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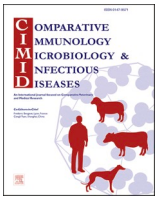

Morphometric, histopathological and molecular findings of *Macracanthorhynchus hirudinaceus* infection in wild boar (*Sus scrofa*) from continental Italy

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ABSTRACT

Although *Macracanthorhynchus hirudinaceus* is a neglected acanthocephalan of suids occasionally responsible for severe infections in humans, the spread of wild boar (*Sus scrofa*) populations in Europe could promote the circulation. Herein, we report the first morphometric, histological and molecular characterization of a severe *M. hirudinaceus* infection in a boar from continental Italy. The boar's intestine displayed granulomatous enteritis due to 24 helminths (14 females, 10 males), identified as adults of *M. hirudinaceus* by a combined morphometric/molecular approach. The phylogenetic analysis of the *cox*1 gene revealed a close relationship of the *M. hirudinaceus* sequence type found herein with those from Hungary and insular Italy. The high haplotype diversity and low nucleotide diversity of *M. hirudinaceus* specimens would suggest its rapid demographic expansion in the Mediterranean basin. More research is needed to assess the presence of *M. hirudinaceus* in susceptible beetle species and the role of boars in the epidemiology of infection.

1. Introduction

Also known as the thorny-headed worm, *Macracanthorhynchus hirudinaceus* (Archiacanthocephala, Oligacanthorhynchida) is a neglected zoonotic acanthocephalan that infects pigs (*Sus scrofa domesticus*) and wild boars (*Sus scrofa*) as definitive hosts and beetles (e.g., coleoptera of the family Scarabaeidae, commonly *Melolontha vulgaris*, *Cetonia aurata*, *Polyphylla fullo*, *Anomalia vitis*) as intermediate hosts [1,2].

Briefly, parasitic eggs containing a first-stage larvae (acanthor) are shed in the faeces of definitive hosts and ingested by intermediate hosts where the larvae mutate into a second-stage (acanthella) that develop to infective-stage (cystacanth); if ingested by the definitive host, the cystacanth becomes an adult attaching to the small intestinal wall [3]. Besides being responsible of economic losses due to the slow growth and weight loss in pigs [4], *M. hirudinaceus* occasionally infects dogs, wild carnivores and humans through the accidental ingestion of insects, especially in young children [5]. Although in the 1800 s human acanthocephaliasis was confined in the Volga Valley of Russia, by frequent consumption of the raw cockchafer beetle (*Melolontha melolontha*) [5], cases of infection have been reported worldwide in the last decades, i.e., in Africa (Madagascar, New Guinea), America (United States, Brazil), Asia (China, Thailand, Vietnam), Europe (Bulgaria, Czech Republic) and Oceania (Australia) [5]. Clinical symptoms of human acanthocephaliasis are often severe, as abdominal pain and digestive complications due to the mechanical damage caused by penetration of the parasitic proboscis inside the wall of the host's intestine [3]. Despite the relevance for public health, few reports are available to date on *M. hirudinaceus* in wild boars, viz. in Turkey [6], Iran [7,8], Belalus [9], Japan [10], Tunisia [11], Bulgaria [12], Ukraine [13], Romania [14] and Spain [15,16]. In Italy, *M. hirudinaceus* has only been found in boars from the islands of Sicily and Sardinia [2,17], except for two reports from central Italy without molecular or histological characterization [18,19]. However,

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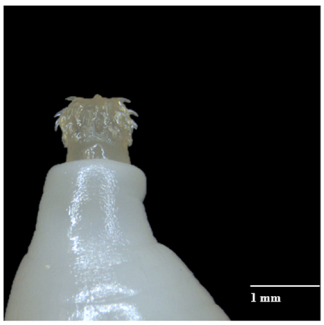

Fig. 1. Cephalic end of *M. hirudinaceus* specimen from the boar's intestine, showing typical retractile proboscis equipped with rows of hooks.

boar populations have increased throughout Europe in recent years invading crops, pastures and peri-urban areas [20,21], where these animals are potentially exposed to the parasite, likely contributing to its maintenance and transmission to animals and humans.

Herein, we report the first morphometric, histological and molecular characterization of *M. hirudinaceus* infection in a *S. scrofa* specimen from continental Italy and discuss the potential role of boars in the epidemiology of this neglected parasite.

