# Testing the 'parasite-mediated domestication' hypothesis: a comparative approach to the wild boar and domestic pig as model species (#100752)

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# Testing the 'parasite-mediated domestication' hypothesis: a comparative approach to the wild boar and domestic pig as model species

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The mechanisms underlying the domestication process have already been well explained. Starting with Belyaev's pioneering experiment on silver foxes, which showed that selection for tameness leads to destabilisation of the regulatory systems that control morphological and behavioural development, resulting in the changes characteristic of the domestication syndrome. Later, the thyroid rhythm hypothesis and the neural crest cell hypothesis provided additional explanations. Recently, the parasite-mediated domestication hypothesis (PMD) has been proposed, suggesting an important role of endoparasites in the domestication process. PMD proposes that the frequency of domesticated syndrome traits is associated with a higher susceptibility to parasites. PMD can be tested either experimentally or comparatively. We followed a systematic comparative approach by analysing the parasite load in wild boar (WB) and free-ranging domestic pig (DP) populations from a comparable environment in a geographically close area. Fresh faeces from WB and DP populations, one each from Slovenia (SI) and Croatia (HR), were sampled. A total of 59 individual faecal samples were collected (SI: 12 WB, 20 DP; HR: 14 WB, 13 DP). Parasitological diagnostics were carried out using the sedimentation and floatation method. Five different taxa were found in WB and seven in DP. Three parasite taxa were found exclusively in DP (Cystoisospora suis, Trichuris sp., Balantidium coli), and one (Strongyloides sp.) only in WB. Of the parasites found in both cohorts, Strongyles/Oesophagostomum sp. were significantly more abundant in DP, while Eimeria sp. was found in equal amounts in both (but in HR only in WB). According to the

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preliminary study presented here, there is evidence to support the PMD baselines in the wild boar – domestic pig association. However, we cannot draw a definitive conclusion as there are many aspects that may bias the interpretation based on parasite load alone, which are also discussed here. Therefore, comparative studies should be supported by a more focussed methodology, including an experimental approach.



1 **Testing the 'parasite-mediated domestication'** 2 hypothesis: a comparative approach to the wild boar 3 and domestic pig as model species 4 5 Oleinic Renat<sup>1</sup>, Posedi Janez<sup>2</sup>, Beck Relia<sup>3</sup>, Šprem Nikica<sup>4</sup>, Škorput Dubravko<sup>5</sup>, Pokorny 6 Boštjan<sup>6,7</sup>, Škorjanc Dejan<sup>1</sup>, Prevolnik Povše Maja<sup>1</sup>, Skok Janko<sup>1\*</sup> 7 8 9 <sup>1</sup> Department of Animal Science, Faculty of Agriculture and Life Sciences, University of Maribor, Maribor, Slovenia 10 11 <sup>2</sup> Unit for Parasitology, Institute of Microbiology and Parasitology, Veterinary Faculty, 12 University of Ljubljana, Ljubljana, Slovenia <sup>3</sup> Laboratory for Parasitology, Croatian Veterinary Institute, Zagreb, Croatia 13 <sup>4</sup> Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, Faculty of 14 Agriculture, University of Zagreb, Zagreb, Croatia 15 <sup>5</sup> Division of Animal Science, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia 16 <sup>6</sup> Faculty of Environmental Protection, Velenje, Slovenia 17 <sup>7</sup> Slovenian Forestry Institute, Ljubljana, Slovenia 18 19 20 Corresponding author: 21 Janko Skok Pivola 10, Hoče, 2311, Slovenia 22 23 Email address: janko.skok@um.si 24 **Abstract** 25 26 The mechanisms underlying the domestication process have already been well explained. Starting with Belyaev's pioneering experiment on silver foxes, which showed that selection for 27 28 tameness leads to destabilisation of the regulatory systems that control morphological and behavioural development, resulting in the changes characteristic of the domestication syndrome. 29 Later, the thyroid rhythm hypothesis and the neural crest cell hypothesis provided additional 30 31 explanations. Recently, the parasite-mediated domestication hypothesis (PMD) has been 32 proposed, suggesting an important role of endoparasites in the domestication process. PMD 33 proposes that the frequency of domesticated syndrome traits is associated with a higher 34 susceptibility to parasites. PMD can be tested either experimentally or comparatively. We followed a systematic comparative approach by analysing the parasite load in wild boar (WB) 35 and free-ranging domestic pig (DP) populations from a comparable environment in a 36 37 geographically close area. Fresh faeces from WB and DP populations, one each from Slovenia

