	140–180	–	140	110–130	100–120	114–160	111–141
Tail, length	200 (n = 1)	196	198–200	–	–	184–245	154–185	180–195	198–225
Buccal cavity, length	160–194	111	151–171	130–170	100–144	100–150	140–150	135–143	154–166
Muscular oesophagus, length	400–460	286	396–499	420–730	320	260–410	250–310	320–337	434–456
Glandular oesophagus, length	930–1,080	720	990–1,044	1,200–1,900**	747	510–830	550–710	670–725	894–988
Cordons, length	250–280	177	280–290	200–330	188–232	130–260	180–230	192–230	251–288
Left spicule, length	205–239	163	178–263	190–200	160–200	174–191	174–183	178–195	195–213
Right spicule, length	125–148	121	105–153	110–140	96–132	117–147	104–132	138–140	129–135
$I_{CL/BL}$	0.027–0.031	0.032	–	–	–	–	–	0.036–0.042	0.036–0.038
$I_{mES/gES}$	0.401–0.455	0.397	–	–	–	–	–	0.454–0.485	0.439–0.510
$I_{ES/BL}$	0.145–0.173	0.183	–	0.167–0.222	–	–	–	0.180–0.195	0.178–0.202
$I_{Lsp/Rsp}$	1.615–1.640	1.347	–	1.400–1.700	–	–	–	1.271–1.393	1.439–1.444
Pairs of postcloacal papillae	6	7	6	7	7	7	7	7	7
Female	**n = 4**	**n = 1**	–	–	–	**n = 11**	**n = 1**	–	**n = 2**
Body length (mm)	22.3–28.3	25.6	16.0–24.0	23.9–28.4	21.0–30.0	17.8–24.6	10	–	30.0–30.8
Maximum body width	137–220	285	170	–	188–248	140–220	130	–	241–259
Tail, length	170–250	308	150–170	–	176	120–210	200	–	188–237
Buccal cavity, length	182–228	188	151–158	160–200	130–200	130–200	160	–	190–219
Muscular oesophagus, length	592–706	740	510–520	600–1,200	360–614	430–660	420	–	762–768
Glandular oesophagus, length	1,174–1,270	1,260	924–1,056	1,500–2,000**	1,129–1,328	700–1,180	900	–	1,340–1,459
Cordons, length	230–380	437	420–480	200–450	200–320	200–390	300	–	366–483
Vulva from anterior end, distance (mm)	11.1–13.1	12.8	8.4–12.8	11.2–14.9	5.0–10.0	6.7–12.2	4.8	–	14.5
Eggs	43–48 × 28	37 × 23	29–35 × 18–22	40–43 × 23–24	32–40 × 20–24	38–42 × 24–26	37–41 × 21–23	–	–
$I_{CL/BL}$	0.010–0.016	0.017	–	–	–	–	0.030	–	0.012–0.016
$I_{mES/gES}$	0.467–0.547	0.587	–	–	–	–	0.467	–	0.522–0.573
$I_{ES/BL}$	0.065–0.091	0.078	–	0.064–0.078	–	–	0.132	–	0.070–0.072

*Identified as *Acuaria subula*; **The length of the entire oesophagus. ***Type series of *Acuaria buttnerae*.

