International Journal for Parasitology: Parasites and Wildlife 22 (2023) 199–204

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Protostrongylus caprae Zdzitowiecki et Boev, 1971 (Nematoda: Protostrongylidae) – First record in Alpine ibex (*Capra ibex* Linnaeus, 1758) from Europe

Mariana Panayotova-Pencheva [a,*], Martin Visser [b], Steffen Rehbein [b]

[a] Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bl. 25, 1113, Sofia, Bulgaria
[b] Boehringer Ingelheim Vetmedica GmbH, Kathrinenhof Research Center, Walchenseestr. 8-12, 83101, Rohrdorf, Germany

ARTICLE INFO

Keywords:
Protostrongylus caprae
Protostrongylidae
Alpine ibex
Europe

ABSTRACT

In the course of a survey of the endoparasites of Alpine ibex (*Capra ibex*) from Austria, examination of the lungs revealed male *Protostrongylus* nematodes presenting morphological characters which differed from those of the three *Protostrongylus* species previously reported from this host. Fragments of 16 adult male and 4 adult female nematodes extracted from the lung tissue of two female ibex following peptic digestion were subjected to close microscopic examination. Based on their morphology, the lungworms were identified as *Protostrongylus caprae* Zdzitowiecki et Boev, 1971. This species was originally described from Siberian ibex (*Capra sibirica*) in Asia and previously only reported from this host from Kazakhstan and Mongolia. The identification of *P. caprae* in Alpine ibex represents a new host and geographical record and reinforces the interest to further study the parasite diversity of wild ungulates for a better understanding of complex host-parasite associations and biogeography.

1. Introduction

Protostrongylids (Nematoda: Protostrongylidae) are parasitic nematodes with an indirect life cycle. Their final hosts are ruminants and lagomorphs and, exceptionally, carnivores while terrestrial molluscs serve as intermediate hosts (Boev, 1975). Protostrongylids are also known as 'small lungworms' because most species reside in the lungs. Many species are found deep in the lung tissue (terminal bronchioles and alveoli - the 'lung parenchyma') causing focal chronic lesions. These host reactions consist usually of a layer of fibrous connective tissue surrounding adult nematodes, larvae, eggs and amorphous debris. Although protostrongylid lungworm infections remain usually subclinical, they have been associated with impaired respiratory function, pneumonia and other negative effects in parasitized hosts (Jenkins et al., 2007; Panayotova-Pencheva, 2008).

Pulmonary protostrongylids are important parasites of ungulates worldwide with a considerable diversity among the Caprinae species (Carreno and Hoberg, 1999). The study of protostrongylids in wild ruminants goes back to the late 19th century when hair-like nematodes were isolated from the lungs of a chamois (*Rupicapra rupicapra*) which has died during a pneumonia epidemics in Bavaria (Mueller, 1889). These lungworms, which earlier had been described from sheep, were given the name *Pseudalius capillaris* (later re-named to *Muellerius capillaris* (Mueller, 1889)). Until now, 19 species of protostrongylid lungworms belonging to six genera (*Cystocaulus*, *Muellerius, Neostrongylus, Protostrongylus, Spiculocaulus*, *Varestrongylus*) have been described parasitizing wild bovids of the subfamily Caprinae (Boev, 1975; Kontrimavichus et al., 1976; Panayotova-Pencheva, 2006).

Alpine ibex (*Capra ibex*) from the Alpine Mountain range in Europe have been reported to be host to eight species of pulmonary protostrongylids, namely *Cystocaulus ocreatus*, *Muellerius capillaris*, *M. tenuispiculatus*, *Neostrongylus linearis*, *Protostrongylus hobmaieri*, *P. rufescens*, *P. rupicaprae* and *Spiculocaulus austriacus* (Rehbein et al., 2009).

The aim of this work is to report, for the first time, the finding of a species of small lungworm of the genus *Protostrongylus*, which is not currently assigned to the parasite fauna of the Alpine ibex.

