Macroparasite community of the Eurasian red squirrel (*Sciurus vulgaris*): poor species richness and diversity

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Abstract The Eurasian red squirrel (*Sciurus vulgaris*) is the only naturally occurring tree squirrel throughout its range. We aim at improving current knowledge on its macroparasite fauna, expecting that it will have a poor parasite diversity because in species that have no sympatric congeners parasite richness should be lower than in hosts sharing their range with several closely related species, where host-switching events and lateral transmission are promoted. We examined gastro-intestinal helminth and ectoparasite communities (excluding mites) of, respectively, 147 and 311 red squirrel roadkills collected in four biogeographic regions in Italy and France. As expected, the macroparasite fauna was poor: we found five species of nematodes and some unidentified cestodes, three fleas, two sucking lice and two hard ticks. The helminth community was dominated by a single species, the oxyurid *Trypanoxyuris* (*Rodentoxyuris*) *sciuri* (prevalence, 87 %; mean abundance, 373±65 worms/host). Its abundance varied among seasons and biogeographic regions and increased with body mass in male hosts while decreased in females. The most prevalent ectoparasites were the flea *Ceratophyllus* (*Monopsyllus*) *sciurorum* (28 %), whose presence was affected by season, and the generalist tick *Ixodes* (*Ixodes*) *ricinus* that was found only in France (34 %). All the other helminths and arthropod species were rare, with prevalence below 10 %. However, the first record of *Strongyloides robustus*, a common nematode of North American Eastern grey squirrels (*S. carolinensis*), in two red squirrels living in areas where this alien species co-inhabits, deserves further attention, since low parasite richness could result in native red squirrels being particularly vulnerable to parasite spillover.

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Introduction

Parasite diversity in host communities is affected by many factors related to environmental, ecological, and evolutionary components of both host and parasite species (Poulin 1997; 2004). One of the observed patterns is the positive relationship between parasite richness in a host and the number of phylogenetically closely related host species living in the same area (Krasnov et al. 2006; Pisanu et al. 2009). Closely related species are likely to have similar immunological and physiological characteristics, thus parasites colonizing related hosts have to cope with a similar set of immune defences requiring less adaptations on their part (Poulin and Mouillot 2004). Moreover, contact and lateral transmission may be again facilitated since phylogenetic relatedness often reflects similar life-history traits, behaviour and ecological requirements (Brooks and McLennan 1991). Hence, in hosts sharing their range with several closely related species, host-switching events and lateral transmission could be promoted and parasite diversity should be higher than in species that have no sympatric congeners. Indeed, a positive relationship between presence of closely related hosts and parasite richness has been observed for example in rodents (Krasnov et al. 2004) and fish (Raibaut et al. 1998; Marques et al. 2011).

The Eurasian red squirrel (*Sciurus vulgaris*) is the only naturally occurring tree squirrel species throughout its range (Lurz et al. 2005). Locally, it shares its ecological niche only with night-active tree-dwelling rodents such as the edible dormouse (*Myoxus glis*) or the Siberian flying squirrel (*Pteromys volans*). Two more congeneric tree squirrels live in Palearctic region, *Sciurus anomalus* and *Sciurus lis*, but their range is restricted respectively to the Caucasian region and Southern Japanese islands (Gurnell 1987). In contrast with the red squirrel, in the Nearctic region, four species of tree squirrels belonging to the genus *Sciurus* (*Sciurus carolinensis*, *Sciurus niger*, *Sciurus griseus* and *Sciurus aberti*) share parts of their range among one another (Steele and Koprowski 2001). Moreover, in some areas, the range of these species extensively overlaps with the range of tree squirrels of the genus *Tamiasciurus*, which is phylogenetically close to *Sciurus* (Mercer and Roth 2003). The presence of closely related squirrels within the same forest results in high parasite richness, with many heteroxenous parasites infecting multiple hosts (see, e.g. Rausch and Tiner 1948 on parasitic helminths of Sciuridae).

Currently, there is little basic information on the macroparasite community of the Eurasian red squirrel. Actual knowledge about its ectoparasites and helminths comes from taxonomic studies about single parasite species or checklists regarding localized populations with small sample size (e.g. Shimalov and Shimalov 2002; Popiolek et al. 2009). Only Feliu et al. (1994) surveyed helminths of the red squirrel in many individuals over a wide area across the Iberian Peninsula.

Here, we aim at improving the knowledge of the Eurasian red squirrel macroparasite fauna, exploring both its ectoparasite and gastro-intestinal helminth fauna over a wide geographical area including diverse habitats and climatic regions. Following predictions by Krasnov et al. (2004, 2006), we expect red squirrels to harbour relatively few parasite species since the species is isolated from closely related squirrels. Moreover, the red squirrel is a solitary, arboreal species that spends a relatively small amount of time on the ground (Wauters and Dhondt 1987), therefore we also expect its helminths to be mainly specialists with direct life cycles. Last, we also explore environmental (biogeographic region and season) and host-related variables (gender and body mass) affecting the distribution and abundance of the dominant ecto- and endoparasites, to identify the major factors influencing the parasite communities of the red squirrel.

Finally, it must be stressed that in some European countries the survival of the red squirrel is at risk due to the introduction of the Eastern grey squirrel, Sciurus carolinensis (Martinoli et al. 2010; Bosch and Lurz 2012). Hence, a poor parasite community in red squirrels could be especially alarming since the species may be particularly vulnerable to spillover of new parasites from the alien congener (Tompkins and Poulin 2006; Pisanu et al. 2007).

Materials and methods

A total of 356 freshly roadkilled red squirrels were collected between 1999 and 2012 in Italy and France from four biogeographic regions (Atlantic, Continental, Alpine and Mediterranean) as defined by EU Habitats Directive (http://ec.europa.eu/environment/nature/natura2000/sites_hab/biogeog_regions/index_en.htm) (Fig. 1). Only complete carcasses were recovered and stored in individual plastic bags and frozen at −20 °C for later examination. For each animal we recorded sex and age class (juvenile or adult, Wauters et al. 1993). The latter was defined according to body mass, weighed to the nearest gram, and hind foot length measured to the nearest millimeter (Wauters and Dhondt 1989; Wauters et al. 2007). Out of the whole sample, 209 squirrels were examined only for ectoparasites, 45 only for gastrointestinal helminths and 102 for both. Ectoparasites (fleas, hard ticks and sucking lice) were collected by repeatedly grooming squirrels with a flea comb and carefully examining the body regions where they most commonly aggregate (e.g. behind the ears). Ectoparasites were then counted and stored in ethanol 70 % for later identification. We searched for gastrointestinal helminths in the stomach, small intestine, large intestine and rectum separately by washing each part with tap water. The content of each tract was then flushed through a 0.04-mm sieve and examined using a stereo-microscope. Helminths were counted and stored in lactophenol or ethanol