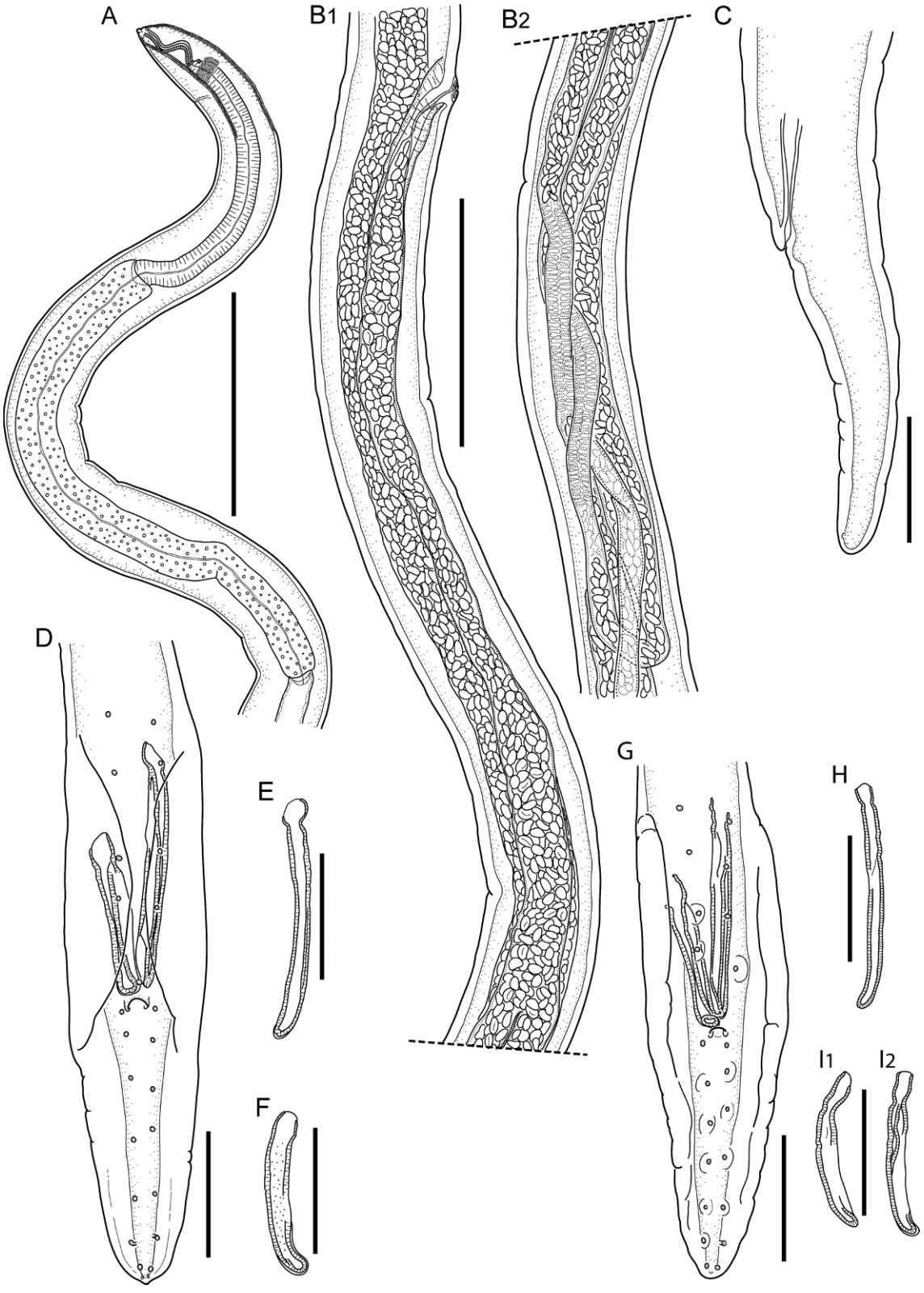

Fig. 4. *Acuaria skrjabini*. **A-C.** Females from *Passer domesticus*. **A.** Anterior end, lateral view. **B1, B2.** Details of female genital system, lateral view. **C.** Posterior end, lateral view; **D-F.** Males from *Passer hispaniolensis*. **D.** Posterior end, ventral view. **E.** Left spicule, sinistral view. **F.** Right spicule, dextral view. **G-I.** Males from *Calandrella brachydactyla* (syntypes of *A. buttnerae*). **G.** Posterior end, ventral view. **H.** Left spicule, sinistral view. **I.** Right spicules, dextral view. Scale bars = 500 μm (A, B); 100 μm (C-I)

characterized by morphological characters similar to those of specimens from *E. rubecula* and appear to represent other reliable host records of *A. subula* (Table I).

According to Sobolev (in Kurashvili 1957 and Skrjabin *et al.* 1965), *Acuaria skrjabini* is a junior synonym of *Acuaria subula*. The measurements supporting his study vary in broad ranges. He reported a body length of 3.4–9.2 mm in males and 15.5–27.0 mm in females, length of the muscular oesophagus 120–456 in males and 360–706 in females, length of glandular oesophagus 400–1,600 in males and 840–1,580 in females, and lengths of spicules 140–230 (left) and 88–132 (right). Skrjabin *et al.* (1965) accepted conditionally *A. subula* and *A. skrjabini* as distinct species. The lack of information about hosts of specimens studied by Sobolev makes these metrical data unreliable. Therefore, the reasons for considering these two species as synonymous remain unclear.

