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# Diversity and host specificity of coccidia (Apicomplexa: Eimeriidae) in native and introduced squirrel species

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## Abstract

Introduction of alien species into new areas can have detrimental effects on native ecosystems and impact the native species. The present study aims to identify coccidia infecting native and introduced squirrels in Italy, to gain insight into possible transmission patterns and role of monoxenous coccidia in mediating the competition between alien and native hosts. We collected 540 faecal samples of native red squirrels, *Sciurus vulgaris*, invasive alien grey squirrels, *S. carolinensis*, and introduced Pallas's squirrels, *Callosciurus erythraeus*. Total prevalence of *Eimeria* spp. was 95.6% in *S. vulgaris*, 95.7% in *S. carolinensis* and only 4.1% in *C. erythraeus*. Morphological examination revealed 3 *Eimeria* morphotypes. Phylogenetic analyses of *Eimeria* DNA based on 18S, ITS, *cox I* markers displayed fairly distinct monophyletic clades in the microscopically indistinguishable E2 morphotype, proving indisputable distinction between the isolates from red and grey squirrels. Grey squirrels successfully introduced *E. lancasterensis* from their native range, but this species does not spill over to native red squirrels. Similarly, there is no evidence for the transmission of *E. sciurorum* from red to grey squirrels. The possible transmission and the potential role of monoxenous coccidia in mediating the competition between native and invasive squirrels in Italy were not confirmed.


***Keywords:*** Competition; *Eimeria*; *Sciurus carolinensis*; *Sciurus vulgaris*; Squirrels

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## Introduction

Biological invasions are among the most prominent threats for biodiversity. Introduction of alien species into new geographic areas can have detrimental effects on native ecosystems and impact the native species both directly (e.g. through predation or introduction of lethal pathogens) or indirectly (e.g. through competition, including the parasite-mediated competition) (Clavero and Garcia-Berthou 2005; Hartigan et al. 2011; Pizzatto and Shine 2011; Zavaleta et al. 2001). Moreover, invasive species and the pathogens they spread represent a threat for human health (Hulme 2014).

Parasites may play a role in biological invasions via three main mechanisms: (i) invaders may lose some of their parasites during translocation, leading to a competitive advantage (Torchin et al. 2003); (ii) invaders may serve as complementary hosts for local parasites, leading to spillback process or dilution effect, depending on their competence as hosts (Kelly et al. 2009); (iii) invaders may introduce with them new parasites, which may spill over to native hosts (Dubey and Shine 2008; Paterson and Gray 1997). Although the majority of parasites are host-specific (Pizzatto and Shine 2011; Poulin 2007), a range of examples of successful invasion (i.a. avian malaria to Hawaii, *Fascioloides magna* to Europe, spreading of chytridiomycosis in amphibian populations) suggests that the interspecific transmission of parasites can occur more frequently than expected (Atkinson et al. 2014; Marzal et al. 2015; Skerratt et al. 2007). In some of these cases, the introduced pathogens seriously impacted the naive host populations leading to their decline or extinction.

Parasite-mediated competition is common in natural populations although difficult to observe (Price et al. 1988). Introduction of novel pathogens and parasites along with their hosts can play an important indirect role in invasion outcome by mediating competitive interactions with susceptible native hosts (Prenter et al. 2004). The phylogenetic relatedness between invaders and native hosts might facilitate the host-switch and spill over of parasites (Torchin and Mitchell 2004). Parasites have the evolutionary advantage of having shorter generation times, which leads to fast adaption to new hosts (Kaltz and Shykoff 1998). Among the others, the squirrel pox virus (SQPV) accelerates replacement of the susceptible native Eurasian red squirrel (*Sciurus vulgaris*) by alien Eastern grey squirrels (*Sciurus carolinensis*), which serve as unaffected reservoir (Collins et al. 2014; Tompkins et al. 2003). The North American Eastern grey squirrels have been repeatedly introduced into Europe (mainly Great Britain, Ireland and Italy) since the end of 19th century and cause the local extinction of the native Eurasian red squirrel mainly through competition for food resources (Gurnell et al. 2004; Wauters et al. 2005). However, in the British Isles, the replacement process is accelerated by the SQPV (Rushton et al. 2005) and recent findings suggest that in Italy, where SQPV does not seem to occur, competition between these two squirrel species might be mediated by a North American nematode, introduced by the alien host (Romeo et al. 2014, 2015). The grey squirrels were introduced into Italy later than Great Britain: they were first reported in Piemont in 1948, but subsequent introductions were reported in Genova-Nervi in 1966 and, since the 1990, in many sites in Lombardy (Bertolino et al. 2014; Martinoli et al. 2010). During the last decade, Pallas's squirrel (*Callosciurus erythraeus*) has been introduced in Lombardy from South-East Asia and established a viable population in the North of the Varese province, co-occurring with native red squirrels (Bertolino and Lurz 2013; Mazzamuto et al. 2016a). Since both species are a threat for the local fauna, and in particular for the native red squirrel (Gurnell et al. 2004, 2015; Wauters et al. 2005; Bertolino et al. 2014) long-term conservation strategies aiming to preserve native biodiversity should not only include intensive control of populations of the alien species, but also surveys of parasites and infectious diseases and disease spread risk assessment (Guberti et al. 2014). As stated above, disease risk for native hosts may be greatly exacerbated by the introduction of alien species, especially when the two are phylogenetically related. Hence, our study focused on these three squirrel species present in Italy (native *S. vulgaris* and alien *S. carolinensis* and *C. erythraeus*) and on coccidia of genus *Eimeria* infecting them. In general, these intestinal protozoan parasites affect individuals with reduced immunocompetence, such as young animals, and may represent an added threat to already endangered populations (Hakkarainen et al. 2007; Levine and Ivens 1965; Winternitz et al. 2012). Although *Eimeria* species are considered highly host-specific, cross-transmission of these species between different hosts has been demonstrated (Levine and Ivens 1988). The present study aims to identify *Eimeria* spp. infecting native and invasive squirrels in Italy to gain insight into possible transmission patterns and, consequently, on the potential role of monoxenous coccidia in mediating the competition between native and invasive hosts.

Tens of *Eimeria* spp. have been described in squirrels (Levine and Ivens 1965) and microscopic examination of oocysts is often insufficient for exact species determination as different *Eimeria* spp. may have morphologically indistinguishable oocysts. This is especially true when, as in our case, data about endogenous stages or experimental infection are unavailable. Hence, when possible, we will make use of molecular tools for specific identification of oocysts.

Finally, we will also explore factors affecting variation of coccidia infections in our host species, to highlight possible differences in host–parasite relationships among the three squirrel species.

## Material and Methods

### Trapping and sample collection

Faecal samples of native red squirrels (*S. vulgaris*) and invasive grey squirrels (*S. carolinensis*) were collected periodically between 2010 and 2014; sampling of Pallas's