Fig. 1 Locations of the 356 red squirrel roadkills collected in France and Italy between 1999 and 2011 in Atlantic, Alpine, Continental and Mediterranean biogeographic region
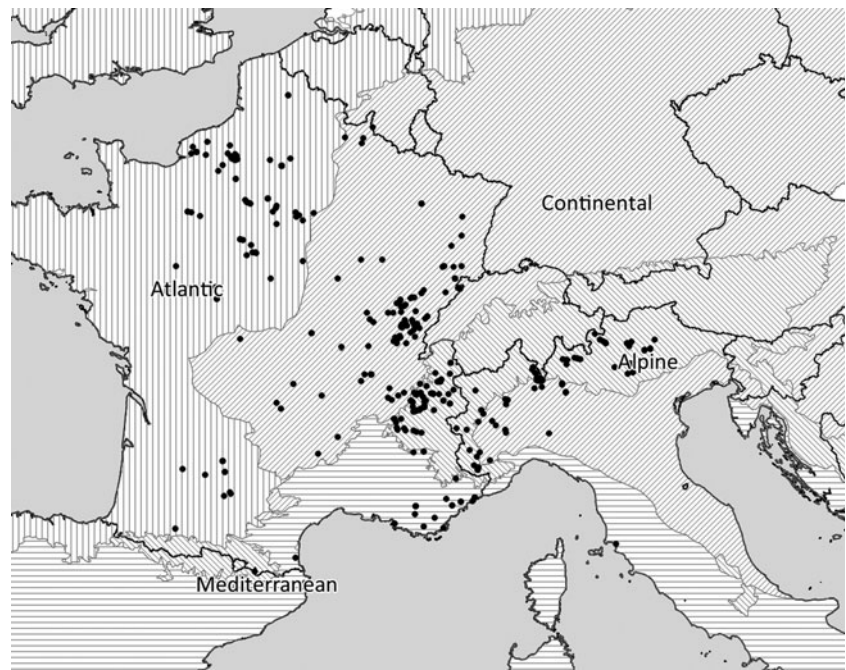
70 % for later identification. Species identification of both ectoparasites and helminths was done morphologically, using a microscope equipped with camera lucida, and was based on the descriptions of Anoploura by Beaucournu (1968) and Beaucournu et al. (2008), of Siphonaptera by Beaucournu and Launay (1990), and of Ixodid ticks by Pérez-Eid (2007). Strongyloidid helminths refer to Chandler (1942) and Sato et al. (2007), Oxyurids to Hugot (1984), Trichostrongyloids genus *Trichostrongylus* Loos, 1905 to Durette-Desset (1983), and species to Audebert et al. (2003). Subfamily of Cyclophyllidean cestodes refers to Khalil et al. (1994).

Statistical analysis

The abundance (number of parasites/host) of the most common helminth and the prevalence (number of infested hosts) of the most common ectoparasite were analysed through generalized linear models to explore the influence of host characteristics and extrinsic factors on their distribution.

To deal with the aggregated distribution of parasites (Shaw et al. 1998), before the analysis the abundance of the dominant helminth was log-transformed (log $(x+1)$). After transformation the variable met the assumption for normality (Shapiro–Wilk test: $W > 0.9$) and was analysed using linear models with Gaussian error. Only five specimens collected in the Mediterranean region were examined for helminths: due to the small sample size, these hosts were excluded from this part of the analysis. Variation in the presence of the most widespread species of ectoparasite was examined using logistic regression with a binomial error distribution and logit link

function. We chose to analyse variation in presence rather than abundance because roadkilled squirrels were sometimes recovered several hours after their death, when part of the ectoparasites could have already left the host, resulting in a potential underestimation of parasite burdens. In all the models, the effects of sex, season, biogeographic region and body mass were explored. We defined season using the same categories, based on temporal changes in squirrel behaviour and food availability, described in previous studies (winter, December–February; spring March–May; summer June–August; autumn September–November, e.g. Wauters et al. 2007; Romeo et al. 2010). Since body mass was used as a parameter to separate juvenile from adult squirrels and since interactions of age with other fixed effects would result in extremely small sample size or missing data for some combinations, we did not include age as a factor. We also could not include year of collection as a fixed effect since sample size in different years was highly unequal. We first fitted saturated models including all fixed effects and their second-order interactions, then we obtained minimum adequate models through backward selection based on $\Delta AICc > 2$ (Burnham and Anderson 2004). Graphical checking showed no evidence of spatial or temporal autocorrelation in variance errors, confirmed variance homogeneity and normality of residuals in linear models with Gaussian error, and no overdispersion in binomial GLM (Zuur et al. 2010). Interpretation of final models was based on differences of least square means (DLSM). Parameter estimates are reported as mean (±SE).

All the statistical analysis were done using SAS/STAT 9.2 software (Copyright © 2009, SAS Institute Inc., Cary, NC, USA).

Results

Helminth community

A total of 5 nematodes species and 13 specimens of unidentified cestodes were found in 147 red squirrels (Table 1). No trematodes or acantocephalan species were found. The number of parasite species per host ranged between 0 and 2 with a mean value of 0.9 species/individual. The most common helminth, which was found in all biogeographic regions, was the oxyurid *Trypanoxyuris* (*Rodentoxyuris*) *sciuri* (Cameron 1932), with a total prevalence of 87.1 % and a mean abundance of 373±65 worms/host. *T.* (*R.*) *sciuri* abundance varied among biogeographic regions and seasons (Table 2). In addition, the effect of sex was found to depend on body mass: *T.* (*R.*) *sciuri* abundance increased with body mass in males (Fig. 2a), while it decreased with body mass in females (Fig. 2b). Squirrels from the Continental region were more infested than those from the Alpine and Atlantic regions (both DLSM: *p*<0.0001) whereas difference between *T.* (*R.*) *sciuri* abundance in the Atlantic and the Alpine region was not significant (*p*=0.14; Fig. 3a). Mean abundance of the parasite was significantly lower in specimens recovered in summer than in spring and autumn (*p*=0.0002 and *p*=0.039, respectively) while there was no difference between the other seasons (all *p*>0.05; Fig. 3b). The other five helminth taxa were rare, with prevalence below 5 %. Immature stages of 13 specimens of Cyclophyllidean cestodes, all belonging to the family Hymenolepididae (Ariola, 1899), were found in the small intestine of five squirrels (3.4 %). Also, an immature stage of a female of Capillariid nematode was found in the stomach of

an adult female (0.7 %). One adult male of *Trichostrongylus vitrinus* (Loos, 1905) and another adult male of *Trichostrongylus* sp. (? *retortaeformis*) were found in the small intestine of two adult red squirrels (both 0.7 %). In Italy, 2 and 20 adult specimens of *Strongyloides robustus* (Chandler 1942) were identified in the small intestine of two adult squirrels (1.4 %).

Ectoparasite community

Seven ectoparasite species were found on 311 squirrels: three fleas, two sucking lice and two hard ticks (Table 3). Ectoparasite species richness ranged from 0 to 4, with a mean value of 0.7 species per host. The flea *Ceratophyllus* (*Monopsyllus*) *sciurorum sciurorum* (Schrank, 1803) was the most widespread parasitic arthropod, found in both countries and in all biogeographic regions, with a total prevalence of 27.6 %. *C.* (*M.*) *sciurorum* presence was affected only by season (χ^2_3=10.83; *p*=0.013): prevalence were significantly lower in winter than in autumn and summer (DLSM: *p*=0.026 and *p*=0.015) and lower in spring than in autumn (*p*=0.041; Fig. 4). Another flea species, *Tarsopsylla octodecimdentata octodecimdentata* (Kolenati, 1863), was found on seven squirrels (1.9 %) collected at altitudinal levels ranging between 740 and 1,220 m a.s.l.. We also found a single specimen of *Dasypsyllus* (*Dasypsyllus*) *gallinulae gallinulae* (Dale, 1878), on a juvenile collected in France (0.3 %). Two species of sucking lice were found, the most common was *Neohaematopinus sciuri* (8.0 %), while *Enderleinellus nitzschi* was found only on three hosts (1.0 %). Finally, we found two species of hard ticks that showed a segregated distribution in the two countries: squirrels collected in France were frequently infested by *Ixodes* (*Ixodes*) *ricinus*

Table 1 Helminth species infecting red squirrels in four biogeographic regions

Helminth species	Continental		Alpine		Mediterranean		Atlantic	
	n (*p*)	mI±SE	*n* (*p*)	mI±SE	*n* (*p*)	mI±SE	*n* (*p*)	mI±SE
Juvenile	*N*=6		*N*=1		*N*=1		*N*=7	
Trypanoxyuris (*R.*) *sciuri*	5 (83 %)	923±232	0	–	1 (100 %)	*20*	3 (43 %)	*350; 406; 1,000*
Adult ♀	*N*=15		*N*=22		*N*=1		*N*=21	
Trypanoxyuris (*R.*) *sciuri*	14 (93 %)	554±300	22 (100 %)	225±96	1 (100 %)	*15*	14 (67 %)	262±161
Strongyloides robustus	1 (7 %)	*2*	0	–	0	–	0	–
Capillariinae [gen. sp.]	0	–	0	–	0	–	1 (4 %)	*1*
Hymnolepididae [gen. sp.]	0	–	0	–	0	–	1 (4 %)	*1*
Adult ♂	*N*=19		*N*=28		*N*=3		*N*=23	
Trypanoxyuris (*R.*) *sciuri*	19 (100 %)	777±281	26 (93 %)	114±41	3 (100 %)	*20; 80; 1867*	20 (87 %)	263±78
Strongyloides robustus	1 (5 %)	*20*	0	–	0	–	0	–
Trichostrongylus sp. (?*retortaeformis*)	0	–	1 (4 %)	*1*	0	–	0	–
Trichostrongylus vitrinus	0	–	0	–	0	–	1 (4 %)	*1*
Hymnolepididae [gen. sp.]	2 (11 %)	*2; 4*	0	–	0	–	2 (9 %)	*3; 3*