(SI) and Croatia (HR), were sampled. A total of 59 individual faecal samples were collected (SI:

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39 12 WB, 20 DP; HR: 14 WB, 13 DP). Parasitological diagnostics were carried out using the sedimentation and floatation method. Five different taxa were found in WB and seven in DP. 40 Three parasite taxa were found exclusively in DP (Cvstoisospora suis, Trichuris sp., Balantidium 41 coli), and one (Strongyloides sp.) only in WB. Of the parasites found in both cohorts, 42 43 strongyles/Oesophagostomum sp. were significantly more abundant in DP, while Eimeria sp. was found in equal amounts in both (but in HR only in WB). According to the preliminary study 44 presented here, there is evidence to support the PMD baselines in the wild boar – domestic pig 45 association. However, we cannot draw a definitive conclusion as there are many aspects that may 46 bias the interpretation based on parasite load alone, which are also discussed here. Therefore, 47 comparative studies should be supported by a more focussed methodology, including an 48 49 experimental approach.

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### Introduction

52 Domesticated animals are characterised by a set of phenotypic traits that are either behavioural or 53 morphological and are referred to as the domestication syndrome. When considering the beginning of the domestication process in animals, tameness is an essential prerequisite. 54 followed by floppy ears, a short and curled tail, piebaldism, depigmentation, short and wide 55 56 skull, reduced size of the adrenal gland, and others. Many of the mechanisms behind the 57 domestication/domestication syndrome have already been well explained. Starting with the pioneering studies on domestication by Dmitry Belyaev, who proposed that the domestication 58 syndrome is genetically linked to genes associated with tameness. His experiment on 59 domestication of silver foxes, which is among the most influential work in this field, showed that 60 selection for tameness (impaired stress response, changes in the 'hypothalamic-pituitary-adrenal 61 62 system' - HPA axis) leads to significant destabilisation of regulatory systems controlling morphological and behavioural development, resulting in changes that are otherwise 63 characteristic of domestication syndrome (Belyaev, 1979). Much later, some other findings and 64 65 hypotheses were added (Wilkins, 2017). In the thyroid rhythm hypothesis, Crockford (2004) 66 proposed that domestication is also driven by genetically controlled changes in the activity 67 rhythm of the thyroid gland, which have a crucial effect on heterochronic changes and thus play an important role in the domestication syndrome (e.g. paedomorphism). Furthermore, Wilkins, 68 Wrangham & Fitch (2014) proposed that the main phenotypic components of domestication 69 70 syndrome are neural crest cells (NCC) derivatives, i.e. a result of a developmental reduction in 71 NCC input for the affected phenotypic traits. Recently, the parasite-mediated domestication hypothesis (PMD) has been proposed (Skok, 2023). Parasites affect literally all the major 72 mechanisms that otherwise underlie the domestication syndrome (HPA, thyroid, NCC -e.g. 73 74 neuroendocrine regulation, hormonal modulation, changes in the host miRNA profile, etc.), and in this way could mediate the domestication process, both directly through manipulation of host 75 behavioural traits (e.g. Poulin, 1994, 2013; Adamo, 2013) and indirectly via genes related to 76 resistance/tolerance to parasites, the role of miRNA in the process of epigenetic inheritance or 77 78 the transgenerational inheritance of stress pathology (see Skok, 2023). Therefore, the PMD



