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Italian wolves (*Canis lupus italicus* Altobello, 1921) and molecular detection of taeniids in the Foreste Casentinesi National Park, Northern Italian Apennines



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ABSTRACT

After centuries of massive decline, the recovery of the wolf (*Canis lupus italicus*) in Italy is a typical conservation success story. To learn more about the possible role of parasites in the wolves' individual and population health and conservation we used non-invasive molecular approaches on fecal samples to identify individual wolves, pack membership, and the taeniids present, some of which are zoonotic. A total of 130 specimens belonging to 54 wolves from eight packs were collected and examined. Taeniid eggs were isolated using a sieving/flotation technique, and the species level was identified by PCR (gene target: 12S rRNA and *nad1*). Taeniid prevalence was 40.7% for *Taenia hydatigena*, 22.2% for *T. krabbei*, 1.8% for *T. polyachanta* and 5.5% for *Echinococcus granulosus*. The prevalence of *E. granulosus* is discussed. Our results show that the taeniid fauna found in wolves from the Foreste Casentinesi National Park is comparable to that described for other domestic and wild Italian canids and provides insights into the wolves' diet and their relationship with the environment.

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1. Introduction

After centuries of massive decline, several populations of large carnivores (brown bear, wolf, lynx, and wolverine) are now recolonizing parts of their historical ranges in many European countries thanks to the implementation of active adaptive conservation efforts (Chapron et al., 2014). The wolf in Italy is a typical conservation success story (Randi, 2011).

At the end of the Second World War, Italian wolves were close to extinction, surviving at their historical minimum population size in two isolated areas in the Southern Apennines (Zimen and Boitani, 1975; Boitani, 1984, 1992). However, since the late eighties socio-ecological changes and the increase in wild ungulates in natural areas have favored a spontaneous re-expansion of Italian wolves along the Apennines to the Western Italian and French Alps

(Breitenmoser, 1998; Boitani, 2000; Valière et al., 2003; Fabbri et al., 2007; Marucco and McIntire, 2010). On one hand, the impact of this rapid recovery can increase conflicts with hunters seeking the same prey, livestock breeders suffering economic losses caused by wolf predation on domestic herds (Milanese et al., 2015), and the general public many of whom have a historical fear of wolves, which are still perceived as a potential threat to human safety (Linnell and Boitani, 2011; Glikman et al., 2012). On the other, the wolf arouses positive harmonies as a flagship species whose biology, ecology and population dynamics remain poorly known in the Italian ecological context.

During the last 40 years, many studies have investigated the distribution and expansion of the Italian wolf population (Zimen and Boitani, 1975; Fabbri et al., 2007), its abundance (Marucco et al., 2009; Caniglia et al., 2012; Galaverni et al., 2016), composition and home ranges of packs (Ciucci et al., 1997; Apollonio et al., 2004; Scandura et al., 2011; Caniglia et al., 2014), its genetic variability (Randi et al., 2000; Randi and Lucchini, 2002; Lucchini et al., 2004), the threat posed by hybridization with domestic dogs

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(Caniglia et al., 2013; Randi et al., 2014) and the impact on wild and domestic ungulates (Gazzola et al., 2005). A number of studies have investigated Italian wolf parasites (Arru et al., 1988; Guberti et al., 1991, 1993, 1998, 2004, 2005; Gori et al., 2015) because of the recognized role of wildlife parasites in shaping individual host fitness (Hudson, 2002) and their public health significance as zoonoses (Thompson, 2013). All these studies paid particular attention to *Echinococcus granulosus* sensu stricto (sheep strain genotype 1), an important emerging and re-emerging zoonotic agent, above all in the Mediterranean basin (Sadjjadi, 2006). *E. granulosus* is a small tapeworm approximately 3 mm in length, endemic in this region since the appearance of sheep farming and hence a close relationship has developed over the centuries between the domestic dog (definitive host) and small ruminants as the main intermediate hosts. The official data of sheep cystic echinococcosis (CE) in Italy is summarized in Deplazes et al. (in press), while the prevalence in adult sheep is at least around 40% (Poglayen et al., 2008a, b). The low prevalence of cystic echinococcosis (CE) in wild ruminants, the main wolf prey, has prevented the establishment of a purely wild animal cycle so far (Guberti et al., 2004). The low number of wolves ($n = 1300\text{--}1800$) (Galaverni et al., 2016), a high prevalence of infected sheep (40%), and many positive dogs, allow the wolf to be considered in a parallel epidemiological context, closely linked to the domestic cycle (Guberti et al., 2004).

The other species of tapeworm give rise to speculation in attempts to understand and know more about the wolf diet, as the larval stage of each cestode has a specific host range (i.e. *Taenia hydatigena*: wild and domestic ungulates; *T. krabbei*: only wild ungulates; *T. polyacantha*: micromammals).

The aim of this molecular study was to evaluate the presence of taeniid tapeworms in the wolves of the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP), Northern Italy. This area provides opportunities to better understand the ongoing expansion of the Italian wolf population as some (Cagnolaro et al., 1974; Apollonio et al., 2004) claim that the wolf never disappeared from the FCNP, which acted as a natural ecological corridor along the Apennines guaranteeing the link between wolves from Central Italy and those of the Western Alps (Fabbri et al., 2007; Caniglia et al., 2014).