2. Materials and methods

In the course of a survey of the endoparasites of Alpine ibex from Austria during the years of 2019–2021, the lungs of 17 ibex from the Pitztal valley in the central Alps in Tyrol (St. Leonhard i. Pitztal, 47° 3′ 58.802″ N, 10° 50′ 50.246″ E) were examined. Lengthwise opening of the

* Corresponding author.
E-mail addresses: marianasp@abv.bg (M. Panayotova-Pencheva), vissermum@gmail.com (M. Visser), Steffen.Rehbein@boehringer-ingelheim.com (S. Rehbein).

https://doi.org/10.1016/j.ijppaw.2023.10.009
Received 27 September 2023; Received in revised form 19 October 2023; Accepted 19 October 2023
Available online 20 October 2023
2213-2244/© 2023 Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

accessible air passages revealed three *D. filaria* nematodes in one ibex. For the recovery of lung parenchyma dwelling protostrongylids, the lung tissue was ground and subjected to peptic digestion (one well-mixed 100 g sample per lung) as described earlier (Rehbein and Visser, 2002). Fragments of protostrongylid nematodes were recovered from digested lung material of 16 ibex and stored in 10% formalin. Based on their morphology they were identified as *M. capillaris* (13 ibex), *N. linearis* (7 ibex), *S. austriacus* (3 ibex), and recovered from six ibex *Protostrongylus* sp. which could not be referred to any of the three *Protostrongylus* species known in Alpine ibex.

For close examination and identification of the *Protostrongylus* sp. nematodes, caudal ends of 20 specimens (16 males and 4 females) obtained from two ibex – one female kid harvested in December 2019 and one 13 years old female in poor health harvested in August 2021 were used. Only *Protostrongylus* sp. were recovered from the digested lung material of the kid while *Protostrongylus* sp. and *M. capillaris* were isolated from the adult female ibex. Specimens used for identification were examined by light microscopy, either native or cleared in lactophenol. The methodology of collection and preservation of the material did not allow for molecular analysis. Identification was done on morphological characters according to the polytomic key for protostrongylids (Kontrimavichus et al., 1976) and descriptions of species by Boev (1975). Imaging and measurements (in micrometers) of relevant structures were performed using a Motic Images Plus 3.0 camera connected to an Amplival microscope, with accompanying software. Morphometric data were processed using Microsoft®Excel. Some specimens, stored in 10% formalin or embedded in glycerin-gelatin (permanent microscopic preparations), were deposited in the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria.

3. Results

Nematodes that could not be referred to any of the three *Protostrongylus* species known in Alpine ibex until now are briefly described below.

Males. Caudal end presented typical structures of nematodes of the family Protostrongylidae including copulatory bursa, two spicules, gubernaculum and telamon. Short lateral alae were also visualized in some specimens (Fig. 1). The copulatory bursa consisted of two symmetrical parts (Fig. 2). Ventral bursal rays begin with a common trunk, split midway along their length, and almost reach the edge of the copulatory bursa. Antero-lateral rays separate, not extending to the bursal margin. Medio- and postero-lateral rays begin with a common trunk, confluent in their first half and almost reach the bursal margin; the postero-lateral rays slightly shorter than medio-lateral rays. Extero-dorsal rays shorter than other rays, separate, and not reaching the margin of the bursa. Dorsal ray is very small with a hemispheric shape. The telamon is well developed and clearly visible (Fig. 3). Spicules consisting of a spongiform stem and comb-like wings (Fig. 4). The distal end of the spicule stems is compact and rounded. At their beginning the wings are narrower and gradually widened. At the distal end of the spicules, the wings become more delicate, separated from the stem, and protruding above it (Fig. 4). Brown gubernaculum, consisting of capitulum, corpus and crura (Fig. 5). Capitulum small, sickle-shaped, with two elongated ears. Corpus built of two peculiarly shaped parts, proximally threadlike, almost transparent and hardly visible, and distally wider, well chitinized and clearly visible. The crura constitute the most massive part of the gubernaculum and presenting two or three teeth of characteristic shape at the distal end.

Females. Posterior end pointed with vulva opens close to it; well-defined sub-vulvar protuberance visible. Provagina is well developed, hood-shaped. Eggs with the shape of an elongated ellipse in the uterus (Fig. 6).

Results of the measurements of morphological characters of the helminths are shown in Table 1.