79 assumes an important role of endoparasites in the process of domestication, especially in the initial phase (proto-domestication). It predicts that the frequency of domestication syndrome 80 traits in the wild population increases with decreasing genetic resistance to parasites and/or with 81 increasing parasite load. PMD can be tested in different ways, either experimentally 82 83 (experimentally parasitised wild population) or comparatively (on the existing wild and domestic populations). Although comparative studies can be problematic for a variety of reasons, they are 84 relatively easy to conduct and are therefore suitable for an initial test of hypothesised PMD 85 baselines. It can be assumed that, under comparable conditions, the parasite load in the domestic 86 population will be higher than in the wild population. Therefore, we tested the PMD in Sus 87 scrofa with a systematic comparative approach by analysing the parasite load in wild boar and 88 free-ranging domestic pigs from a comparable environment. We selected animals from two 89 regions, one in Slovenia and the other in Croatia. In each region, samples were collected from 90 91 wild and domestic cohort in a relatively narrow geographical area. According to PMD, we 92 proposed a higher parasite load in the domestic pig compared to its wild counterpart, both in terms of diversity of parasite taxa and quantitatively (severity of infection). 93

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### **Materials & Methods**

- 96 Study sites and sampling period
- 97 The study took place in Croatia and Slovenia. The samples in both countries consisted of two
- 98 cohorts, wild boar and domestic pigs. In both countries, the wild boar and domestic pig
- 99 populations were located about 15-20 km apart, so they had similar chances of being exposed to
- the local parasitofauna. In Croatia, the wild boar samples were collected on 3-4 November 2023
- in the Prolom hunting ground (village of Buzeta, Glina municipality, Sisak-Moslavina County)
- and the domestic pigs on 10 November 2023 in the free-range enclosure of the autochthonous
- Banija spotted pig (Banijska šara) in the village of Srednje Mokrice (Petrinja municipality,
- 104 Sisak-Moslavina County). In Slovenia, wild boar samples were collected on 10 December 2023
- in the Stoperce hunting ground (village of Stoperce, municipality of Majšperk, Štajerska region)
- and domestic pigs on 11 December 2023 in the free-range enclosure of the autochthonous
- 107 Krškopolje pig or black-belted pig (Krškopoljski prašič) in the village of Zgornje Laže
- 108 (municipality of Slovenjske Konjice, Štajerska region).

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- 110 Animals and faeces collection
- Wild boar populations and free-ranging (grazing) domestic pig populations, one each from
- 112 Slovenia (SI) and Croatia (HR), were included in the study. Fresh faeces were collected from the
- rectum of hunted wild boar or immediately after the defecation of domestic pigs. Each individual
- sample was collected in plastic cups with lids the rubber gloves were changed before each
- faecal collection. The samples were then stored in a refrigerator at a temperature of 4°C and
- analysed up to 48 hours after sampling. A total of 59 individual faecal samples (SI: 12 wild boar,
- 117 20 domestic pig; HR: 14 wild boar, 13 domestic pig) were examined for endoparasites. Domestic
- 118 pigs were not threated against parasites.