Most of the studies on wildlife intestinal parasites depend on standard methodologies based on post-mortem examination (Wobeser, 2007). As the wolf is a protected and elusive species these techniques are not a feasible option, so we used fecal analyses (Carbonell and Rodriguez, 1998) combining parasitological analysis with individual host genotyping based on fecal DNA (e.g. Zhang et al., 2011). This approach allowed us to identify each fecal sample's taxonomic affiliation (e.g. wolf, dog or hybrid), genetic profile, sex and, thanks to the pedigree reconstruction, the family group to which it belonged (Lucchini et al., 2002; Fabbri et al., 2007; Marucco et al., 2012; Caniglia et al., 2014).

2. Materials and methods

2.1. Study area

The study area includes the Foreste Casentinesi, Monte Falterona e Campigna National Park (FCNP) located in the Northern Italian Apennines ($43^{\circ}51'34.26''\text{N}$; $11^{\circ}44'38.39''\text{E}$) and covers a surface of about 36,000ha, ranging from 400 to 1658 m a.s.l. (Fig. 1). Much of the area is woodland, characterized by some of the oldest European secular forests of silver fir (*Abies alba* Miller, 1759) and deciduous mixed woods of oak (*Quercus* spp.), beech (*Fagus sylvatica* L.), sycamore (*Acer pseudoplatanus* L.) and chestnut (*Castanea sativa* Miller). The area is densely populated by wild ungulates,

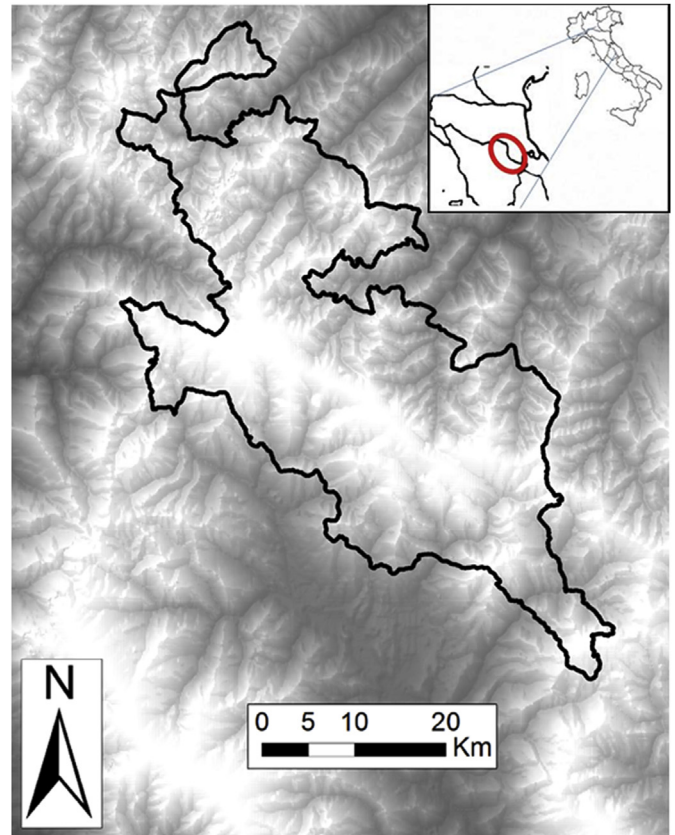


Fig. 1. The study area is located on the two sides of the Apennine watershed between Romagna and Tuscany, including the whole territory of the Foreste Casentinesi, Monte Falterona and Campigna National Park (FCNP).

including wild boar (*Sus scrofa* L., 1758), red deer (*Cervus elaphus* L., 1758), roe deer (*Capreolus capreolus* L., 1758), fallow deer (*Dama dama* L., 1758) and mouflon (*Ovis musimon* Pallas, 1762). The park lies between two regions, Emilia-Romagna and Tuscany. The protected area includes roads and 13 villages, with an average human density of 41.05 people/km². Few domestic ungulates (cattle, sheep and goats) are reared inside the park and hunting is strictly forbidden.

2.2. Sample collection and individual genotyping analysis

From 2001 to 2008 the Environmental Section (CTA) of the Italian Forestry Corp (CFS) and Institute for Environmental Protection and Research (ISPRA) started an intensive genetic monitoring program based on the non-invasive collection of scat samples to investigate the presence, status and distribution of the wolf population in the FCNP. The project was carried out in the framework of a wider regional study, whose results are reported in Caniglia et al. (2014).

During the genetic monitoring project, 1433 non-invasive presumed wolf biological samples were collected in the FCNP and analyzed at the ISPRA Genetic Laboratory to identify the genetic profile of individual wolves. Feces were collected along trails or country roads chosen opportunistically to maximize the probability of finding fresh samples and covering the entire study area. Roads and trails were surveyed at least once per month and the geographic coordinates of every sample were recorded by GPS.

Small samples from the external portions of scats were individually stored in 10 vials of 95% ethanol. Before any manipulation,

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