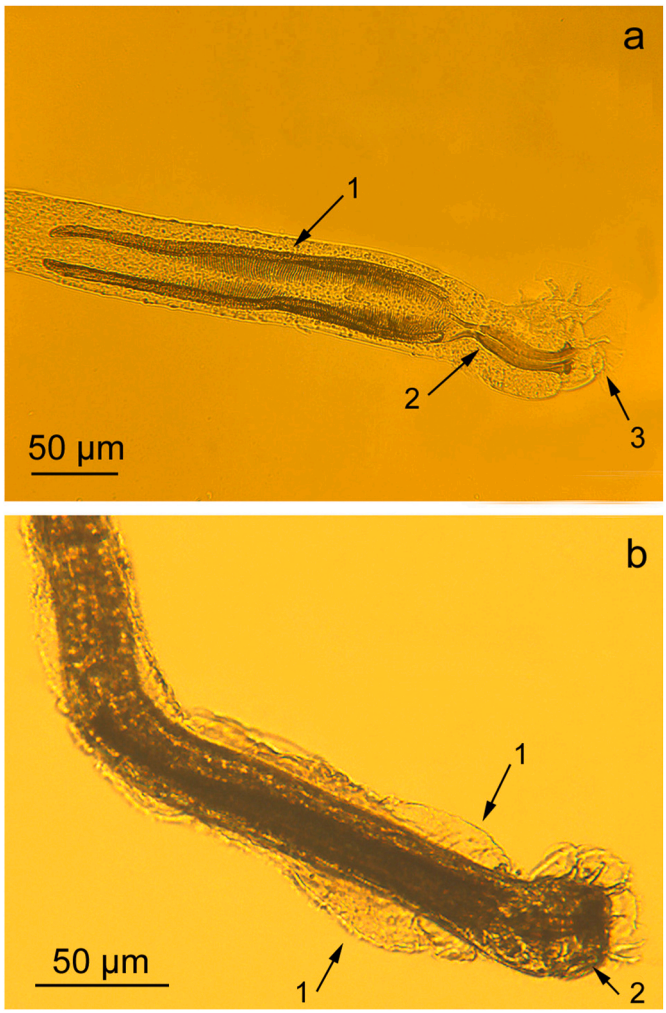

Fig. 1. Male *P. caprae* from an Alpine ibex from Austria: a) Ventral view: 1 — spicules, 2 —gubernaculum, 3 — copulatory bursa. b) Dorsal view: 1 — lateral alae, 2 — telamon. (original pictures).

Comparing the morphological characters of the nematodes studied with those of the previously described protostrongylid species (Boev, 1975), including the original description of *Protostrongylus caprae* by Zdzitowiecki and Boev (1971), and considering the protostrongylid differentiation key pointed out by Kontrimavichus et al. (1976), the protostrongylids recovered from the lungs of Alpine ibex from the Pitztal valley in Austria were determined as *P. caprae*.

4. Discussion

To the best knowledge of the authors, the finding of *P. caprae* lungworms in the ibex from Austria constitutes the first record of this parasite in the Alpine ibex and in Europe and thus represents a new host and geographical record for this protostrongylid species. *Protostrongylus caprae* was described as a new species based on nematodes isolated from the lungs of Siberian ibex from Kazakhstan Tian Shan Mountains (Zailiiskii Ala-Tau and Djungarskii Ala-Tau) by Zdzitowiecki and Boev (1971). Zdzitowiecki and Boev (1971) separated *P. caprae* from *P. davtiani* (Savina, 1940; Davtian, 1949 which is primarily a parasite of domestic sheep and goats, and argali (*Ovis ammon*) in central Asia. The authors considered the Siberian ibex to be the type-host and only host of *P. caprae*. Ten years after the first description, *P. caprae* was reported from Siberian ibex from Mongolia (Tazieva et al., 1981; reviewed recently by Sharhuu and Sharkhuu (2004)). However, no *P. caprae* were identified from recently studied Siberian ibex in Uzbekistan (Kuchboev