Baron (1967) reported *A. subula* from *P. domesticus* in England. The specimens from these samples differ from those described from *E. rubecula* by their larger body dimension (7.0–9.8 vs 3.8–7.0 mm in males and 16.0–24.0 vs 8.6–18.0 mm in females). However, they have a shorter glandular oesophagus (990–1,044 vs 400–1,600 mm in males and 924–1,056 vs 1,233–1,923 in females). In our opinion, the nematodes from *P. domesticus* from England identified by Baron (1967) as *A. subula* possess a morphology corresponding to *A. skrjabini* (Table II).

Bashkirova (1960) reported *A. subula* as a parasite of *Passer montanus*, *Streptopelia decaocto* and *Picus canus*. These samples are characterized by very long cordons (1,800 in males and 2,500 in females), which do not correspond to those of *A. subula* (Table I). Therefore, we consider these identifications as requiring further study.

The specimens from Curonian Spit and Bulgaria possess smaller body dimensions than those from the original description of *Acuaria subula* (Table I). However, the lengths of their spicules and the spicule length ratio are comparable with the description by Dujardin (1845). The specimens of *A. subula* studied are in the known range of morphological variation of this species. On the basis of these comparisons, we identify specimens described here as *A. subula*.

***Acuaria skrjabini* (Ozerskaya, 1926) Cram, 1927** (Figs 4–5)
Syns: *Cheilospirura skrjabini* Ozerskaya, 1926; *Acuaria buttnerae* Chabaud et Petter, 1961, new synonymy

Material studied

From *Passer hispaniolensis* (Temminck), vicinity of Nova Cherna (44°01′N, 26°26′E), Silistra Region, NE Bulgaria, 2 July 2005, IBER-BAS N000.940 (2 males); from *Passer domesticus* (L.), vicinity of Nova Cherna (44°01′N, 26°26′E), Silistra Region, NE Bulgaria, 11 November 2007, 2 females, IBER-BAS N001.047 (1 female), N001.056 SEM stub (1 female); another host specimen with the same collection data, IBER-BAS N001.048 (1 female);

MNHN no. 15C (syntypes), 3 males of *Acuaria buttnerae* Chabaud et Petter, 1961, from *Calandrella brachydactyla* (Leisler) (Passeriformes, Alaudidae), Banyuls-sur-Mer, France.

Description (based on specimens from *Passer* spp. from Bulgaria). Medium-sized acuariid nematodes. Anterior end with 2 triangular pseudolabia, each bearing single amphid and pair of papillae (Fig. 5A, B). Cordons four, arise dorsally and ventrally between pseudolabia, extending laterally in longitudinal direction to level of first and second quarter of muscular oesophagus (Fig. 4A). Each cordon consisting of two rows of cuticular plates differing by size and shape (Fig. 5C, D). Plates of inner row with rectangular shape, shorter than distance between cuticular striations. Plates of outer row with more heterogeneous shape, longer than distance between cuticular striations. Both cuticular rows delimiting longitudinal canal. Deirids small, bifurcate (Fig. 5C, E), situated anteriorly to nerve ring. Cuticular striations present, interrupted along lateral sides of body (Fig. 5F). Excretory pore posterior to nerve ring. Buccal cavity long. Phasmids subterminal.

Male (from *Passer hispaniolensis*, n = 2, see Table II for other measurements): Maximum width about mid-body; body width at level of cloaca 59–70. Cordons 9 wide. Deirids and excretory pore at 182–213 and 274–304, respectively, from anterior body end. Buccal cavity 10–12 wide. Muscular oesophagus 26–32 wide. Glandular oesophagus 136–152 wide. Nerve ring at 183–185 from anterior body length. Cuticle 11–12 thick; distance between cuticular striations about 5 μm. Caudal alae 438–465 long. Caudal papillae 7 pairs (4 precloacal and 7 postcloacal); one single ventral precloacal papilla (Fig. 4D). Left spicule 12 wide (Fig. 4E). Right spicule 18–19 wide (Fig. 4F).