N number of host examined; *n* number of infected hosts; *p* prevalence; *mI* mean intensity (*n* parasites/infected hosts; when number of infected hosts<5, worm counts in italic)

Table 2 Generalized linear model exploring effects of host characteristics and environmental variables on *T. (R.) sciuri* abundance. Parameter estimates were significantly different from 0 (both |*t*|>2.8; *p*<0.006)

Source of variation	F	df	p	Parameter estimate (±SE)
Sex	17.9	1, 131	<0.0001	
Body mass	0.04	1, 131	0.8	
Season	4.9	3, 131	0.003	
Biogeographic region	14.7	2, 131	<0.0001	
Sex × body mass	19.2	1, 131	<0.0001	
M				0.014 (±0.004)
F				−0.013 (±0.005)

(prevalence in France: 34.0 %), whereas in Italy we only found specimens of *I. (I.) acuminatus*, on three adult hosts (prevalence in Italy: 6.5 %).

Discussion

As expected, Eurasian red squirrels have a poor macroparasite assemblage, with only three dominant species: one gastro-intestinal nematode and two arthropods. The composition of macroparasite communities is consistent across biogeographic regions/habitats.

In particular, the gastro-intestinal helminth community is dominated by a single species, *T. (R.) sciuri*, an oxyurid nematode specific to the red squirrel (Hugot 1984). As predicted, the host arboreal ecology seems to prevent infestation by helminths with indirect life-cycles and/or free-living stages. Most oxyurids are characterised by an over-infestation strategy, and are mainly vertically transmitted through the population, although some horizontal transmission can occur via physical contact or environmental contamination (Anderson 2000). Red squirrels are solitary and frequent contact between individuals occurs almost exclusively during the mating season (between January and May, Wauters and Dhondt 1989; Wauters et al. 1990). Moreover, horizontal transmission can occur in dreys, since the same nest can be used by different

individuals on consecutive nights (Wauters and Dhondt 1990; Lurz et al. 2005). These changes in the probability of horizontal transmission could be the reason for the observed seasonal variation in levels of infestation by *T. (R.) sciuri*, with the highest peak in spring and the lowest abundance in summer, when contacts between individuals are rarer and squirrels spend less time in nests (Wauters and Dhondt 1987; Wauters 2000). This helminth was common in all the habitats, but its abundance was higher in the Continental biogeographic region. This could be related to red squirrels occurring at higher densities in deciduous or mixed broadleaf-pine woods with more predictable and higher food availability, and less harsh weather conditions, than in conifer forests in the Alpine region (Wauters et al. 2004, 2008; Lurz et al. 2005). The Continental region is also the most urbanized area in Europe and home range overlap between individual squirrels can increase in fragmented landscapes with small woodland patches (e.g. Verbeylen et al. 2009), possibly increasing the frequency of horizontal transmission of *T. (R.) sciuri*. Moreover, individuals living in disturbed habitats might be more susceptible to parasite infections because they are more stressed (Christe et al. 2006). Finally, gender differences in parasite infestations are commonly observed in many higher vertebrates due to sexual size dimorphism, testosterone immunodepressive effect and/or behavioural and ecological differences between males and females (see Poulin 1996; Shalk and Forbes 1997; Ferrari

Fig. 2 Relationship between *T. (R.) sciuri* abundance and body mass in male (**a**) and female (**b**) squirrels
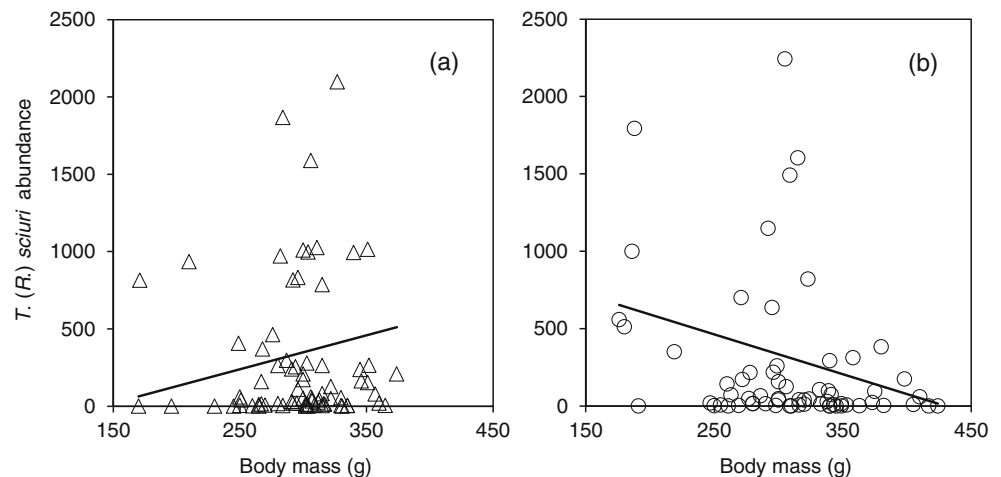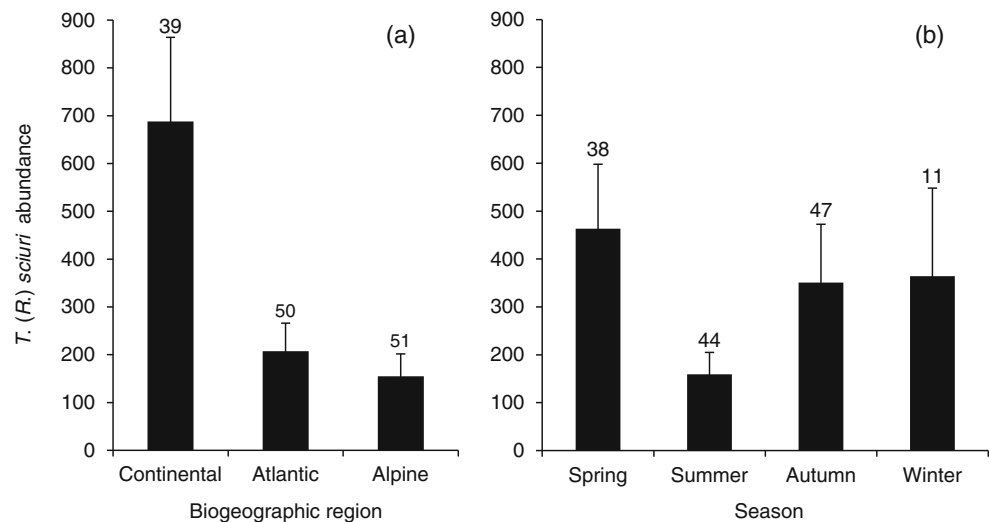
Fig. 3 Mean abundance (±SE) of the helminth *T. (R.) sciuri* by biogeographic region (**a**) and by season (**b**). *Sample size above error bars*

et al. 2007, 2010). For example, in several species of polygynous desert rodents, Krasnov et al. (2005) observed male-biased flea abundances during the mating season, when males' mobility and testosterone levels increase. In our case, although polygynous red squirrel males increase their mobility and home-range size during the mating season (Wauters et al. 1990; Di Pierro et al. 2008; Romeo et al. 2010), we did not observe any general nor seasonal differences in infestation between sexes. Yet, we found that the abundance of *T. (R.)*

sciuri increased with body mass in males whereas females showed the opposite relationship. This interesting pattern could be linked to gender differences in behaviour or immune function and should be investigated more deeply.