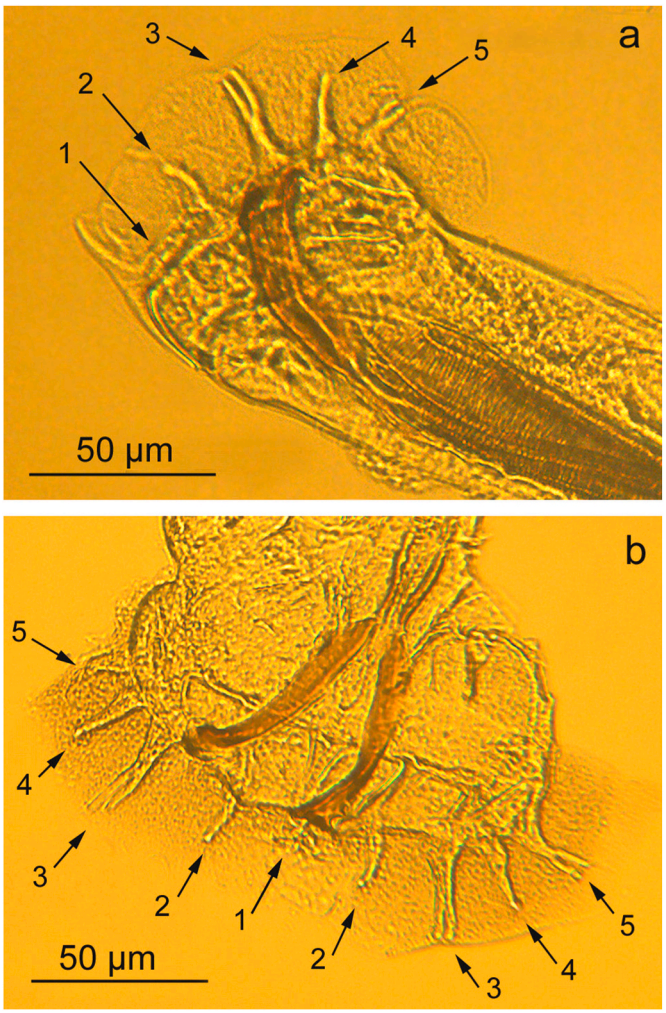

Fig. 2. Male *P. caprae* from an Alpine ibex from Austria: a) Dorsolateral view of copulatory bursa: 1 — dorsal ray, 2 — exterodorsal ray, 3 — postero-lateral and medio-lateral rays, 4 — antero-lateral ray, 5 — ventral rays. b) Two symmetrical parts of copulatory bursa: 1 — dorsal ray, 2 — exterodorsal ray, 3 — postero-lateral and medio-lateral rays, 4 — anterolateral ray, 5 — ventral rays. (original pictures).

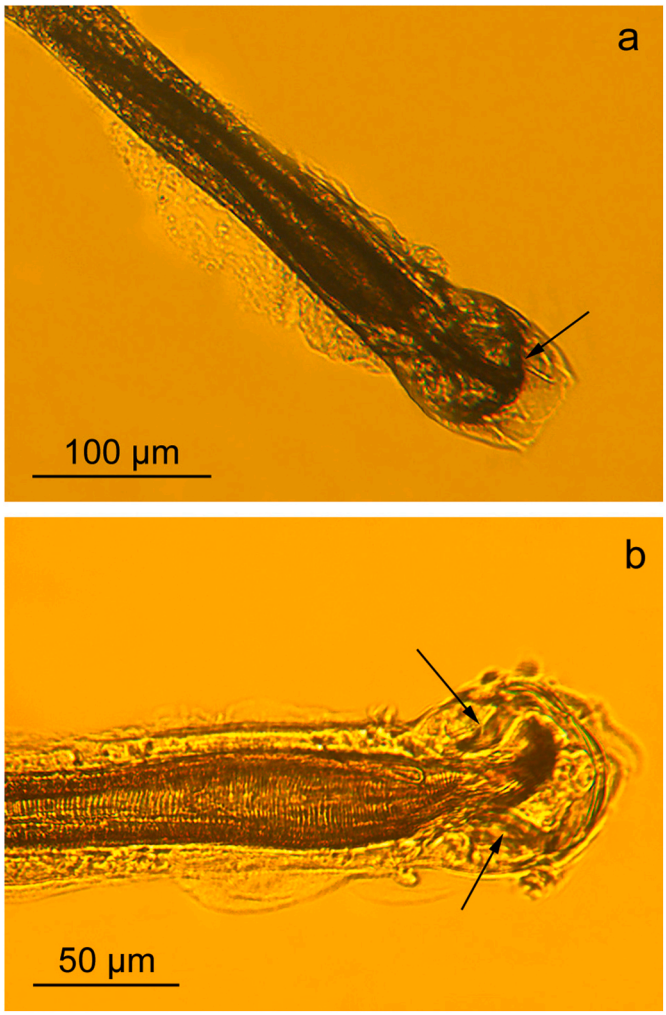

Fig. 3. Male *P. caprae* from an Alpine ibex from Austria: a) Dorsal view: basal and lateral plates of telamon. b) Ventral view: ventral plates of telamon. (original pictures).

et al., 2017).

As summarized by Rehbein et al. (2009) Alpine ibex have been reported previously to be host of eight species of small lungworms including three of the genus *Protostrongylus*: *P. hobmaieri*, *P. rufescens* and *P. rupicaprae*. *Protostrongylus hobmaieri* and *P. rufescens*, both dwelling the airways of the lungs, were reported from ibex in Italy or from ibex in France, Italy, Switzerland and Austria, respectively; *P. rupicaprae*, dwelling the airways and lung parenchyma, was reported from ibex in France (Rehbein et al., 2009). Recently conducted studies in Switzerland and Italy also reported *Protostrongylus* sp. infection in Alpine ibex, however they were based on fecal larval recovery and conventional microscopical diagnosis which does not allow identification of the nematodes to the species level (Zumbach et al., 1991; Marreros et al., 2012; Cassini et al., 2015; Zanet et al., 2021).