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120	Diagnosis of parasitic infections
121	Due to the occurrence of African swine fever (ASF) in Croatia (the first occurrence of the virus
122	was confirmed in domestic pigs on June 26, 2023), which also hindered the sampling process in
123	general, all samples remained in the country of origin and were analysed there. The Croatian
124	samples were analysed in the Laboratory for Parasitology of the Croatian Veterinary Institute in
125	Zagreb. Slovenian samples were analysed in the Unit for Parasitology of the Institute of
126	Microbiology and Parasitology at the Veterinary Faculty of the University of Ljubljana.
127	The faecal samples were examined for the presence of endoparasites using the flotation or
128	McMaster method and the sedimentation method according to Thienpont, Rochette & Vanparijs
129	(2003). A saturated salt solution (density 1.20 at 20°C) was used to float nematode and cestode
130	eggs, while tap water was used for sedimentation. Taxon determination under the microscope
131	was based on the different morphotypes of the eggs/(oo)cysts. The assessment of parasite load
132	differs between national laboratories, but this was irrelevant for the reliability of the PMD
133	testing, as we primarily aimed to compare wild and domestic populations within the selected area
134	(country) and not between countries. In its standard methodology, the Slovenian laboratory uses
135	5 classes for the intensity of parasite load, defined by the number of eggs/(oo)cysts present in the
136	sample/smear. The classes were defined as follows: Negative = 0; Individual egg/oocyte = 3-5; +
137	= 5-10; $++$ = 10-20; $+++ \ge 21$ . In the Croatian laboratory, the total number of eggs/(00)cysts per
138	gram in the sample of a given quantity (approx. 2-5 g) was defined. For this reason, the results
139	are presented here separately for each country and with a different scale for the figures.
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141	Data analyses
142	Due to the restriction with regard to ASF and the separate diagnostics within the country from
143	which the samples originate, the estimates of the parasite load differ between the two data sets
144	and were therefore also statistically analysed differently. For the Slovenian data, where the
145	parasite load estimators were reported as classes, the non-parametric Mann-Whitney U-test (also
146	known as Wilcoxon rank sum test) was used to compare the parasite load between the domestic
147	and wild cohorts. The Croatian estimator was a continuous variable. However, as it was not
148	normally distributed, the non-parametric Independent-Samples Wald-Wolfowitz Run test was
149	used to compare the parasite load between the domestic and wild cohorts.
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151	Results
152	As shown in Table 1, a total of five different parasite taxa were found in the HR samples. Wild
153	and domestic populations were each infected with three different parasite taxa (wild boar:
154	Eimeria sp., Strongyle type eggs, and Strongyloides sp.; domestic pig: Cystoisospora suis,
155	strongyle type eggs, and <i>Trichuris</i> sp.). A total of four different parasite taxa were found in SI

samples. Only two of them were found in wild boar, Eimeria sp., and Oesophagostomum sp.

(strongyle), but all four (Eimeria sp., Balantidium coli, Oesophagostomum sp. (strongyle), and

Trichuris sp.) were found in domestic pig. Strongyloides sp. was the only parasite taxon found

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- only in wild boar but not in domestic pigs, all others were found either exclusively in domestic
- pigs or in both cohorts.
- 161 In the Croatian samples, the only taxon present in both cohorts were strongyles, the eggs of
- which were significantly more abundant in domestic pigs than in wild boar. Of the others,
- 163 Cystoisospora suis and Trichuris sp. were found exclusively in domestic pigs, while Eimeria sp.
- and *Strongyloides* sp. were only found in wild boar (Figure 1).
- In the Slovenian samples there were two parasite taxa, *Trichuris* sp. and *Balantidium coli*, which
- were found exclusively in domestic pigs, while the other two were found in both cohorts. For
- two matching parasites, the load of *Oesophagostomum* sp. (strongyle) was significantly higher in
- domestic pig than in wild boar, while *Eimeria* sp. was present to the same extent in both cohorts
- 169 (Figure 2).

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### **Discussion**

- Our results are in favour of PMD, as domestic pigs generally had a significantly higher parasite
- 173 load (taxon richness) than their wild counterparts from the same area. According to PMD
- baselines, domestic pigs descend from parasite-susceptible ancestors, with susceptibility to
- parasites, which presumably trigger the domestication syndrome, being passed on/inherited
- through generations of progeny after the domestication process. Rare earlier comparative studies
- on parasite load in Sus scrofa have shown mixed results, some of which contradict ours –
- 178 although it should be emphasised that the sampling in previous comparative studies was not as
- 179 systematically targeted as ours. For example, Ineson (1954) found differences in parasite load
- depending on parasite species, with domestic pigs in New Zealand having a higher overall
- parasite load (richness) than wild boar. In contrast, Alwin et al. (2015) found that parasite loads
- were higher in the wild boar than in domestic pig in semi-free range and on farms in India.
- 183 Based on our results, we cannot draw a definitive conclusion because, apart from the fact that
- this is only a very preliminary attempt at a systematic comparison to test PMD, there are many
- aspects that could confound interpretation.
- 186 The first aspect concerns the possible artificial (either intentional or spontaneous) selection of
- resistant/tolerant animals aimed at increasing resistance (McManus et al., 2014) and possibly
- leading to a deceptively high resistance/tolerance to parasites in the domestic population, which
- was certainly not the case in our study. In fact, none of the breeds included in our study had been
- 190 historically subjected to methodical selection for parasite resistance. Furthermore, selection for
- parasite resistance/tolerance in pigs has generally not received much attention in contrast to
- sheep, although the heritability of host resistance to certain parasites is so high that breeding for
- resistant pigs could be a possibility (Roepstorff et al., 2011).
- The second aspect, which can lead to the opposite conclusion, concerns relaxed selection, in
- which the source of selection that was previously important for the maintenance of a particular
- trait is weakened or even eliminated (Lahti et al., 2009). Indeed, selection pressure from parasites
- may have been reduced during the domestication process. Since domestication, animals have
- been confined, at least to a certain extent, which prevents them from being exposed to the full