Ectoparasite assemblage was richer than helminths assemblage, still it was dominated only by two species: the flea *C. (M.) sciurorum* and the tick *I. (I.) ricinus*. *C. (M.) sciurorum* is distributed throughout Europe, and its primary hosts are red squirrels (Beaucournu and Launay 1990), along with other

Table 3 Infestation by arthropod species in red squirrels from four biogeographic regions

Arthropod species	Continental		Alpine		Mediterranean		Atlantic	
	n	*p*	*n*	*p*	*n*	*p*	*n*	*p*
Juvenile	*N*=31		*N*=6		*N*=1		*N*=14	
Ceratophyllus (M.)sciurorum	8	26 %	4	67 %	0	–	6	43 %
Tarsopsylla o. octodecimdentata	0	–	1	17 %	0	–	0	–
Dasypsyllus (D.) gallinulae	0	–	0	–	0	–	1	7 %
Neohaematopinus sciuri	2	6 %	0	–	0	–	4	29 %
Ixodes (I.) ricinus	5	16 %	0	–	0	–	3	21 %
Adult ♀	*N*=45		*N*=29		*N*=11		*N*=31	
Ceratophyllus (M.)sciurorum	9	20 %	10	38 %	1	9 %	9	29 %
Neohaematopinus sciuri	1	2 %	2	7 %	0	–	4	13 %
Enderleinellus nitzschi	0	–	0	–	2	18 %	0	–
Ixodes (I.) ricinus	12	27 %	6	21 %	1	9 %	16	52 %
Ixodes (I.) acuminatus	0	–	1	3 %	0	–	0	–
Adult ♂	*N*=60		*N*=42		*N*=6		*N*=35	
Ceratophyllus (M.)sciurorum	15	25 %	10	24 %	1	17 %	12	34 %
Tarsopsylla o. octodecimdentata	1	2 %	3	7 %	1	17 %	0	–
Neohaematopinus sciuri	1	2 %	3	7 %	0	–	8	23 %
Enderleinellus nitzschi	0	–	1	2 %	0	–	0	–
Ixodes (I.) ricinus	16	27 %	10	24 %	1	17 %	20	57 %
Ixodes (I.) acuminatus	2	3 %	0	–	0	–	0	–

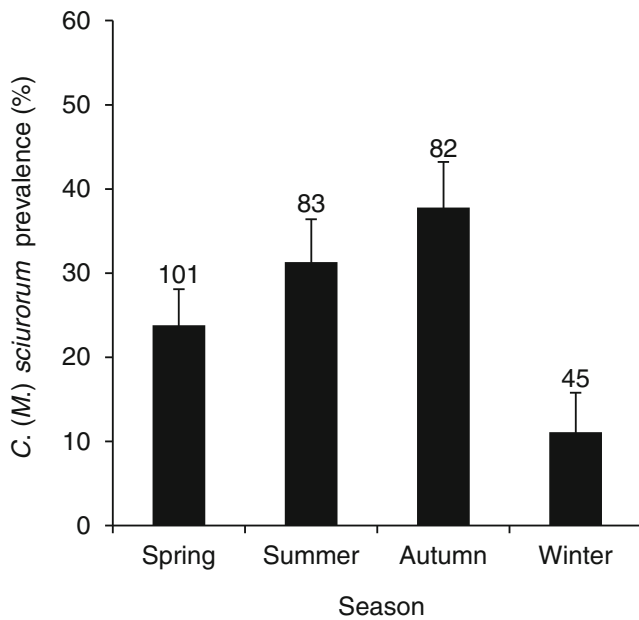*N* number of host examined; *n* number of infested hosts; *p* prevalence

Fig. 4 Prevalence (±SE) of the flea *C.* (*M.*) *sciurorum* in different seasons. *Sample size above error bars*

aerial nesting mammals such as the edible dormouse (*Myoxus glis*) or the common dormouse (*Muscardinus avellanarius*) (Beaucournu and Launay 1990; Traub et al. 1983; Trilar et al. 1994). This flea has been found also on Siberian flying squirrels (*Pteromys volans*, Haukisalmi and Hanski 2007), on introduced grey squirrels in Britain (Smit 1957) and Siberian chipmunk in France (Pisanu et al. 2008), and occasionally on martens (*Martes* spp.) and several other small carnivores and birds (Beaucournu and Launay 1990; Smit 1957; Traub et al. 1983). Infestation by *C.* (*M.*) *sciurorum* did not show any spatial variability, but prevalence varied seasonally, with lowest values in winter. Lower levels of infestation in winter could be a consequence of reduced host density and activity, preventing flea transmission (Krasnov et al. 2002). The other main flea, *T. o. octodecimdentata*, is a Paleartic subspecies infecting mainly red squirrels, but also Siberian flying squirrels (Haukisalmi and Hanski 2007). This species is well adapted to cold climates; in fact, it replaces *C.* (*M.*) *sciurorum* at high altitudes in the Alps (Beaucournu and Launay 1990). It is a nest flea, thus usually found on hosts in small numbers (Smit 1957). Of the two sucking lice we found, *E. niztschi* is monoxenous whereas *N. sciuri* is a Holarctic species mainly occurring on Eurasian red and North American squirrels (Durden and Musser 1994). The only generalist parasite for which the red squirrel seems an important feeding host, at least in France, is the tick *I.* (*I.*) *ricinus*. Surprisingly, this species was found only in France, despite being known to occur in various habitats also in Italy (e.g. Dantras-Torres and Otranto 2013a; Dantas-Torres and Otranto 2013b). This dissimilarity may be due to differences in ungulate presence between collection sites in the two countries, since deer are

primary hosts for the reproduction of *I.* (*I.*) *ricinus* (Pérez-Eid 2007). On specimens collected in Italy infestation by ticks was not relevant: red squirrels were only rarely infested by *I.* (*I.*) *acuminatus*, whose life-cycle takes place almost entirely inside burrows of ground-dwelling small mammals (Pérez-Eid 2007), limiting the opportunities for transmission to squirrels. The use of roadkilled animals may have led to an underestimation of ectoparasite richness, because some species leave carcasses earlier than others (e.g. Westrom and Yescot 1975), but ectoparasite screening on live-trapped red squirrels confirms that their macroparasite community in our study area is composed only by the above-mentioned arthropods (Romeo et al., unpublished data).

All other helminth and arthropod species found were rare and can be considered accidental (e.g. the nematode *T. vitrinus* and the flea *D. gallinulae*, specific to sheep and passerine birds, respectively), but particularly meaningful is the first record of *S. robustus* in Europe. This species was found in two roadkills collected from an area in Northern Italy where the introduced Eastern grey squirrel is present. This nematode is a common parasite of North American squirrels, mainly grey squirrels (e.g. Davidson 1976; Conti et al. 1984); hence, our finding suggests that this species may spillover from the alien species towards red squirrels.

In general, we found that the red squirrel's parasite fauna is composed by a limited number of species. This result is consistent with previous findings (Feliu et al. 1994) on helminths of the red squirrel in Spain, where, based on a large sample (N=248), the helminth community was also found to be dominated by auto-infective oxyurids: *T.* (*R.*) *sciuri* (prevalence: 17.8 %) and *Syphabulea mascomai* (39.3 %). The absence of *S. mascomai* in our survey seems to confirm that this species is endemic of the Iberian Peninsula (Hugot and Feliu 1990).

Ectoparasite richness in the Eurasian red squirrel is similar to the parasite diversity observed in congeners in the Nearctic region (e.g. Parker 1971; Coyner et al. 1996; Durden et al. 2004). However, the number of gastro-intestinal helminths species is much lower. For example, even excluding potentially accidental species (i.e. prevalence<5 %), Eastern grey squirrels (*S. carolinensis*) and fox squirrels (*S. niger*) in their native range are known to be infested by respectively 7 and 4 species of gastro-intestinal helminths (Chandler 1942; Rausch and Tiner 1948; Parker 1971; Davidson 1976; Conti et al. 1984; Coyner et al. 1996). In addition, most of these parasites are shared between these two hosts and also with other closely related tree squirrels such as the American red squirrel (*Tamiasciurus hudsonicus*) (Rausch and Tiner 1948; Eckerlin 1974; Flyer and Gates 1982). Unfortunately, we could not directly test the hypothesis that a poor parasite community is related to a poor host community because in Europe there are no areas where the species is (naturally) sympatric with other tree squirrels. However, the fact that the extension of the range and the diversity of habitats exploited by the Eurasian red

squirrel and the North American species are similar, suggests that this difference in parasite richness is a specific result of the different structure of host communities in the two regions: one "isolated" host in the Palearctic and many closely related squirrels in the Nearctic. Hence, the comparison of our data with that of Nearctic squirrels, seems to support the hypothesis of a positive relationship between parasite richness in a host and the number of phylogenetically closely related host species living in the same area.