2. Methods

2.1. Study area and sampling

In December 2022, a two-years-old female wild boar was culled within an official hunting area of southern Italy (Caserta province - 41.297580°N, 14.244231°E). The carcass was collected by hunters supported in the field by an official veterinarian of the University of Naples Federico II (Naples, Italy), under the frame of a ministerial monitoring plan for zoonoses in wildlife of southern Italy (authorization: "Decreto Dirigenziale 210-Piano B7 DPAR"). The animal was aged by using the examination of the teeth [22] and the whole carcass was delivered to the wildlife disease unit of the Department of Animal Health, Experimental Zooprophylactic Institute of southern Italy (Portici, Italy). During necropsy, helminths were found inside the small intestine of the boar with presence of deep nodular-like lesions inside the intestinal wall. Helminths and intestinal tracts were conferred to the units of Parasitology and Histology for insights, whereas a part of helminths collected in 96% ethanol was sent to the Department of Veterinary Medicine, University of Sassari (Sassari, Italy), for molecular analyses.

2.2. Morphometric analysis of specimens

Structural features of the helminths were evaluated by optical stereomicroscopy (Leica DM-LB2) with a digital camera software (Leica Las version 4.5.0) (Leica Microsystems, Wetzlar, Germany); all measurements obtained were compared to those available in the literature [2,7,23].

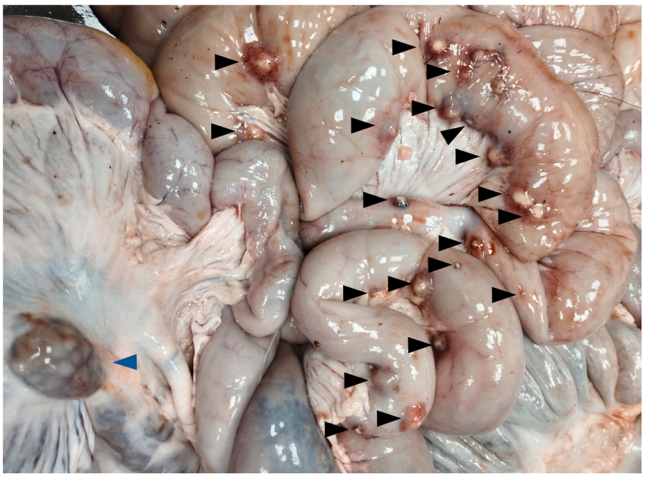

Fig. 2. Multiple granulomas (black arrowheads) in the boar's small intestine caused by *M. hirudinaceus* specimens with presence of enlarged mediastinal lymph nodes (blue arrowhead).

2.3. Histological analysis

Helminths and intestinal tracts of the boar were fixed in 10% buffered formalin, embedded in paraffin and 3 μm-thick sections were set up and stained with haematoxylin and eosin, according to D'Aquino et al. [24].

2.4. DNA extraction and PCR

DNA was extracted from helminths using G-spin™ Total DNA Extraction kit (iNtRON Biotechnology, Korea), according to the manufacturer's instructions. PCR amplification of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1) was performed using primers Acanth_Cox1-F1 (5′-TTTGGAGGGGTTTGATGGGT-3′) and Acanth_Cox1-R4 (5′-CCCCAGACATTCTTCTCTCCA-3′) with cycling conditions according to Kamimura et al. [10]. Amplicons were purified using Nucleospin Gel and PCR Clean Up (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

2.5. Sequencing, polymorphism analyses and phylogenesis

Amplicons were sequenced in both directions with primers Acanth_cox1-F4 (5′-TTGGGTATTTGGGCATGGTG-3′) and Acanth_cox1-R2 (5′-CACCATGCCCAAATACCCAA-3′) [10]. Sequences were aligned via BioEdit software (version 7.7), trimmed to median length (947 bp) and then exported to DnaSP software (version 6) for the computation of polymorphisms [25] with Tajima's [26] and Fu's Fs tests [27]. A maximum-likelihood tree of the *cox*1 fragments obtained was generated by MEGA-X software with 1000 bootstrap replications [28], including homologous sequences from GenBank and a sequence of *Monoliformis monoliformis* (OK415026) as outgroup.

Table 1
Morphometric features (reported in mm) of female and male *M. hirudinaceus* specimens ($n = 10$ for each) from a wild boar of southern Italy. Standard deviation, *SD*.