199 spectrum of parasites that could infect/infest them in their natural environment. This, together with "stringent" artificial selection for desirable traits, could influence (jeopardise) the evolution 200 of genetic resistance in (parasite-naïve) domestic animals (Eizaguirre et al., 2012; Smallbone et 201 202 al., 2021). With regard to genetic parasite resistance, the major histocompatibility complex (MHC) and its 203 variability is often considered one of the functionally most important loci associated with 204 parasite resistance (Sommer, 2005; Axtner & Sommer, 2012; Arbanasić et al. 2019). MHC is a 205 large locus of genes responsible for the adaptive immune system, helping to activate not only 206 appropriate T cells that help the organism to eliminate intracellular pathogens (e.g. viruses), but 207 also B cells involved in the elimination or neutralisation of extracellular pathogens, including 208 parasites (Janeway et al., 2001; McManus et al., 2014). It is assumed that animals with limited 209 MHC gene diversity also have low resistance to parasites. 210 211 Indeed, constant selection pressure by various parasites leads to rapid adaptive evolutionary 212 changes that result in an adaptive shift in allele frequency, implying that parasite-mediated selection directly contributes to the maintenance of MHC polymorphism (Eizaguirre et al., 213 2012). Accordingly, Smallbone et al. (2021) found a significantly lower number of MHC alleles 214 and MHC supertypes per individual in domesticated guppies (*Poecilia reticulata*) compared to 215 the wild ones. They pointed out that artificial selection for desirable, economically important 216 traits may lead to the loss of key immune functional alleles and thus reduce the immunogenetic 217 diversity that protects populations from the risk of parasite infection/infestation, which could 218 significantly impair host resistance to parasites. Similar results have been shown for the red 219 junglefowl (Gallus gallus) compared to the domestic chicken (Nguyen-Phuc, Fulton, Berres, 220 221 2016). In contrast, studies on the pig did not follow the same premise and showed that domestication does not appear to act as a bottleneck limiting MHC diversity (Moutou et al., 222 223 2013), and the same was found in the zebra finch (*Taeniopygia guttata*), for example (Newhouse & Balakrishnan, 2015). 224 225 However, MHC diversity does not necessarily imply higher functional resistance, but may simply be the signature of the pathogens and parasites that the animal has been confronted with 226 during evolution (Mikko et al., 1999; see also Portanier et al., 2019, showing that MHC diversity 227 in mouflons (Ovis gmelini musimon) is associated with resistance to nematodes but not to 228 229 coccidia). In addition to MHC, there are other quantitative trait loci for parasite resistance (McManus et al., 2014), which makes this aspect of PMD testing even more challenging. 230 Testing PMD based on parasite load/resistance/tolerance in existing populations of wild or 231 domestic animals and simply comparing these populations is therefore interpretatively quite 232 complex, although relatively easy to perform. Comparative studies, such as the present one, 233 234 should be supported by a more focussed methodology. Either to examine the frequency of domestication syndrome traits in the wild population in relation to their parasite resistance/load. 235 or to examine the parasite resistance/load of wild animals showing signs of domestication 236 237 syndrome (e.g. tameness) in comparison to completely wild animals of the same population, as 238 originally proposed (Skok, 2023). The other and probably most reliable way to test PMD would