Female (from *Passer domesticus*, n = 2 except otherwise stated; see Table II for other measurements): Maximum body width at vulva; body width at anus 75–100. Cordons 11–12 wide. Deirids and excretory pore at 201–237 and 329–349, respectively, from anterior end. Buccal cavity 14–15 wide. Muscular oesophagus 43–54 wide. Glandular oesophagus 93–100 wide. Nerve ring at 237 (n = 1) from anterior body end. Cuticle 21–23 thick; distance between cuticular striations 7–8. Circular cuticular folds surround vulva (Fig. 5G). Female reproductive system didelphic. Vagina simple, posteriorly directed, with circular musculature, passing into long ovejector with thin muscular walls, filled with eggs (Fig. 4B). Uteri two; their terminal parts lined with epithelium consisting of tall, narrow cells. $I_{V/BL}$ 0.431–0.492.

Observations on the type material of Acuaria buttnerae

Metrical data for these specimens are presented in Table II. Details of their morphology are illustrated in Fig. 4G-I.

Host range and geographical distribution

Records: [1] Ozerskaya (1926); [2] Singh (1948); [3] Mason *et al.* (1978); [4] Mawson (1972); [5] Smogorzhevskaya *et al.*

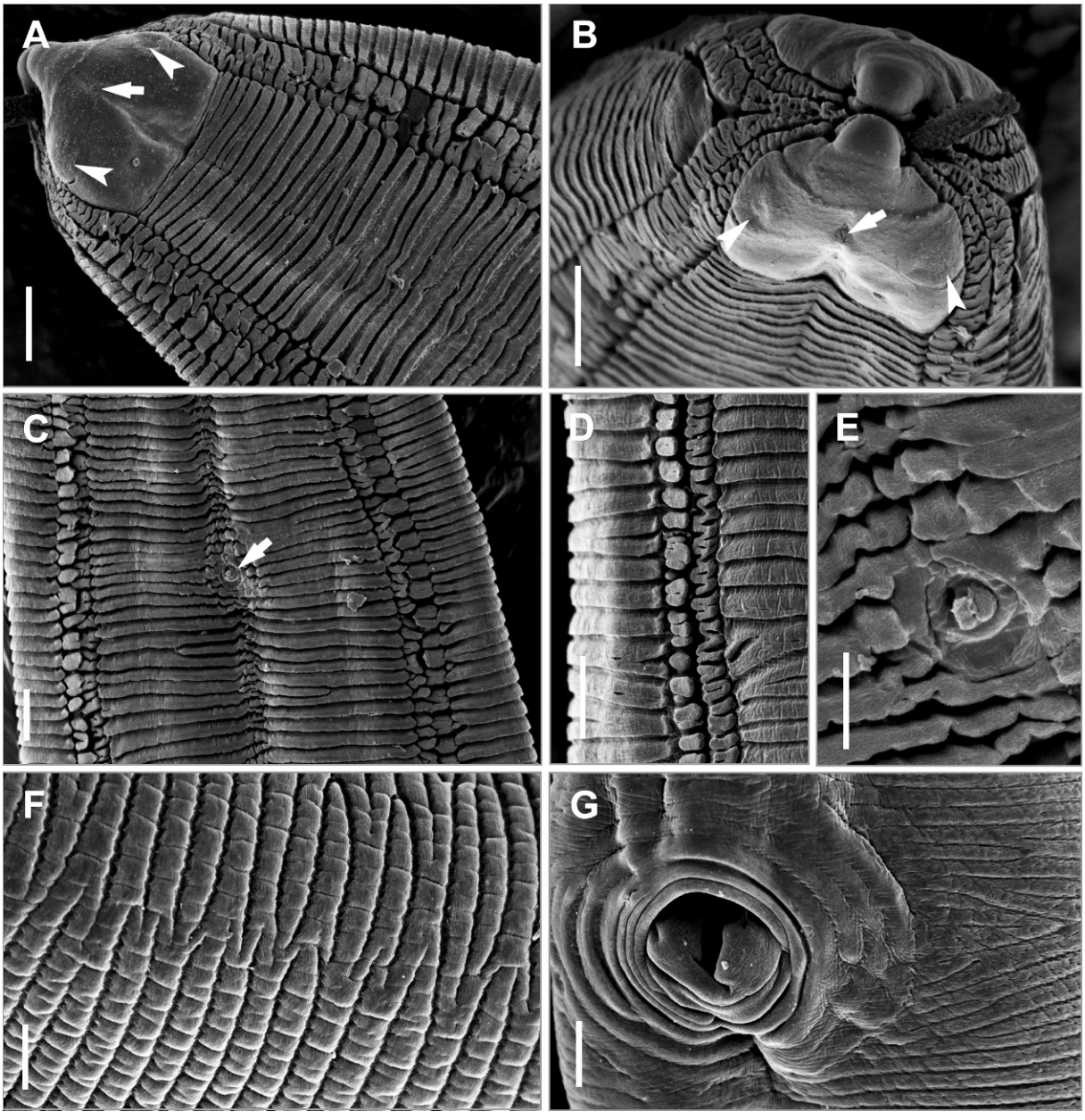

Fig. 5. A-G. *Acuaria skrjabini*, female, SEM. **A.** Anterior end, lateral view; note cephalic papillae (arrowheads) and amphid (arrow). **B.** Anterior end, apical view; note cephalic papillae (arrowheads) and amphid (arrow). **C**. Cordons and deirid (arrow). **D**. Detail of cordon structure. **E.** Deirid. **F**. Cuticle striation posterior to cordons, lateral body surface. **G.** Vulva. Scale bars = 10 μm (A-D, F, G); 5 μm (E)

(1978); [6] McOrist *et al.* (1982); [7] Petrova (1984); [8] Sato *et al.* (2005); [9] present study.

Descriptions: 1, 2, 4, 5, 8, 9; see also Skrjabin *et al.* (1965).

Type-host: *Passer domesticus* (L.) [1, 2, 5, 9] (Passeriformes, Passeridae).

Other hosts: Passeriformes, Alaudidae: *Calandrella brachydactyla* (Leisler) [9]; Estrildidae: *Erythrura psittacea* (Gmelin) [6], *Estrilda melpoda* (Vieillot) [4], *Lonchura malacca* (L.) [4], *Neochmia modesta* (Gould) [6], *Poephila acuticauda* (Gould) [6], *Uraeginthus bengalus* (L.); Passeridae: *Passer hispaniolensis* (Temminck, 1820) [9], *Passer montanus* (L.) [8]; Prunellidae: *Prunella collaris* (Scopoli, 1769) [8]; Sturnidae: *Sturnus cineraceus* Temminck [8]; Thraupidae: *Tiaris canorus* (Gmelin) [4].