Feature	Females (n=10) min - max	Mean ± *SD*	Males (n=10) min - max	Mean ± *SD*
Total body length	123.1–348.4	235.7 ± 96.8	54–87	66.6 ± 13.3
Mid - body width	3–6	4.58 ± 1.17	2–3	2.78 ± 0.38
Proboscis length	0.71–0.85	0.79 ± 0.07	0. 56–0.82	0.67 ± 0.11
No. rows in proboscis	5–6	6.2 ± 0.84	5–6	6.2 ± 0.84
No. hooks per row	6–8	7.0 ± 1	6–7	6.4 ± 0.5
First hook length	0.186–0.291	0.231 ± 0.05	0.132–0.210	0.174 ± 0.03

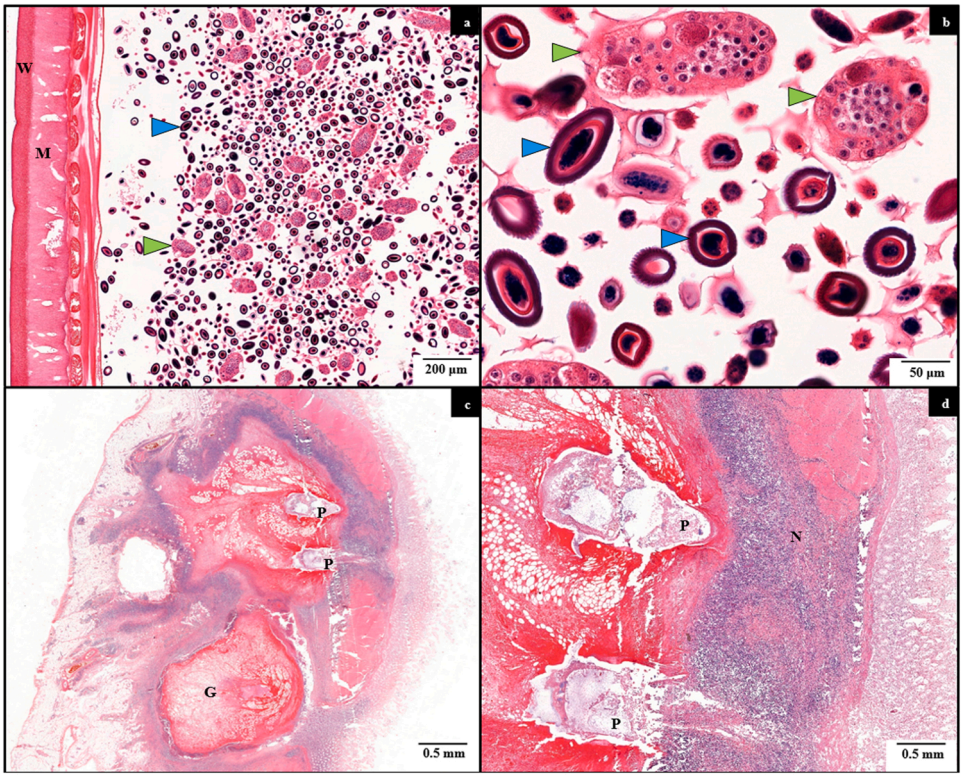

Fig. 3. Histological findings with HE-staining of a *M. hirudinaceus* female and lesions of the boar's small intestine: (a) longitudinal section of the parasitic wall (W), muscle (M), ovarian balls (green arrowhead) and eggs (blue arrowhead); (b) details of *M. hirudinaceus* metasomal body cavity with ovarian balls (green arrowhead) and developing eggs (blue arrowhead) in longitudinal and transversal section; (c) boar's intestine showing multiple granulomas (G) and two parasitic proboscises (P); (d) details of severe enteritis with diffused necrosis (N), circled by neutrophilic granulocytes in apoptosis, eosinophilic granulocytes, epithelioid macrophages and two parasitic proboscises invading the mucosa (P).