- be an experimental approach, i.e. experimental proto-domestication, such as the Belyaev fox
- experiment (Belyaev, 1979). However, instead of selecting animals for tameness, an
- 241 experimental population of the wild counterpart of domestic animals would be experimentally
- 242 exposed to parasites, selected against parasite resistance (whereby more infected/infested, less
- resistant individuals would be selected), and analysed over generations for the frequency of
- 244 domestication syndrome traits in the population. It is predicted that the frequency of typical
- 245 domestication syndrome traits (tameness, depigmentation, floppy ears, etc.) would increase in a
- 246 parasite-susceptible population.
- 247 It should also be considered that the influence of parasites on the domestication process cannot
- simply be generalised to the entire parasite load in the host, as it is also possible that only certain
- 249 parasites play a mediating role in the domestication process.
- 250 To mediate domestication, direct (i.e., without an intermediate host) interspecific transmission
- 251 including a possible zoonotic potential of the parasite seems to be a necessary criterion. Of the
- 252 parasite taxa found in our study, all parasites found exclusively or predominantly in domestic pig
- 253 (Balantidium coli, Strongyle/ Oesophagostomum sp. and Trichuris sp.) fulfil both criteria, but
- 254 not Cystoisospora suis, which was only found in domestic pig and is species-specific. In
- addition, the only parasite taxa found exclusively in wild boar was *Strongyloides* sp. of which
- 256 none of the known species infecting pigs were found to be zoonotic. Furthermore, *Strongyloides*
- 257 sp. have been shown to be both parthenogenetic and autoinfectious (Moore, 2002), making them
- 258 less reliant on intensive transmission between hosts.
- 259 Further, the PMD predicts that parasites should increase the occurrence of domestication
- 260 syndrome traits. For the parasites found in our study, there is a significant lack of specific
- information on their potential effects on phenotypic traits of interest in the context of PMD.
- However, of the target traits, mainly behavioural changes/correlates of the host have been
- 263 investigated so far.
- Studies on the impact of the endoparasites taxa relevant (found) in our study on host behaviour
- 265 usually deal with either parasite load in relation to a specific personality type or social behaviour
- 266 (e.g. Côté & Poulin, 1995; Ezenwa, 2004; Melfi & Poyser, 2007; Müller-Klein et al., 2019;
- 267 Santicchia et al., 2019), avoidance/sickness behaviour within a social group (e.g. Ghai et al.,
- 268 2015; Chapman et al., 2016; Friant, Ziegler & Goldberg, 2016; Wren et al., 2021), changes in the
- 269 complexity and dynamics of a social group (Burgunder et al., 2017) and the fear response to
- predators (Moore, 2002; Barber & Dingemanse, 2010; Kaushik, Lamberton & Webster, 2012).
- 271 Indeed, behaviours that are (in)directly indicative of the animals' degree of tameness, which is
- otherwise a pivotal trait of the domestication syndrome, are primarily of PMD interest.
- 273 Tameness in the context of domestication can be defined as reduced fear of humans, as well as
- 274 increased boldness and reduced aggression, which have been shown to cause the development of
- 275 many aspects of the domestication syndrome (Belvaev, 1979; Agnvall et al., 2015). With regard
- 276 to PMD, the influence of parasites on behaviours such as reduced fear of novelty or of other
- 277 species, including humans, is therefore of particular interest. However, such studies are relatively
- 278 rare, and the results are often inconclusive.