Finally, the low parasite richness observed in the gastrointestinal helminth fauna of the red squirrel could be particularly meaningful since impoverished parasite communities may show less plasticity towards environmental changes (Hudson et al. 2006) and be highly vulnerable to spillover from introduced alien species (especially closely related invaders). In recent years, there is growing concern about the role played by parasites in invasions by exotic plants and animals (e.g. Daszak et al. 2000; Cunningham et al. 2003; Prenter et al. 2004; Tompkins and Poulin 2006; Dunn 2009). One of the most cited examples concerns precisely the role of Squirrelpoxvirus in mediating the competition between Eurasian red squirrels and Eastern grey squirrels introduced to Great Britain and Ireland (Tompkins et al. 2002; Rushton et al. 2006). Considering that, apart from the grey squirrel, several squirrels species have been recently introduced to Europe (e.g. *Tamias sibiricus* and *Callosciurus* spp. in France and Italy, Chapuis 2005; Bertolino and Genovesi 2005; Bertolino and Lurz 2013), investigating the role of parasite-mediated competition in the interaction of this native species with other squirrels should be a priority in future research.

References

Anderson RC (2000) Nematode parasites of vertebrates: their development and transmission, 2nd edn. CABI Publishing, Wallingford

Audebert F, Cassone J, Kerboeuf D, Durette-Desset M-C (2003) Development of *Trichostrongylus colubriformis* and *Trichostrongylus vitrinus*, parasites of ruminants in the rabbit and comparison with *Trichostrongylus retortaeformis*. Parasitol Res 90:57–63

Beaucournu J-C (1968) Les Anoploures de Lagomorphes, Rongeurs et Insectivores dans la région paléarctique occidentale et en particulier en France. Ann Parasit Hum Comp 43:201–271

Beaucournu J-C, Launay H (1990) Les puces de France et du bassin méditerranéen occidental. Faune de France 76. Fédération Française des Sociétés de Sciences Naturelles, Paris

Beaucournu J-C, Pisanu B, Chapuis J-L (2008) *Enderleinellus tamiasis* Fahrenholz, 1916 (Anoploura: Enderleinellidae), espèce importée, implantée et nouvelle pour la faune de France. Parasite 15:175–178

Bertolino S, Genovesi P (2005) The application of the European strategy on invasive alien species: an example with introduced squirrels. Hystrix It J Mamm 16:59–69

Bertolino S, Lurz PWW (2013) *Callosciurus* squirrels: worldwide introductions, ecological impacts and recommendations to prevent the establishment of new invasive populations. Mammal rev 43:22–33

Bosch S, Lurz PWW (2012) The Eurasian red squirrel. Westarp Wissenschaften, Hohenwarsleben

Brooks DR, McLennan DA (1991) Phylogeny, ecology, and behavior. A research program in comparative biology. The University of Chicago Press, Chicago

Burnham KP, Anderson DA (2004) Multi-model inference: understanding AIC and BIC in model selection. Sociol Method Res 33:261–304

Cameron TW (1932) On a new species of *Oxyuris* from the grey squirrel in Scotland. J Helminthol 10:29–32

Chandler AC (1942) Helminths of tree squirrels in Southeast Texas. J Parasitol 28:135–140

Chapuis JL (2005) Répartition en France d'un animal de compagnie naturalisé, le tamia de sibérie (*Tamias sibiricus*). Rev Ecol (Terre Vie) 60:239–253

Christe P, Morand S, Michauux J (2006) Biological conservation and parasitism. In: Morand S, Krasnov BR, Poulin R (eds) Micromammals and macroparasites. Springer, Tokyo, pp 593–613

Conti JA, Forrester DJ, Frohlich RK, Hoff GL, Bigler WJ (1984) Helminths of urban gray squirrels in Florida. J Parasitol 70:143–144

Coyner DF, Wooding JB, Forrester DJ (1996) A comparison of parasitic helminths and arthropods from two species of fox squirrels (*Sciurus niger*) in Florida. J Wildlife Dis 32:492–497

Cunningham AA, Daszak P, Rodriguez JP (2003) Pathogen pollution: defining a parasitological threat to biodiversity conservation. J Parasitol 89:S78–S83

Dantas-Torres F, Otranto D (2013a) Seasonal dynamics of *Ixodes ricinus* on ground level and higher vegetation in a preserved wooded area in southern Europe. Vet Parasitol 192:253–258

Dantas-Torres F, Otranto D (2013b) Species diversity and abundance of ticks in three habitats in southern Italy. Ticks Tick-Borne Dis 4:251–255

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287:443–449

Davidson WR (1976) Endoparasites of selected populations of Gray Squirrels (*Sciurus carolinensis*) in the southeastern United States. Proc Helminthol Soc Wash 43:211–217

Di Pierro E, Molinari A, Tosi G, Wauters LA (2008) Exclusive core areas and intrasexual territoriality in Eurasian red squirrels (*Sciurus vulgaris*) revealed by incremental cluster polygon analysis. Ecol Res 23:529–542

Dunn AM (2009) Parasites and biological invasions. Adv Parasitol 68:161–184

Durden LA, Musser GG (1994) The sucking lice (Insecta, Anoplura) of the world: a taxonomic checklist with records of mammalian hosts and geographical distributions. Bull Am Mus Nat Hist 218:1–90

Durden LA, Ellis BA, Bank CW, Crowe JD, Oliver JH Jr (2004) Ectoparasites of gray squirrels in two different habitats and screening of selected ectoparasites for bartonellae. J Parasitol 90:485–489

Durette-Desset M-C (1983) Keys to genera of the Superfamily *Trichostrongyloidea*. In: Anderson RC, Chabaud AG (eds) CIH Keys to the nematodes parasites of vertebrates. Cambridge Agricultural Bureau, Cambridge, pp 1–86

Eckerlin RP (1974) Studies on the life-cycle of *Strongyloides robustus* Chandler, 1942, and a survey of the helminths of Connecticut sciurids. PhD Dissertation, University of Connecticut

Feliu C, Torres J, Miquel J, Casanova JC (1994) The helminthfaunas of rodents of the Iberian Peninsula in relation to continental rodents: the case of *Sciurus vulgaris* Linnaeus, 1758 (Sciuridae). Res Rev Parasitol 54:125–127

Ferrari N, Rosà R, Pugliese A, Hudson PJ (2007) The role of sex in parasite dynamics: model simulations on transmission of *Heligmosomoides polygyrus* in populations of yellow-necked mice, *Apodemus flavicollis*. Int J Parasitol 37:341–349

Ferrari N, Rosà R, Lanfranchi P, Ruckstuhl KE (2010) Effect of sexual segregation on host–parasite interaction: model simulation for abomasal parasite dynamics in alpine ibex (*Capra ibex*). Int J Parasitol 40:1285–1293

Flyger V, Gates JE (1982) Fox and gray squirrel, *Sciurus niger*, *Sciurus caroliensis* and allies. Pine squirrell, *Tamiasciurus hudsonicus* and *T. douglasii*. In: Chapman JA, Feldhammer GA (eds) Wild mammals of North America: biology, management, and economics. The Johns Hopkins University Press, Baltimore, pp 209–238

Gurnell J (1987) The natural history of squirrels. Helm, London

Haukisalmi V, Hanski IK (2007) Contrasting seasonal dynamics in fleas of the Siberian flying squirrel (*Pteromys volans*) in Finland. Ecol Entomol 32:333–337

Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? TRENDS Ecol Evol 21:381–385

Hugot JP (1984) Sur le genre *Trypanoxyuris* (Oxyuridae, Nematoda) I. Parasites de Sciuriés: sous-genre *Rodentoxyuris*. Bull Mus Natl Hist Nat 6:711–720

Hugot JP, Feliu C (1990) Description de *Syphabulea mascomai* n. sp. et analyse du genre *Syphabulea*. Syst Parasitol 17:219–230

Khalil LF, Jones A, Bray RA (1994) Keys to the cestode parasites of vertebrates. CAB International, Wallingford

Krasnov BR, Khokhlova IS, Shenbrot GI (2002) The effect of host density on ectoparasite distribution: an example of a rodent parasitized by fleas. Ecology 83:164–175

Krasnov BR, Shenbrot GI, Khokhlova IS, Degen AA (2004) Flea species richness and parameters of host body, host geography and host 'milieu'. J Anim Ecol 73:1121–1128

Krasnov BR, Morand S, Hawlena H, Khokhlova IS, Shenbrot GI (2005) Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. Oecologia 146:209–217

Krasnov BR, Poulin R, Morand S (2006) Patterns of macroparasite diversity in small mammals. In: Morand S, Krasnov BR, Poulin R (eds) Micromammmals and macroparasites. From evolutionary ecology to management. Springer, Tokyo, pp 197–231

Lurz PWW, Gurnell J, Magris L (2005) *Sciurus vulgaris*. Mammalian Species 769:1–10

Marques JF, Santos MJ, Teixeira CM, Batista MI, Cabral HN (2011) Host–parasite relationships in flatfish (Pleuronectiformes)—the relative importance of host biology, ecology and phylogeny. Parasitol 138:107–121

Martinoli A, Bertolino S, Preatoni DG, Balduzzi A, Marsan A, Genovesi P, Tosi G, Wauters LA (2010) Headcount 2010: the multiplication of the grey squirrel introduced in Italy. Hystrix It J Mamm 21:127–136

Mercer JM, Roth VL (2003) The effects of Cenozoic global change on squirrel phylogeny. Science 299:1568–1572

Parker JC (1971) Protozoan, helminth and arthropod parasites of the grey squirrel in south-western Virginia. PhD Dissertation, Virginia Polytechnic Institute

Pérez-Eid C (2007) Les tiques: identification, biologie, importance médicale et vétérinaire. Lavoisier, Paris

Pisanu B, Jerusalem C, Huchery C, Marmet J, Chapuis J-L (2007) Helminth fauna of the Siberian chipmunk, *Tamias sibiricus* Laxmann (Rodentia, Sciuridae) introduced in suburban French forests. Parasitol Res 100:1375–1379

Pisanu B, Marmet J, Beaucournu J-C, Chapuis J-L (2008) Diversité du cortège en Siphonaptères chez le Tamia de Sibérie (*Tamias sibiricus* Laxmann) introduit en forêt de Sénart (Ile-de-France). Parasite 15:35–43

Pisanu B, Lebailleux L, Chapuis J-L (2009) Why do Siberian chipmunks *Tamias sibiricus* (Sciuridae) introduced in French forests acquired so few intestinal helminth species from native sympatric Murids? Parasitol Res 104:709–714

Popiolek M, Hildebrand J, Zalesny J (2009) Morphology and taxonomy of *Rodentoxyuris sciuri* Quentin et Tenora, 1974 (Nematoda: Oxyurida: Enterobiinae) with notes on molecular phylogeny. Ann Zool 59:415–421

Poulin R (1996) Sexual inequalities in helminth infections: a cost of being male? Am Nat 147:287–295

Poulin R (1997) Species richness of parasite assemblages: evolution and patterns. Annu Rev Ecol Syst 28:341–358

Poulin R (2004) Macroecological patterns of species richness in parasite assemblages. Basic Appl Ecol 5:423–434

Poulin R, Mouillot D (2004) The relationship between specialization and local abundance: the case of helminth parasites of birds. Oecologia 140:372–378

Prenter J, MacNeil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasions. TRENDS Ecol Evol 19:385–389

Raibaut A, Combes C, Benoit F (1998) Analysis of the parasitic copepod species richness among Mediterranean fish. J Mar Syst 15:185–206

Rausch R, Tiner JD (1948) Studies on the parasitic helminths of the North Central States. I. Helminths of Sciuridae. Am Midl Nat 39:728–747

Romeo C, Wauters LA, Preatoni D, Tosi G, Martinoli A (2010) Living on the edge: space use of Eurasian red squirrels in marginal high-elevation habitat. Acta Oecol 36:604–610

Rushton SP, Lurz PWW, Gurnell J, Nettleton P, Bruemmer C, Shirley MDF, Sainsbury AW (2006) Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. Epid Infect 134:521–533

Sato H, Torii H, Une Y, Ooi H-K (2007) A new Rhabditoid nematode species in Asian sciurids, distinct from *Strongyloides robustus* in North American Sciurids. J Parasitol 93:1476–1486

Schalk G, Forbes MR (1997) Male biases in parasitism of mammals: effects of study type, host age and parasite taxon. Oikos 78:67–74

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Shimalov VV, Shimalov VT (2002) Helminth fauna of the red squirrel (*Sciurus vulgaris* Linnaeus, 1758) in Belorussian Polesie. Parasitol Res 88:1008

Smit FGAM (1957) Handbook for the Identification of British Insects, 1. Royal Entomological Society, London

Steele MA, Koprowski JL (2001) North American tree squirrels. Smithsonian Institution, Washington

Tompkins DM, Poulin R (2006) Parasites and biological invasions. In: Aleen RB, Lee WG (eds) Biological Invasions in New Zealand. Springer, Berlin, pp 67–82

Tompkins DM, Sainsbury AW, Nettleton P, Buxton J, Gurnell J (2002) Parapoxvirus causes a deleterious diseases in red squirrels associated with UK population declines. Proc Roy Soc B 269:529–533

Traub R, Rothschild M, Haddow JF (1983) The Rothschild collection of fleas. The Ceratophyllidae: key to the genera and host relationships. With notes on their evolution, zoogeography and medical importance. Academic, Orlando

Trilar T, Radulovic S, Walker DH (1994) Identification of anatural cycle involving *Rickettsia typhi* infection of *Monopsyllus sciurorum*

sciurorum fleas from the nests of the fat dormouse (*Glis glis*). Eur J Epidemiol 10:757–62

Verbeylen G, Wauters LA, De Bruyn L, Matthysen E (2009) Woodland fragmentation affects space use of Eurasian red squirrels. Acta Oecol 35:94–103

Wauters LA (2000) Squirrels—medium-sized Granivores in Woodland Habitats. In: Halle S, Stenseth NC (eds) Activity patterns in small mammals: a comparative ecological approach. ecological Studies 141, Springer, Heidelberg, pp 131–143

Wauters LA, Dhondt AA (1987) Activity budget and foraging behaviour of the red squirrel (*Sciurus vulgaris*, Linnaeus, 1758) in a coniferous habitat. Mamm Biol 52:341–352

Wauters LA, Dhondt AA (1989) Body weight, longevity and reproductive success in red squirrels (*Sciurus vulgaris*). J Anim Ecol 58:637–651

Wauters LA, Dhondt AA (1990) Nest-use by red squirrels (*Sciurus vulgaris* Linnaeus, 1758). Mammalia 54:377–389

Wauters LA, De Vos R, Dhondt AA (1990) Factors affecting male mating success in red squirrels (*Sciurus vulgaris*). Ethol Ecol Evol 2:195–204

Wauters LA, Bijnens L, Dhondt AA (1993) Body mass at weaning and juvenile recruitment in the red squirrel. J Anim Ecol 62:280–286

Wauters LA, Matthysen E, Adriaensen F, Tosi G (2004) Within sex-density dependence and population dynamics of red squirrels *Sciurus vulgaris*. J Anim Ecol 73:11–25

Wauters LA, Vermeulen M, Van Dongen S, Bertolino S, Molinari A, Tosi G, Matthysen E (2007) Effects of spatio-temporal variation in food supply on red squirrel *Sciurus vulgaris* body size and body mass and its consequences for some fitness components. Ecography 30:51–65

Wauters LA, Githiru M, Bertolino S, Molinari A, Tosi G, Lens L (2008) Demography of alpine red squirrel populations in relation to fluctuations in seed crop size. Ecography 31:104–114

Westrom D, Yescott R (1975) Emigration of ectoparasites from dead California ground squirrels *Spermophilus beecheyi* (Richardson). California Vector News: 97–103

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Meth Ecol Evol 1:3–14

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Online resource 1

Data exploration, choice and validation of statistical models

The following online resource aims at describing the process of data exploration and model checking applied to avoid misinterpretation of the statistical analyses performed in our paper. The process is inspired by recommendations and detailed methods and computation scripts provided by Zuur *et al.* (2009, 2010, 2012, 2013). Analyses were performed using packages provided in the R 2.15.2 software (R Core Team, 2012).