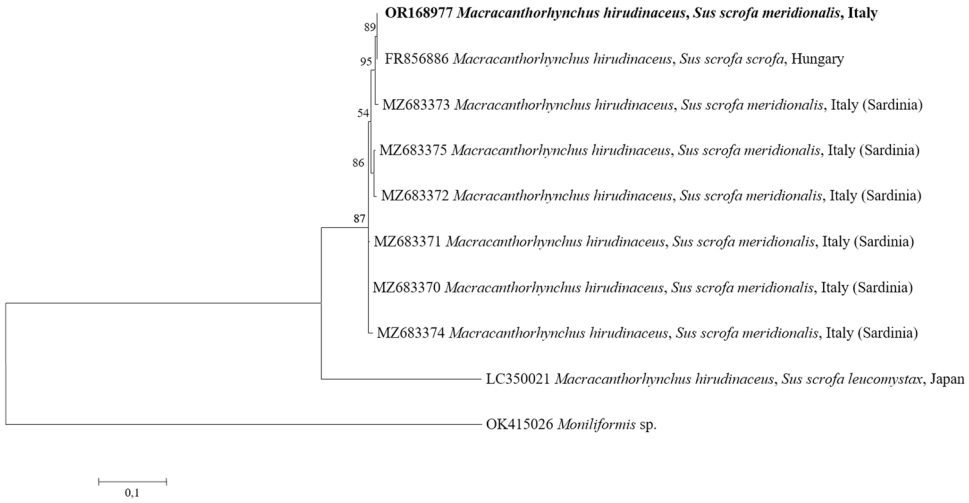

Fig. 4. Phylogenetic relationship of *M. hirudinaceus* sequences in this study (bold) and those available in literature. Phylogenesis was based on 947 bp of the *cox*1 partial gene and inferred by the Maximum Likelihood method based on Tamura 3-parameter model and Gamma distribution used to model evolutionary rate differences among sites (+G) selected by best-fit model. Evolutionary analyses were conducted on 1000 bootstrap replications using MEGA X software. Homologous sequence from *M. moniliformis* (OK415026) was used as outgroup.

2.6. Statistical analysis

A standard deviation (*SD*) was calculated for the mean morphometric values between female and male helminths found herein. The statistical analysis was performed with the online software GraphPad QuickCalcs website software [29].

3. Results

A total number of 24 helminths (i.e., 14 females and 10 males) was retrieved from the small intestine of the wild boar. By morphology, all helminths displayed white-reddish color, a transversely wrinkled cuticle and a retractile proboscis equipped with a variable number of rows of hooks characterized by decreasing size posteriorly (Fig. 1). The mean

Table 2
Diversity and neutrality indices of M. hirudinaceus cox1 sequences from this study and insular Italy. Polymorphic (segregating) sites, S; number of mutations, Eta; average pairwise nucleotide differences, k; nucleotide diversity, Pi; number of haplotypes, Hn; haplotype diversity, Hd; variance of haplotype diversity, πd; P-value, p.

S	*Eta*	*k*	*Pi*	*Hn*	*Hd*	*πd*	Tajima's D	*p*	Fu's Fs	*p*
17	17	6.38095	0.00674	7	1.000	0.0583	-0.44763	> 0.10	-2.449	> 0.10

values of total body length and mid-body width were 235.7 mm and 4.6 mm for females, whereas 66.6 mm and 2.8 mm for males, respectively; details of morphometric features and body measurements of the helminths are reported in Table 1.

At macroscopic examination, the intestinal tract of the boar displayed miliary to button-like inflammatory neoformations (Fig. 2), hard in consistency and clear in shear section. Histologically, a parasitic granulomatous enteritis was detected with multicentric inflammatory granulomas characterized by necrosis centers, cellular debris and multiple mineralization in the mucosa and surrounding hyper-segmented eosinophilic and neutrophilic granulocytes. Clusters of bacteria, epithelioid macrophages and activated fibroblasts circumscribed the areas of injury. Parasitic eggs found at histological examination appeared ovoid and thick-shelled with a mamillated outer membrane and measured 98–102 μm in length and 54–62 μm in width (Fig. 3).

Molecular analysis of the *cox*1 partial gene sequences confirmed the morphometric identification of *M. hirudinaceus*, displaying 100% query coverage and 99–100% nucleotide identity compared to those available in the GenBank database. The sequences of 20 *M. hirudinaceus* specimens obtained from the boar of this study were identical and clustered with high bootstrap values with those from Hungary and Sardinia (Fig. 4). The *M. hirudinaceus* sequence found herein was deposited in GenBank under the accession number OR168977. The computation of polymorphisms revealed high haplotype diversity and low nucleotide diversity of *M. hirudinaceus* from this study and insular Italy (Sardinia), with not significantly negative values of Tajima's D and Fu's Fs tests (Table 2).

4. Discussion

This is the first morphometric, histopathological and molecular case of a severe *M. hirudinaceus* infection in a wild boar from continental Italy.

The infection has been previously reported only in two cases from central areas of the peninsula without molecular or histological insights [18,19]. Although morphometric features of *M. hirudinaceus* specimens (Fig. 1, Table 1) are in accordance with those of the literature [2,7,23,30], a molecular confirmation is always recommended considering the possible misidentification of species via morphology [31] and underestimation of coprological examinations [15].