To differentiate the *Protostrongylus* nematodes recovered from the Alpine ibex from Austria from the other lungworms of the family Protostrongylidae, the criteria described in the polytomic key of Kontrimavichus et al. (1976) were considered, specifically the morphological features of the male nematodes (copulatory bursa, telamon, dorsal ray of copulatory bursa, postero-lateral ray of copulatory bursa, length of spicules, general appearance of gubernaculum, capitulum of gubernaculum, shape of gubernaculum's corpus, length of gubernaculum's corpus, appearance of gubernaculum's crura, length of gubernaculum's crura), geographical distribution, and range of the hosts.

The morphology of the gubernaculum, especially its crura, is the most noticeable differential character of *P. caprae* to the three *Protostrongylus* species reported from Alpine ibex previously. In contrast to the gubernaculum's crura of *P. caprae,* which present two or three large teeth of characteristic shape near the distal end, gubernaculum's crura of *P. hobmaieri* are sledge runners-like, smooth, without teeth (Panayotova-Pencheva, 2006). Those of *P. rupicaprae* are elongated and smooth (Panayotova-Pencheva, 2008). The gubernaculum's crura of *P. rufescens* are not smooth; however, unlike those of *P. caprae* the teeth of *P. rufescens* are small and in greater number (Panayotova-Pencheva, 2011).

There are no morphometric data of *P. caprae* other than those provided in the original description of the species (Zdzitowiecki and Boev, 1971). The comparison of the data of *P. caprae* nematodes from Alpine ibex and of *P. caprae* nematodes from Siberian ibex (Table 1) shows that measurements for multiple characters overlap. However, length of spicules and gubernaculum's crura, and length of the provagina of the specimens isolated from Alpine ibex exceeded those of nematodes recovered from Siberian ibex while the opposite was the case for the distance vulva to anus and the length of the intrauterine eggs. Such differences may be attributable to host parameters but also procedures of collection, preservation and processing of the material.

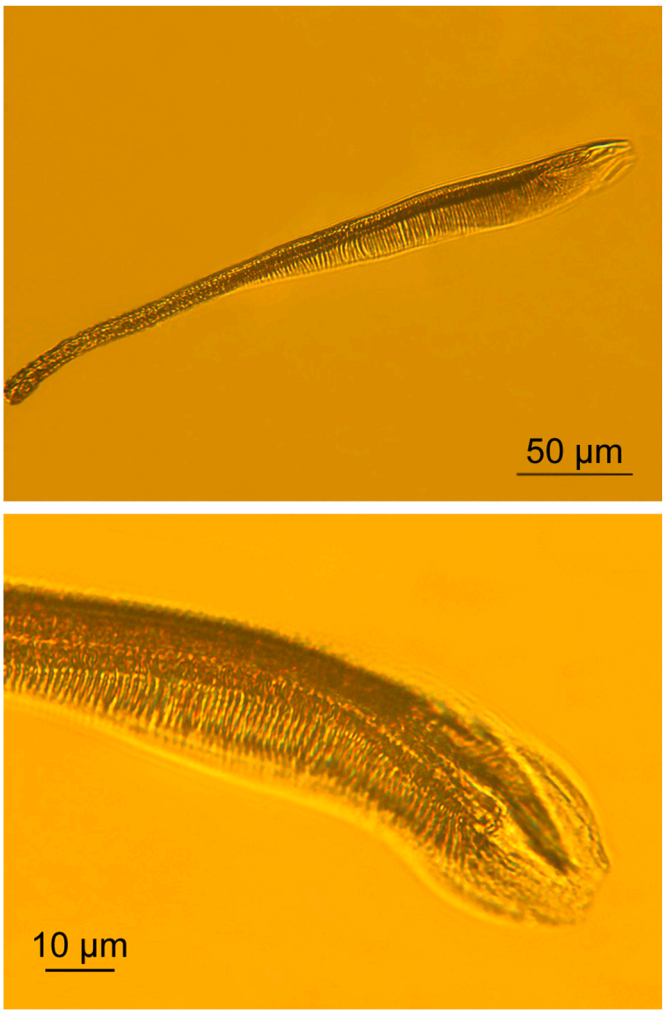

Fig. 4. Male *P. caprae* from an Alpine ibex from Austria: Spicules. (original pictures).