A. Linear model exploring variation in log(+1) transformed abundance of the pinworm *Trypanoxyuris* (*R.*) *sciuri*

a. Initial model

Worm counts in host individuals form a discrete variable that may be best described by a theoretical Poisson distribution. Running a Generalized Linear Model with a Poisson error and a logarithm link function yielded in considerable overdispersion: 1224.7, indicating an excess of variability in the data and a poor fit to a Poisson error model; using a Negative binomial error (and a log-link function) dropped the dispersion parameter down an acceptable value of 1.266, indicating an apparent overdispersion of the Poisson model rather than a true one. However, checking the validity of the initial model indicated a poor fit due to extreme fitted values associated with low Pearson's residuals.
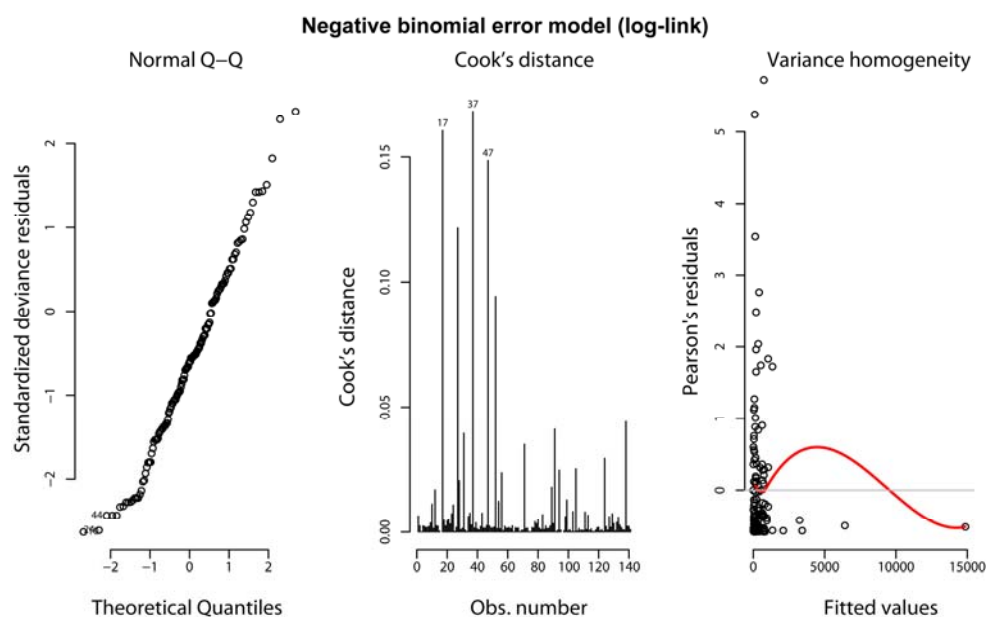

We found no reason to involve other source of heterogeneity (*i.e.*, missing co-variates or interactions between covariates, non-linear effect of covariates, zero-inflation or dependencies in the data such as

temporal or spatial autocorrelation between observations) than the one generated by outliers. The simplest approach to our dataset was to log(+1) transform the counts of *Trypanoxyuris* and use it as the response variable in a simple linear model, which yielded in a much better fit in term of variance homogeneity, with an absence of temporal or spatial dependencies (see below).
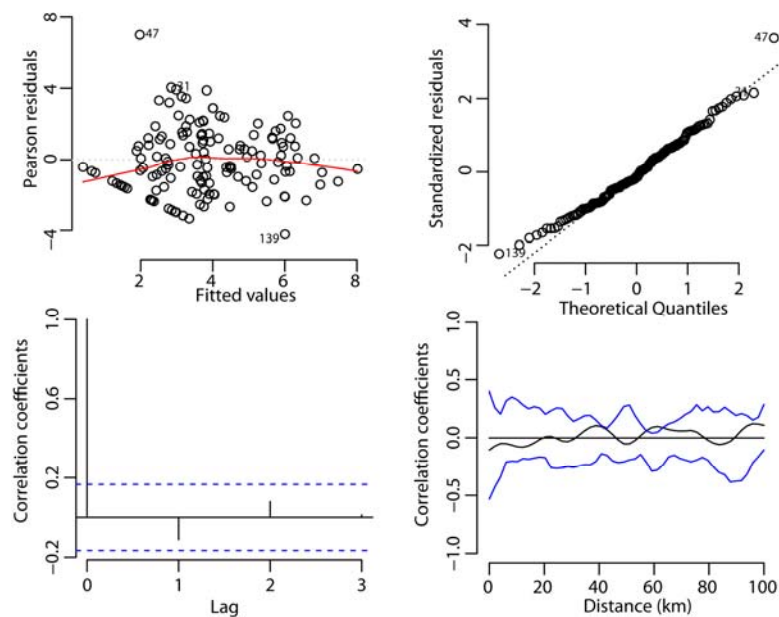
b. Final model

The graphical validation of the final model (*i.e.*, after AICc-based backward selection process) is given below: it does not show deviation from assumptions needed to validate a simple linear model.
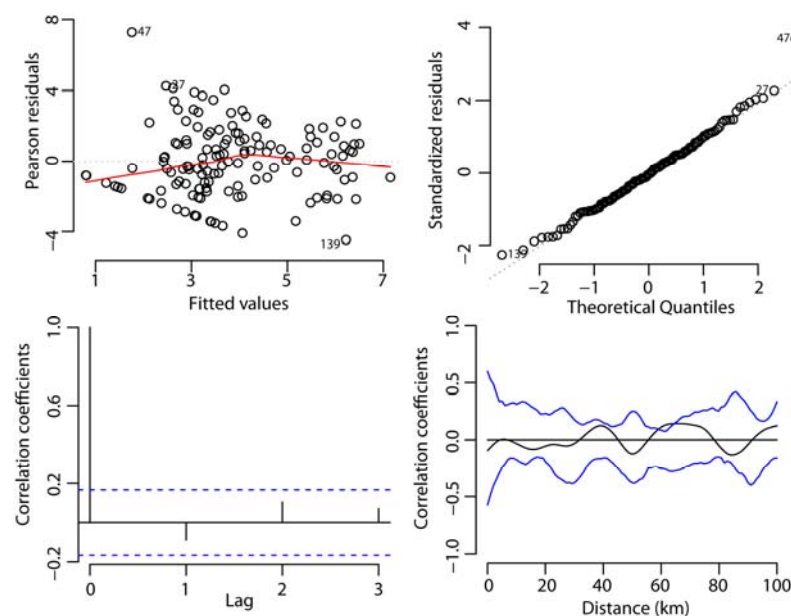
It should be noted however that an outlier is present (observation n° 47), corresponding to an adult female collected in an urban park near Paris (Parc Henri Sellier, Le Plessis-Robinson; 48°46'49.5"N-2°15'58.5"W) in June 2010 with an extreme worm burden (*i.e.*, 8000 worms).
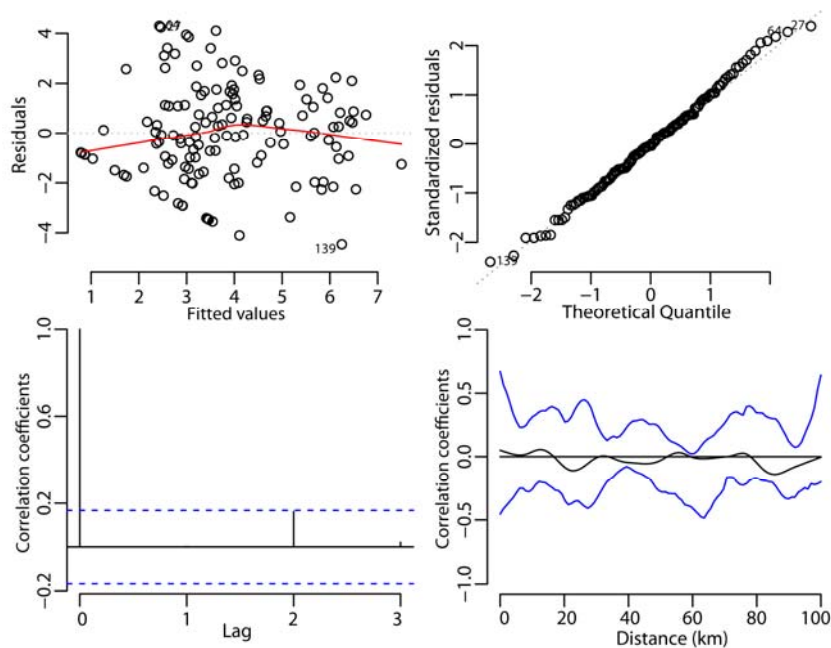
c. Dealing with the outlier n°47