The severe infection found may be the result of repeated ingestion of infected beetles, which is not surprising as these insects feed on faeces and plant roots (up to 20 cm deep) in forests, parks and gardens [32] and may therefore be ingested through the typical coprophagous and rooting (up to 40 cm deep) behaviors of boars [33,34]. Despite the great biodiversity of Coleoptera species acting as competent vectors for *M. hirudinaceus*, the one responsible for infection in the study area may be larvae of the cockchafer *M. melolontha*, widely spread in the Mediterranean basin where up to 20 million specimens per 18 km^2 have been estimated [32].

The presence of multicentric granulomas (Fig. 2 and Fig. 3) is in line with previous studies [2,7,17,35] and suggests the importance of considering these lesions at necropsy in the differential diagnosis of tuberculosis, especially where the latter is endemic in boars [36,37]. In addition, future studies are needed to assess the potential correlation between *M. hirudinaceus* and other parasitic, viral and bacterial co-infections in terms of host's health status. This hypothesis is not be ruled out considering the impact of such neglected parasites on the body condition variations of hosts co-infected with other pathogens [38] and the positive correlation between helminths and tuberculosis severity [39].

From a public health perspective, the high density of boars in Europe [20], including Italy [21], enhanced during the Covid-19 lockdown [40], could drive the rise in the environment of contaminated faeces available to coprophagous beetles, increasing the risk of accidental infection to humans. During the lockdown, the reduction of artificial light at night (i.e., "ALAN"), which is a well-known factor of insect decline [41], may have been a trigger for the spread of beetles. This hypothesis finds support in a recent study showing the increased abundance and distribution of some beetle species during the lockdown [42]. In addition, the high survival ability of parasitic eggs in the environment, which remain infectious up to 3 years tolerating drought and below-zero temperatures [43], poses a further risk of infection to beetles [17]. From an epidemiological perspective, although the circulation of *M. hirudinaceus* has been reduced in domestic pigs due to improved management of extensive [10] and industrial production systems [4]. However, hygiene safety assurances in domestic animals do not apply to free-ranging wildlife. Indeed, high prevalence is reported in boars from central (i.e., 9.4%) [19] and insular Italy (11.1% in Sicily, 12.1% in Sardinia) [2,17], as well as in canids that commonly share with these ungulates same rural/peri-urban areas, such as stray dogs (4.3–4.8%) red foxes (*Vulpes vulpes*) (22.7–43.8%) [44,45] and golden jackals (*Canis aureus*) (3.2%) [46]. These findings underline the importance to improve the surveillance of boars and also other wildlife for assessing the diffusion of this zoonotic acanthocephalan in southern Italy.

The phylogenetic analysis of the *cox*1 gene reveals a close relationship of the *M. hirudinaceus* sequence found in this study with those from Hungary and insular Italy (Fig. 4). The high haplotype diversity and low nucleotide diversity of *M. hirudinaceus* (Table 2) is indicative of a rapid demographic expansion of the parasite. Although not statistically significant, the negative values of Tajima's D and Fu's Fs suggest an excess of rare polymorphic sites, feature of recent population expansion, and presence of rare haplotypes compared to what is expected under neutrality, pointing to past bottleneck and/or purifying selection events.

Future studies are needed to clarify the beetle species involved in the biological cycle of *M. hirudinaceus* in Italy and the role of boars and wild canids in the maintenance and transmission of this neglected parasite in urban and peri urban environment.

CRediT authorship contribution statement

Giovanni Sgroi: Conceptualization, Investigation, Writing - original draft. **Nicola D'Alessio:** Investigation, Project administration, Data curation, Formal analysis. **Antonio Varcasia**: Methodology. **Barbara Degli Uberti**: Methodology. **Caterina Fani**: **Michele Trotta**: **Giovanna Fusco**: Resources, Validation. **Kandai Doi**: Writing - review & editing. **Vincenzo Veneziano**: Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available on

request from the corresponding author.

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References

[1] I.N. Pavlović, Z.B. Kulišić, Z. Tambur, N.M. Protić, Scarabidae: intermediate host for *Macracanthorhynchus hirudinaceus*, Proc. Nat. Sci. Matica Srp. Novi Sad. (119) (2020) 89–95, https://doi.org/10.2298/ZMSPN1019089P.

[2] G. Dessì, P. Cabras, N. Mehmood, F. Ahmed, F. Porcu, V. Veneziano, G.P. Burrai, C. Tamponi, A. Scala, A. Varcasia, First molecular description of *Macracanthorhynchus hirudinaceus* in wild boars from Italy with pathomorphological and epidemiological insights, Parasitol. Res. 121 (2022) 197–204, https://doi.org/10.1007/s00436-021-07386-3.