Distribution: Russia, Rostovskaya Oblast' (type locality) [1], France [9], England [9], Bulgaria [7, 9], Ukraine [5], India [2], Japan [8], New Zealand [3], Australia [4, 6].

Remarks

The above-described males from *P. hispaniolensis* have smaller body dimensions than those from the original description (Oz-

erskaya 1926). However, they resemble it by the length of the buccal cavity, the lengths of the muscular and glandular oesophagus and the ratio between them, the length of the cordons and the lengths of the spicules (Table II). The females from *P. domesticus* from Bulgaria differ from those originally described by Ozerskaya (1926) by their longer body, longer muscular and glandular oesophagus and longer cordons; however, these samples are similar by the ratio between lengths of the muscular and glandular oesophagus, the ratio between lengths of the entire oesophagus and the length of the body, and the ratio between the length of the cordon and the length of the body (Table II). The observed metrical differences are in the limits of the known intraspecific variation of acuariid species.

Previous articles have also reported substantial morphological variability for *A. skrjabini*. The males from the original description (Ozerskaya 1926) differ from those reported subsequently from *Passer* spp. (Singh 1948, Smogorzhevskaya 1990, Sato *et al.* 2005) by their greater body dimensions, longer left and right spicules, greater ratio between spicules and presence of six (instead of seven) pairs of postcloacal papillae (Table II). However, the females of these records closely resemble the original description (Table II) and we believe that the above-mentioned differences reflect the infraspecific variation of *A. skrjabini*. Moreover, the samples from birds in captivity (*Lonchura malacca*, *Tiaris canorus* and *Estrilda melpoda*) from Australia (Mawson 1972) exhibit a wide range of variation relative to the lengths of body, oesophagus and spicules as well as the spicule ratio (Table II).

As discussed in the remarks on *A. subula*, the specimens identified as *A. subula* from *P. domesticus* in England (Baron 1967) morphologically correspond to *A. skrjabini*.