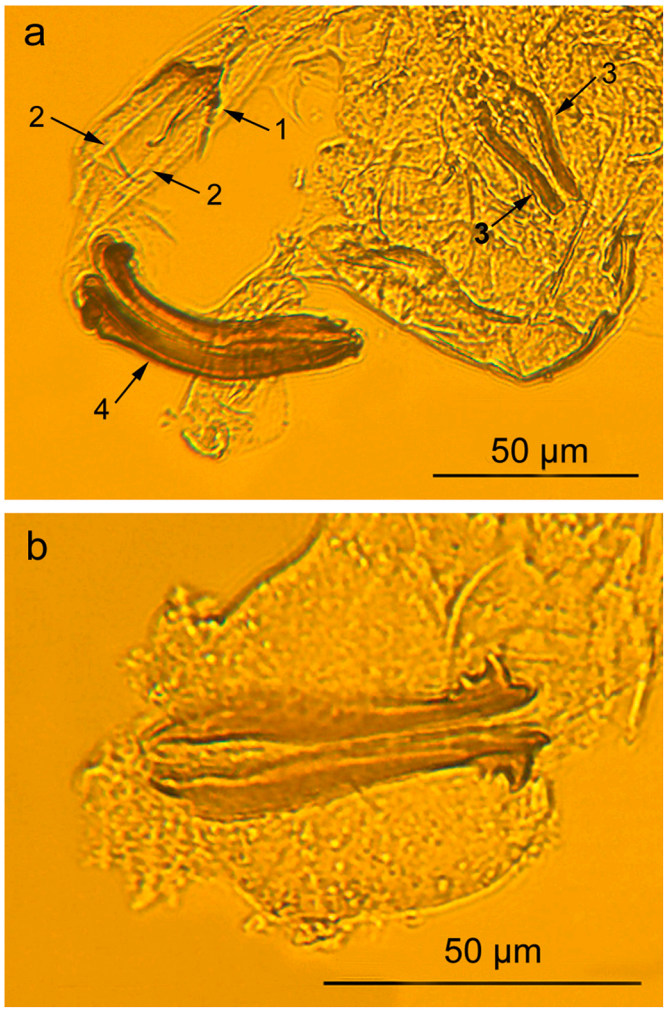

Fig. 5. Male *P. caprae* from an Alpine ibex from Austria: a) Parts of gubernaculum: 1 — capitulum, 2 — proximal parts of corpus, 3 — distal parts of corpus, 4 — crura. b) Crura of gubernaculum. (original pictures).

Unfortunately, Zdzitowiecki and Boev (1971) provided no information on age, sex and number of the ibex from which the *P. caprae* specimens were obtained nor on the preservation of the material and isolation of the parasites.

It is suggested that the genus *Capra* originates from Central Asia and spread westwards with ibex expanding into the Eastern Mediterranean during the Pleistocene ice ages and that the ancestors of the Alpine ibex migrated into Europe from Central Asia (Ahmad et al., 2022; Brambilla et al., 2020). All currently Alpine ibex in the European Alps have their roots in a small number of animals, most likely less than 100, that survived in the first half of the 19th century in the Gran Paradiso Massif of the western Alps and formed the basis for the restoration of the species in the entire Alpine Mountain range starting in 1911 (Parrini et al., 2009; Brambilla et al., 2020). The occurrence of the lungworm, *P. caprae*, in both Siberian ibex and Alpine ibex may be an indicator of the biogeographical relationship between these two widely separated caprine species.

5. Conclusion

The finding of *P. caprae* in Alpine ibex in Europe indicates that the knowledge of the parasite fauna of the wild Caprinae is not complete. Continuing systematic research is demanded for the documentation of biodiversity, correct description and identification, definition of the distribution and historical links of host – parasite relationships. This is of special importance for the tiny lung tissue dwelling protostrongylids as

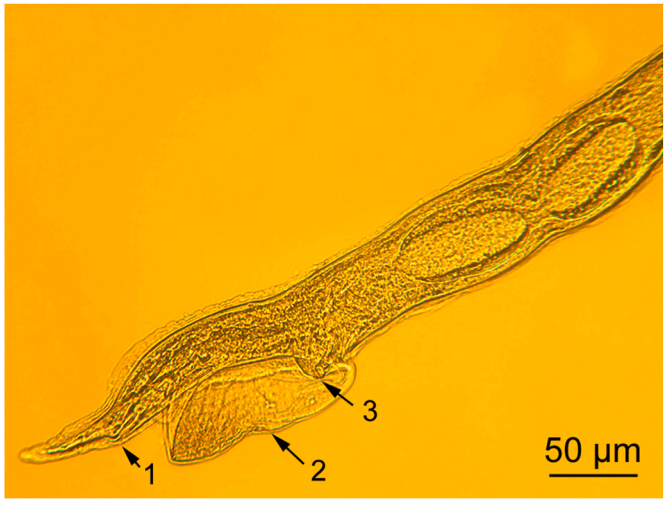

Fig. 6. Female *P. caprae* from an Alpine ibex from Austria: 1 — anus, 2 —provagina, 3 — vulva.