[3] Centers for Disease Control and Prevention, Global Health, Division of Parasitic Diseases and Malaria. ⟨https://www.cdc.gov/dpdx/acanthocephaliasis/index.html⟩, 2019 (Accessed 28 July 2023).

[4] J.D. Barbosa, J.B. Silva, A.R.C.S. Reis, H.A. Bomjardim, D. Driemeier, F. M. Salvarani, C.H.S. de Oliveira, C.M. Oliveira, M. Brito, Identification of *Macracanthorhynchus hirudinaceus*, *Stephanurus dentatus* and *Trichuris suis* in native pigs on Marajo island, J. Vet. Sci. Med. Diagn. 6 (4) (2017), https://doi.org/10.4172/2325-9590.1000237.

[5] B.A. Mathison, N. Mehta, M.R. Couturier, Human Acanthocephaliasis: a thorn in the side of parasite diagnostics, J. Clin. Microbiol. 59 (2021), e0269120, https://doi.org/10.1128/JCM.02691-20.

[6] B. Senlik, V.Y. Cirak, O. Girisgin, C.V. Akyol, Helminth infections of wild boars (*Sus scrofa*) in the Bursa province of Turkey, J. Helminthol. 85 (2011) 404–408, https://doi.org/10.1017/S0022149X1000074X.

[7] B. Sarkari, M. Mansouri, M. Najjari, A. Derakhshanfar, G. Mowlavi, *Macracanthorhynchus hirudinaceus*: the most common helminthic infection of wild boars in southwestern Iran, J. Parasit. Dis. 40 (2016) 1563–1566, https://doi.org/10.1007/s12639-015-0728-3.

[8] S. Dodangeh, D. Azami, A. Daryani, S. Gholami, M. Sharif, I. Mobedi, S. Sarvi, E. Soleymani, M.T. Rahimi, M. Pirestani, S. Gohardehi, R. Bastani, Parasitic helminths in wild boars (*Sus scrofa*) in Mazandaran province, northern Iran, Iran. J. Parasitol. 13 (2018) 416–422.

[9] S. Polaz, A. Anisimova, P. Labanouskaya, A. Viarbitskaya, V. Kudzelich, The role of wild and domestic ungulates in forming the helminth fauna of European bison in Belarus, EBCC. 10 (2017) 79–86.

[10] K. Kamimura, K. Yonemitsu, K. Maeda, S. Sakaguchi, A. Setsuda, A. Varcasia, H. Sato, An unexpected case of a Japanese wild boar (*Sus scrofa leucomystax*) infected with the giant thorny-headed worm (*Macracanthorhynchus hirudinaceus*) on the mainland of Japan (Honshu), Parasitol. Res. 117 (2018) 2315–2322, https://doi.org/10.1007/s00436-018-5922-7.

[11] S. Lahmar, P.R. Torgerson, H. Mhemmed, L. Tizaoui, N. Mhadhbi, A. Bani, H. Driss, N. Ghrissi, M. Makhzoumi, A. Ben Houidi, M. Dhibi, Y. Said, E. Pozio, B. Boufana, Cystic echinococcosis and other helminth infections of wild boar in northeastern and northwestern regions of Tunisia, Parasitology 146 (2019) 1263–1274, https://doi.org/10.1017/S0031182019000532.

[12] M. Panayotova-Pencheva, K. Todorova, V. Dakova, Pathomorphological studies on wild boars infected with *Metastrongylus* spp., *Ascarops Strongylina*, and *Macracanthorhynchus hirudinaceus*, J. Vet. Res. 63 (2019) 191–195, https://doi.org/10.2478/jvetres-2019-0037.

[13] O.M. Amin, R.A. Heckmann, S. Dallarés, M. Constenla, T. Kuzmina, New morphological and molecular perspectives about *Macracanthorhynchus hirudinaceus* (Acanthocephala: Oligacanthorhynchidae) from wild boar, *Sus scrofa* Linn., in Ukraine, J. Helminthol. 95 (2021), e73, https://doi.org/10.1017/S0022149X21000675.

[14] G. Dărăbuș, F.S. Hora, N. Mederle, S. Morariu, M. Ilie, T. Suici, M. Imre, Prevalence and intensity of digestive and pulmonary parasites in wild boars in Romania, J. Zoo. Wildl. Med. 50 (2019) 270–273, https://doi.org/10.1638/2018-0040.