In the course of the present study, we re-examined the type material of *A. buttnerae* Chabaud et Petter, 1961 from *Calandrella brachydactyla* from France. These specimens are characterized by smaller body dimensions compared to the original description of *A. skrjabini* (Table II). However, they are in the range of variation of *A. skrjabini* and resemble the other descriptions of this species, especially relative to the length of muscular and glandular oesophagus, length of cordons, length of left and right spicules as well as by the ratios $I_{CL/BL}$, $I_{mES/gES}$, $I_{ES/BL}$ and $I_{Lsp/Rsp}$. The specimens from *C. brachydactyla* correspond to those from *Passer* spp. described in the present article by the detailed structure of the cordons and deirids. Therefore, we recognize *A. buttnerae* as a junior synonym of *A. skrjabini*.

Discussion

In contrast to previous opinions (Skrjabin *et al.* 1965, Sonin *et al.* 1983), the present study demonstrates that *Acuaria subula* and *A. skrjabini* are distinct species. The males of *A. subula* and *A. skrjabini* are similar relative to their body dimensions and the length of the spicules (Tables I and II). However, *A. subula* can be distinguished from *A. skrjabini* on the basis of the following characters of males: larger ratio of the length of cordons to the length of body (0.049–0.078 vs 0.027–0.042), smaller ratio of the length of muscular oesophagus to the length of glandular oesophagus (0.253–0.345 vs 0.397–0.510) and larger ratio of the total length of oesophagus to the length of body (0.319–0.476 vs 0.145–0.222) (values of ratios are summarized from the known ranges of variation, see Tables I and II).

Females of *A. skrjabini* reach larger body dimensions than those of *A. subula* (Tables I and II). The females of *A. subula* can be differentiated from those of *A. skrjabini* by the smaller ratio of the length of muscular oesophagus to the length of glandular oesophagus (0.259–0.340 vs 0.467–0.573) and the greater ratio of total length of oesophagus to the body length (0.129–0.280 vs 0.064–0.132). The ratio of the length of cordons to the length of body of females of *A. subula* and *A. skrjabini* can also be a useful distinguishing character, though exhibiting substantial overlapping (0.022–0.078 vs 0.010–0.030, respectively), which is probably due to measuring females at various degree of maturation (Tables I and II). In addition, the cordons of *A. skrjabini* are only half the length of the muscular oesophagus (Fig. 4A) while those of *A. subula* reach the end of the oesophagus (Fig. 1A).

The plates forming the cordons in these two species exhibit a different morphology. They are rather uniform, with the posterior rim of each plate overlapping the anterior part of the adjacent posterior plate in *A. subula* (Fig. 2D) while, in *A. skrjabini*, the plates of the inner row are different in shape and size from those of the outer row (Fig. 5D). The deirids of *A. subula* are cylindrical (Fig. 2E) while those of *A. skrjabini* possess a split distal part (Fig. 5E). Another difference between the two species is associated with the particular irregular mosaic ornamentation of the cuticle in the ventral and lateral sides of body around the region of vulva of *A. subula* (Fig. 3A, C) and its absence in *A. skrjabini*.

The detailed structure of the cordons was revealed as an important and reliable character for the taxonomy of the genus *Acuaria* (see Williams 1929, Baruš and Garrido 1968, Mawson 1972, Mutafchiev *et al.* 2012). The present study confirms that the pattern of the cordon structure is a constant character at the species level and, together with other morphometric characters, is important for the species identification. We also confirm the usefulness and necessity of usage of morphometric indices for distinguishing species of the genus *Acuaria* as previously suggested by Williams (1929), Mawson (1972), Zhang *et al.* (2003) and Mutafchiev *et al.* (2012).

The terminal part of the female reproductive system of *A. subula* and *A. skrjabini* resembles that described for *Acuaria paraguayensis* Mutafchiev, Mariaux et Georgiev, 2012 and *Acuaria mamillaris* (Molin, 1860) (see Mutafchiev *et al.* 2012). It is characterised by a simple vagina, a long ovejector with thin muscular walls and terminal parts of uteri lined by tall, narrow epithelium cells.

Published data suggest a low level of host specificity and wide geographical ranges for both *A. subula* and *A. skrjabini*.

The morphological differences described in the present article can be used as a basis for further studies on their specificity and distribution.

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