Table 1
Comparative morphometry of *Protostrongylus caprae* nematodes from Alpine ibex (present investigation) and Siberian ibex (Zdzitowiecki and Boev, 1971) (in micrometers).

	Parameter	Alpine ibex		Siberian ibex
		Range[a]	Mean ± SD	Range
Adult male nematodes (n = 16)	Body width directly anterior to the copulatory bursa	40.89–66.12	54.32 ± 6.17	48–68
	Common length of spicules	248.04–283.73	269.53 ± 8.85	199–247
	Length of spicule stems	235.12–267.16	259.27 ± 8.74	183–226
	BSS–BSW	55.97–86.94	77.46 ± 10.70	–
	Protrudion of the spicule wings above the stems	12.67–22	17.91 ± 2.93	16–22
	Common gubernaculum length	107.59–158.47	141.59 ± 14.78	107–151
	Length of gubernaculum's crura	54.56–72.2	64.62 ± 5.25	43–59
	Common length of gubernaculum's corpus	78.83–93.24	84.46 ± 6.83	65–97
	Length of proximal parts of gubernaculum's corpus	34–60.9	43.19 ± 10.38	32–65
	Length of distal parts of gubernaculum's corpus	35.47–41.23	38.11 ± 1.69	22–43
	Length of gubernaculum's ears	13.6–20.47	16.84 ± 2.50	13–19
	Distance between the ends of gubernaculum's ears	10.31–21.67	16.79 ± 4.15	16–22
	Length of lateral alae in the posterior end	434.18–667.95	569.60 ± 95.54	–
Adult female nematodes (n = 4)	Body width in the area of vulva	49.01–53.18	51.49 ± 2.03	43–59
	Body width in the area of anus	22.08–22.67	22.49 ± 0.28	16–22
	Distance anus – tail tip	91.38–117.24	99.66 ± 12.05	–
	Length of provagina	111.15–118.77	115.55 ± 3.25	48–81
	Distance vulva - anus	52.82–58.87	55.93 ± 2.61	59–91
	Length of eggs	65.13–96.7	79.89 ± 10.25	91–129
	Width of eggs	32.15–41.1	36.50 ± 2.75	32–48

BSS-BSW – Distance from the beginning of spicule stems to the beginning of spicule wings.
[a] All minimum values for adult males were obtained from the one specimen isolated from the kid ibex.

the recovery of adult specimens from altered lung tissue requires specific efforts. Future studies should combine classical and molecular methods as molecular markers are not only a powerful tool for the speciation of adult nematodes but may also allow for the identification of fecal *Protostrongylus*-type larvae to species level.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

The research did not receive any specific funding.

Availability of data and materials

All data is provided within the manuscript.

Authors' contributions

Conceptualization: MPP, SR; Formal analysis: MPP, SR; Investigation: MPP, MV; Writing - original draft, reviewing and editing: MPP, SR. All authors read and approved the final manuscript.

Disclaimer

This document is provided for scientific purposes only. Any reference to a brand or trademark herein is for informational purposes only and is not intended for commercial purpose or to dilute the rights of the respective owner(s) of the brand(s) or trademark(s).