[15] D. Gassó, E. Serrano, R. Castillo-Contreras, X.F. Aguilar, A.C. Cadena, R. Velarde, G. Mentaberre, J.R. López-Olvera, D. Risco, P. Gonçalves, S. Lavín, P. Fernandez-Llário, J. Segalés, D. Ferrer, Coprological tests underestimate *Macracanthorhynchus hirudinaceus* burden in wild boar, Parasitol. Res. 115 (2016) 2103–2105, https://doi.org/10.1007/s00436-016-4976-7.

[16] V. Lizana, C. Gortazar, R. Prats, M.A. Sáchez-Isarria, M.J. Carrión, J. Cardells, *Macracanthorhynchus hirudinaceus* in expanding wild boar (*Sus scrofa*) populations in eastern Spain, Parasitol. Res. 120 (2021) 919–927, https://doi.org/10.1007/s00436-020-06975-y.

[17] S. Migliore, R. Puleio, G. Gaglio, D. Vicari, S. Seminara, E.R. Sicilia, P. Galluzzo, V. Cumbo, G.R. Loria, Neglected Parasite: *Macracanthorhynchus hirudinaceus*, first report in feral pigs in a natural park of Sicily (southern Italy), Front. Vet. Sci. 8 (2021), 659306, https://doi.org/10.3389/fvets.2021.659306.

[18] M. Magi, M. Bertani, M. Dell'Omodarme, M.C. Prati, Necropsy and coprology in wild boar (*Sus scrofa*) in Livorno mountain park (Tuscany, central Italy), Parassitologia 46 (2004) 311–313.

[19] R.A. Papini, S. Vannucci, G. Rocchigiani, S. Nardoni, F. Mancianti, Prevalence of *Toxoplasma gondii* and potentially zoonotic helminths in wild boars (*Sus scrofa*) hunted in central Italy, Mac. Vet. Rev. 41 (2018) 83–93, https://doi.org/10.2478/macvetrev-2018-0012.

[20] C. Pittiglio, S. Khomenko, D. Beltran-Alcrudo, Wild boar mapping using population-density statistics: from polygons to high resolution raster maps, PLoS One 13 (2018), e0193295, https://doi.org/10.1371/journal.pone.0193295.

[21] D. Fulgione, M. Buglione, The boar war: five hot factors unleashing boar expansion and related emergency, Land 11 (2022) 887, https://doi.org/10.3390/land11060887.

[22] Istituto Nazionale per la Fauna Selvatica, Biologia e gestione del cinghiale. ⟨https://www.isprambiente.gov.it/files/pubblicazioni/documenti-tecnici/6651_DOC_TEC_5.pdf⟩ (Accessed 13 June 2023).

[23] D.J. Richardson, Identification of cystacanths and adults of Oligacanthorhynchus tortuosa, Macracanthorhynchus ingens, and Macracanthorhynchus hirudinaceus based on probiscis and hook morphometrics, JAAS 59 (2005) 205–209.

[24] I. D'Aquino, I. D'Aquino, G. Piegari, S.M. Casciaro, F. Prisco, G. Rosato, P. Silvestre, B. Degli Uberti, M. Capasso, P. Laricchiuta, O. Paciello, V. Russo, An overview of neoplasia in captive wild felids in southern Italy zoos, Front. Vet. Sci. 9 (2022), 899481, https://doi.org/10.3389/fvets.2022.899481.

[25] J. Rozas, A. Ferrer-Mata, J.C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins, A. Sánchez-Gracia, DnaSPv6, DNA sequence polymorphism analyses of large datasets, Mol. Biol. Evol. 34 (2017) 3299–3302, https://doi.org/10.1093/molbev/msx248.

[26] F. Tajima, Statistical method for testing the neutral mutation hypothesis by DNA polymorphism, Genetics 123 (1989) 585–595, https://doi.org/10.1093/genetics/123.3.585.

[27] Y.X. Fu, Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection, Genetics 147 (1997) 915–925, https://doi.org/10.1093/genetics/147.2.915.

[28] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547–1549, https://doi.org/10.1093/molbev/msy096.

[29] H. Motulsky, GraphPad QuickCalcs web software, Dotmatics, 2023. ⟨https://www.graphpad.com/quickcalcs/⟩.

[30] M.A. Taylor, R.L. Coop, R.L. Wall, Parassitologia e malattie parassitarie degli animali, fourth ed., Edra-Masson, Milan, 2022.

[31] D.C. Huston, T.H. Cribb, L.R. Smales, Molecular characterisation of acanthocephalans from Australian marine teleosts: proposal of a new family, synonymy of another and transfer of taxa between orders, Syst. Parasitol. 97 (2020) 1–23, https://doi.org/10.1007/s11230-019-09896-2.