We eliminated the outlier and re-ran the complete procedure. Both initial and final model yielded in same validation pattern:
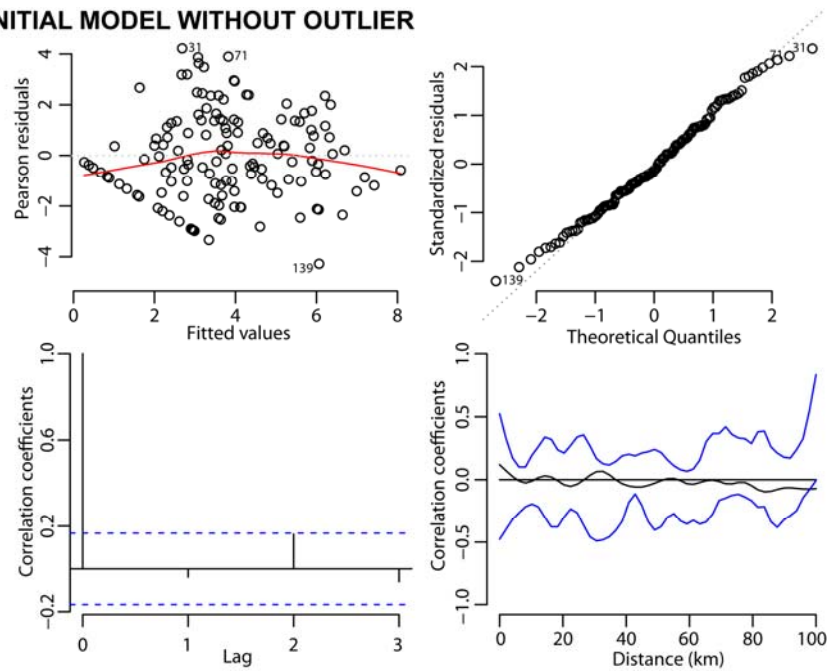
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Eliminating the outlier didn't change the result of the model selection, and parameter estimates were improved in the final model; the outlier was therefore eliminated in the presentation of the analysis of our paper.

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B. Generalized linear model with binomial error and logit link exploring the variation in prevalence of the flea *Ceratophyllus* (*M.*) *s. sciurorum*

In Binomial GLMs, a first step is to investigate for apparent overdispersion, which occurs when the variation in the data exceeds the theoretical amount of variability assumed by the Binomial distribution (Zuur *et al.*, 2013). A perfect fit of the data to the expected distribution yields in a dispersion parameter equal to 1.0; there is no reason to look forward at problems that may have been generated by outliers, missing co-variates or interactions between covariates included in the model, non-linear effect of covariates, zero-inflation or dependencies in the data such as temporal or spatial autocorrelation between observations.

a. Apparent overdispersion in the full model

Dispersion parameter for quasibinomial family taken to be 1.049; There is no apparent overdispersion.

b. Apparent overdispersion in the selected model

Dispersion parameter for quasibinomial family taken to be 1.020; There is no apparent overdispersion.

An absence of overdispersion makes us confident with the validity of the biological interpretation inferred from the Binomial model exploring the variation of the prevalence of *C.* (*M.*) *s. sciurorum*. Below is a graphical checking for validation of the selected model, showing variance homogeneity and few outliers, absence of temporal and spatial dependencies.
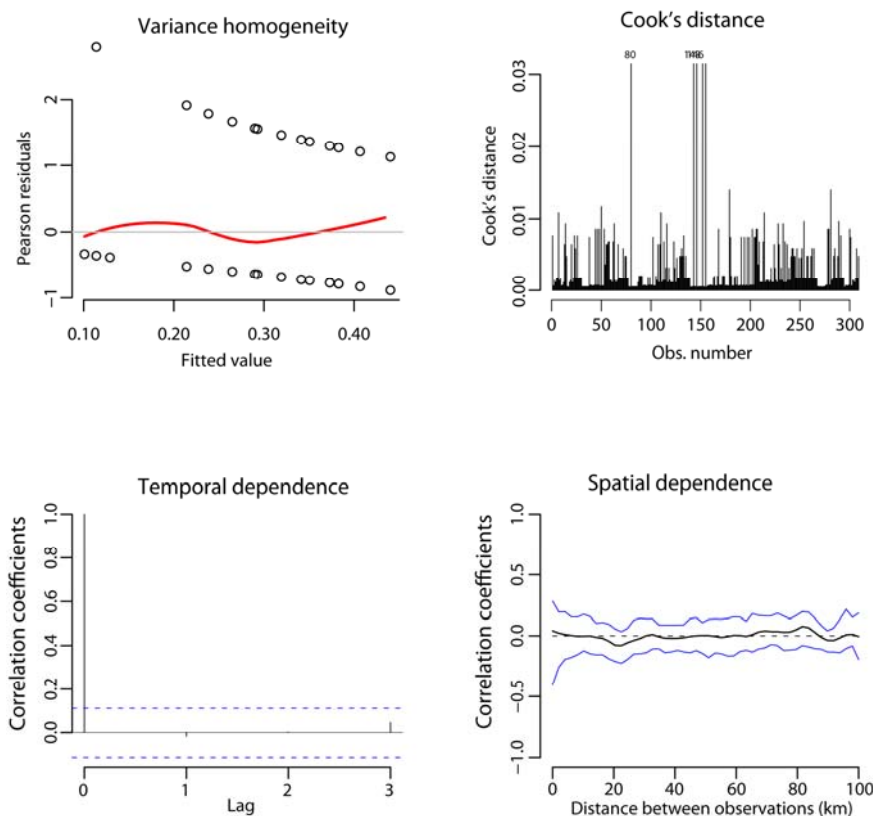
Romeo C., et al. - Macroparasite community of the Eurasian red squirrel (*Sciurus vulgaris*): poor species richness and diversity - *Parasitology Research*

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References

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM 2009 Mixed Effects Models and Extensions in Ecology with R. Springer, New York

Zuur AF, Ieno EN, Elphick CS 2010 A protocol for data exploration to avoid common statistical problems. *Meth. Ecol. Evol.* **1**, 3–14

Zuur AF, Ieno EN, Saveliev AA 2012 Zero Inflated Models and Generalized Linear Mixed Models with R. Highland Statistics Ltd., Newburgh, UK

Zuur AF, Hilbe JM, Ieno EN 2013 A Beginner's Guide to GLM and GLMM with R. Highland Statistics Ltd., Newburgh, UK

R Core Team 2012 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ [R version 2.15.2 (2012-10-26) -- "Trick or Treat"]