279 Fear of predators or novelty (neophobia) as well as boldness and exploratory behaviour appear to be suitable candidates as indicators of tameability. Of the parasites found in the present study, 280 some (Eimeria sp., Strongyles, Strongyloides sp., Trichuris sp.) were associated with host 281 behaviour, but the results are not conclusive. *Eimeria* sp., for example, has generally been shown 282 283 to reduce fear of predators (reviewed in Moore, 2002; Kaushik, Lamberton & Webster, 2012). However, when looking at neophobia/boldness, for example, it was found that rural and urban 284 rats, the latter being considered less neophobic (bolder), did not differ in *Eimeria* sp. load, but 285 were more infected with *Trichuris* sp. (Battersby Parsons & Webster, 2002). In addition, bolder 286 and more exploratory individuals of grey squirrels (Sciurus carolinensis) were shown to be more 287 heavily infected with Strongyloides sp. (S. robustus), but this was considered to be a cause rather 288 than a consequence of infection severity (e.g. Santicchia et al., 2019). Given the apparent species 289 specificity, potential studies in our model species (Sus scrofa) are of particular importance, but 290 they are scarce. In wild boar, a study was recently conducted on parasite load in relation to the 291 292 degree of urbanisation of the area in which they forage. No general difference in endoparasite load (including one strongyle species, Oesophagostomum dentatum) was found between wild 293 boar foraging in suburban areas and those foraging in urban areas – except for *Eimeria* sp., the 294 prevalence of which was significantly higher in individuals foraging in urban areas, i.e. those 295 that were less afraid of the anthropogenic environment/humans (Pilarczyk et al., 2024). 296 Therefore, Eimeria sp. appear to be associated with tame-like behaviour in pigs and could 297 therefore also be one of the candidate parasites associated with PMD. 298 Although not of paramount importance in the context of PMD, intraspecific interactions among 299 members of the same social group have also been frequently studied and should therefore be 300 301 considered. Indeed, literally all domesticated animals except the cat are social, with the dominant status of an individual indirectly indicating the degree of tameness. In general, subordinate 302 animals are considered less aggressive/tameable (e.g. Blanchard et al., 1988; Holekamp & 303 Strauss, 2016) and (with some exceptions) less neophobic, i.e. they are bolder and tend to 304 305 explore new environments and objects (King, 1973; Robertson, 1982; Johnson & Balph, 1990; Darrow & Shivik, 2009; but see also Mettler & Shivik, 2007; Schaffer et al., 2021). Among the 306 parasites found in our study, susceptibility to infection with some of them was also found to be 307 related to individual social status (dominant vs. subordinate). In plain zebras (Equus quagga), for 308 309 example, it was found that subordinate individuals, which could also be considered tamer, were more susceptible to infection with strongyles as well as Strongyloides sp. (Fugazzola & 310 Stancampiano, 2012; Joly et al., 2023). In contrast, in bovids (several species of gazelles), 311 territorial males, which can be considered dominant, have been found to have a higher strongyle 312 and coccidia (including *Eimeria* sp.) load (Ezenwa, 2004). 313 However, our results do not fit easily into this picture of previous findings on the relationship 314 315 between specific parasite and tameness, as we found *Eimeria* sp. to the same extent in the domestic and wild cohort; moreover, in the Croatian sample it was found only in the wild cohort, 316 317 while strongyle/Oesophagostomum sp. and Trichuris sp. were found in domestic pig to a



significantly higher extent or exclusively in the domestic cohort, and *Strongyloides* sp. was found only in wild boar.

320 321

### Conclusions

- 322 The main findings of the present study are consistent with the PMD in terms of overall parasite
- 323 load/richness, as more parasite taxa were found in the domestic pig, whereby of the parasites
- found in both cohorts strongyle/Oesophagostomum sp., but not Eimeria sp. (the same), was
- 325 significantly higher in the domestic pig. However, when considering the influence of a particular
- parasite on the traits of the domestication syndrome, no clear conclusions can be drawn as the
- 327 relevant studies are limited to (indirect) behavioural traits only and some of the parasites found
- 328 have not been studied at all in this regard. In addition, the studies to date suggest that the
- 329 influence of a particular parasite may even be species-specific, but the studies on pig (Sus scrofa)
- are sparse (and only indirect). Therefore, we can only infer partial and indirect correlations,
- which are ultimately inconclusive. Nevertheless, the present study provides some indices in
- 332 favour of PMD, but no definitive answers. This was neither possible nor expected given the
- and a nature of the study, as it was a preliminary test of the hypothesised baselines of PMD indeed,
- no other than an experimental approach can provide a definitive answer.