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

Ahmad, S., Strelnikov, I.I., Ahmad, A., Rajpar, M.N., Kahn, M.Z., Wanghe, K., Ahmad, I. M., Nabi, G., Li, D., 2022. Recent advances in ecological research on Asiatic ibex (Capra sibirica): a critical ungulate species of highland landscapes. Global Ecol. Cons. 35, e2105 https://doi.org/10.1016/j.gecco.2022.e02105.
Boev, S.N., 1975. Protostrongilidy. Osnovy Nematodologii. Tom 25. Izdatelstvo Nauka, Moskva (In Russian).
Brambilla, A., von Hardenberg, A., Nelli, L., Bassano, B., 2020. Distribution, status, and recent population dynamics of Alpine ibex *Capra ibex* in Europe. Mammal. Rev. 50, 267–277. https://doi.org/10.1111/mam.12194.
Carreno, R.A., Hoberg, E.P., 1999. Evolutionary relationships among the Protostrongylidae (Nematoda, Metastrongyloidea) as inferred from morphological characters, with consideration of parasite-host co-evaluation. J. Parasitol. 85, 638–648. https://doi.org/10.2307/3285736.
Cassini, R., Párraga, M.A., Signorini, M., Frangipane di Regalbono, A., Sturato, E., Rossi, L., Ramanzin, M., 2015. Lungworms in Alpine ibex (*Capra ibex*) in the eastern Alps, Italy: an ecological approach. Vet. Parasitol. 214, 132–138. https://doi.org/10.1016/j.vetpar.2015.09.026.
Jenkins, E.J., Veitch, A.M., Kutz, S.J., Bollinger, T.K., Chirino-Trejo, J.M., Elkin, B.T., West, K.H., Hoberg, E.P., Polley, L., 2007. Protostrongylid parasites and pneumonia in captive and wild thinhorn sheep (*Ovis dalli*). J. Wildl. Dis. 43, 189–205. https://doi.org/10.7589/0090-3558-43.2.189. PMID: 17495303.
Kontrimavichus, V.L., Delamure, S.L., Boev, S.N., 1976. Metastrongyloidei domashnih i dikih zhivotnih. Osnovy nematodologii. In: Ryzhikov, K.M. (Ed.), Izd. Nauka, vol. 26. Moskva. (In Russian).
Kuchboev, A.E., Krücken, J., Karimova, R.R., Ruziev, B.H., Pazilov, A., 2017. Infection levels of protostrongylid nematodes in definitive caprine and intermediate gastropod hosts from Uzbekistan. J. Helminthol. 91, 236–243. https://doi.org/10.1017/S0022149X16000171.
Marreros, N., Frey, C.F., Willisch, C.S., Signer, C., Ryser-Degiorgis, M.P., 2012. Coprological analyses on apparently healthy Alpine ibex (*Capra ibex*) from two Swiss colonies. Vet. Parasitol. 186, 382–389. https://doi.org/10.1016/j.vetpar.2011.11.009.
Mueller, A., 1889. Die Nematoden der Säugethierlungen und die Lungenwurmkrankheit. Dtsch. Zschr. Thiermed. Vergl. Pathol. 15, 261–321.
Panayotova-Pencheva, M., 2006. New records of protostrongylid lungworms from wild ruminants in Bulgaria. Vet. Med. (Prague) 51, 477–484. https://doi.org/10.17221/5581-VETMED.
Panayotova-Pencheva, M., 2008. Morphological data on two protostrongylid species, etiological agents of pulmonary helminthoses in wild ruminants (materials from Bulgaria). Eur. J. Wildl. Res. 54, 285–292. https://doi.org/10.1007/s10344-007-0143-x.
Panayotova-Pencheva, M., 2011. Species composition and morphology of protostrongylids (Nematoda: Protostrongylidae) in ruminants from Bulgaria. Parasitol. Res. 109, 1015–1020. https://doi.org/10.1007/s00436-011-2337-0.
Parrini, F., Cain, J.W., Krausman, P.R., 2009. *Capra ibex* (Artiodactyla: bovidae). Mamm. Species 830, 1–12. https://doi.org/10.1644/830.1.

Rehbein, S., Visser, M., 2002. Efficacy of ivermectin delivered via a controlled-release capsule against small lungworms (Protostrongylidae) in sheep. J. Vet. Med. B 49, 313–316. https://doi.org/10.1046/j.1439-0450.2002.00576.x.

Rehbein, S., Visser, M., Hamel, D., Messner, C., 2009. Beitrag zur Kenntnis der Parasiten des Steinwildes in Deutschland. Beitr. Jagd. Wildforsch. 34, 427–439.

Sharhuu, G., Sharkhuu, T., 2004. The helminth fauna of wild and domestic ruminants in Mongolia – a review. Eur. J. Wildl. Res. 50, 150–156. https://doi.org/10.1007/s10344-004-0050-3.

Tazieva, Z.H., Isabaev, M.I., Šarhuu, G., 1981. Legočnye nematody kopytnyh mongolii. Materialy Naučn konf vsesoûzn obŝestva gel'mintol, Akad Nauk SSSR. Lab Gel'mintol, Vyp 33, 74–76 (In Russian).

Zanet, S., Ferroglio, E., Orlandini, F., Bassano, B., Battisti, E., Brambilla, A., 2021. Bronchopulmonary nematodes in Alpine ibex: shedding of first stage larvae analyzed at the individual host level. Front. Vet. Sci. 8, 663268 https://doi.org/10.3389/fvets.2021.663268.

Zdzitowiecki, K., Boev, S.N., 1971. Remarks on the classification and morphology of the nematodes of the subgenus *Davtianostrongylus* Boev, 1950, with a description of a new species. Acta Parasitol. Pol. 19, 237–246.

Zumbach, S., Kipfer, H., Lüps, P., Pfister, K., 1991. Untersuchungen zum Endoparasitenbefall von Steinböcken (*Capra i. ibex*) im Berner Oberland (Schweiz). Wien. Tierärztl. Monatsschr. 78, 383–386.