[32] D. Alderton, Animals, Amber Books LTD, London, 2009.

[33] A. Scandurra, L. Magliozzi, D. Fulgione, M. Aria, B. D'Aniello, Lepidoptera Papilionoidea communities as a sentinel of biodiversity threat: the case of wild boar rooting in a Mediterranean habitat, J. Insect Conserv. 20 (2016) 353–362, https://doi.org/10.1007/s10841-016-9863-0.

[34] G. Sgroi, A. Varcasia, G. Dessì, N. D'Alessio, L. Pacifico, F. Buono, B. Neola, G. Fusco, M. Santoro, V. Toscano, A. Fioretti, V. Veneziano, Massive *Taenia hydatigena* cysticercosis in a wild boar (*Sus scrofa*) from Italy, Acta Parasitol. 64 (2019) 938–941, https://doi.org/10.2478/s11686-019-00110-30.

[35] G. Mowlavi, J. Massoud, I. Mobedi, S. Solaymani-Mohammadi, M.J. Gharagozlou, S. Mas-Coma, Very highly prevalent *Macracanthorhynchus hirudinaceus* infection of wild boar *Sus scrofa* in Khuzestan province, south-western Iran, Helminthologia 43 (2006) 86–91, https://doi.org/10.2478/s11687-006-0017-x.

[36] J. Thomas, A. Balseiro, A. Gortázar, M.A. Risalde, Diagnosis of tuberculosis in wildlife: a systematic review, Vet. Res. 52 (2021), 31, https://doi.org/10.1186/s13567-020-00881-y.

[37] V. Iovane, G. Ferrara, A. Petruccelli, V. Veneziano, N. D'Alessio, R. Ciarcia, A. Fioretti, U. Pagnini, S. Montagnaro, Prevalence of serum antibodies against the *Mycobacterium tuberculosis* complex in wild boar in Campania region, Italy, Eur. J. Wildl. Res. 66 (2020), 20, https://doi.org/10.1007/s10344-019-1359-2.

[38] E. Serrano, J. Millán, What is the price of neglecting parasite groups when assessing the cost of co-infection? Epidemiol. Infect. 142 (2014) 1533–1540, https://doi.org/10.1017/S0950268813002100.

[39] D. Risco, E. Serrano, P. Fernández-Llario, J.M. Cuesta, P. Gonçalves, W.L. García-Jiménez, R. Martínez, R. Cerrato, R. Velarde, L. Gómez, J. Segalés, J. Hermoso de Mendoza, Severity of bovine tuberculosis is associated with co-infection with common pathogens in wild boar, PLoS One 9 (2014), e110123, https://doi.org/10.1371/journal.pone.0110123.

[40] Forbes, Animals roam freely in Italian cities with humans in lockdown. ⟨https://www.forbes.com/sites/davidbressan/2020/03/20/animals-roam-freely-in-italian-cities-with-humans-in-lockdown/?sh=c08302179565⟩, Accessed 13 June 2023).

[41] A.C.S. Owens, P. Cochard, J. Durrant, B. Farnworth, E.K. Perkin, B. Seymoure, Light pollution is a driver of insect declines, Biol. Conserv. 241 (2020), 108259, https://doi.org/10.1016/j.biocon.2019.108259.

[42] P. Brimblecombe, P. Querner, Changing insect catch in Viennese museums during COVID-19, Heritage 6 (2023) 2809–2821, https://doi.org/10.3390/heritage6030150 (2023).

[43] W.C. Marquardt, R.S. Demaree, R.B. Grieve. Parasitology and Vector Biology, second ed..,, Harcoart Academic Press, New York, 2000.

[44] A. Dalimi, A. Sattari, G. Motamedi, A study on intestinal helminthes of dogs, foxes and jackals in the western part of Iran, Vet. Parasitol. 142 (2006) 129–133, https://doi.org/10.1016/j.vetpar.2006.06.024.
[45] M. Zare-Bidaki, I. Mobedi, S.S. Ahari, S. Habibizadeh, S. Naddaf, M. Siavashi, Prevalence of zoonotic intestinal helminths of canids in Moghan Plain, northwestern Iran, Iran. J. Parasitol. 5 (2010) 42–51.
[46] S. Lahmar, B. Boufana, S. Ben Boubaker, F. Landolsi, Intestinal helminths of golden jackals and red foxes from Tunisia, Vet. Parasitol. 204 (2014) 297–303, https://doi.org/10.10.1016/j.vetpar.2014.05.038.