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336

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### Table 1(on next page)

Description of the parasites found in the study.

Parasite taxa found in the study with summarised taxa richness (S) for each cohort. † – only domestic cohort infected, †† – only wild cohort infected, HR – Croatia, SI – Slovenia.



- 1 Table 1: Description of the parasites found in the study.
- 2 Parasite taxa found in the study with summarised taxa richness (S) for each cohort. † only
- 3 domestic cohort infected, †† only wild cohort infected, HR Croatia, SI Slovenia.

		Domestic pig	Wild boar	S
HR		Cystoisospora suis †	Eimeria sp.	5
		Strongyle type eggs	Strongyle type eggs	
		Trichuris sp. †	Strongyloides sp. ††	
SI		Eimeria sp.	Eimeria sp.	4
	O	esophagostomum sp. (strongyle)	Oesophagostomum sp. (strongyle)	
		<i>Trichuris</i> sp. <sup>†</sup>		
		Balantidium coli †		
	S	7	5	

4

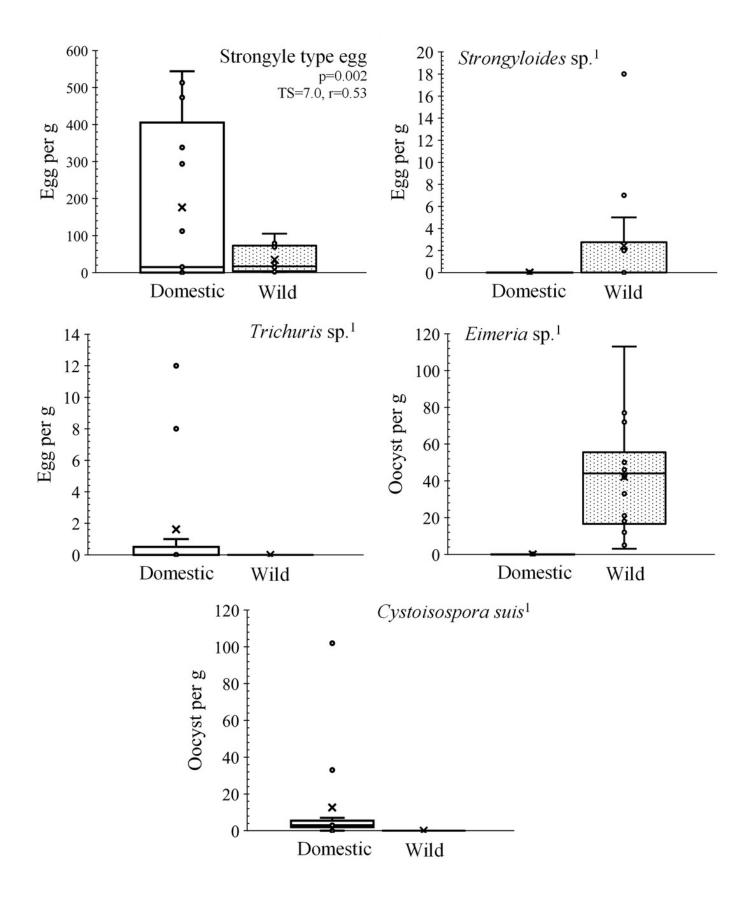
5



### Figure 1

Figure 1: Parasite load in the HR wild boar - domestic pig association.

Comparison of the intensity of infection with certain parasite taxa between wild boar and domestic pig cohorts in Croatia. The centre lines of the boxplots indicate the medians, the edges of the boxes represent the 25th and 75th percentiles. <sup>1</sup> indicates that only one cohort was infected, TS - test statistics, r - effect size.



### Figure 2

Figure 2: Parasite load in the SI wild boar - domestic pig association.

Comparison of the intensity of infection with certain parasite taxa between wild boar and domestic pig cohorts in Slovenia. The centre lines of the boxplots indicate the medians, the edges of the boxes represent the 25th and 75th percentiles. <sup>1</sup> indicates that only one cohort was infected, U - test statistics, r - effect size.

