The gastrointestinal parasitic helminth fauna of 55 alien Pallas's squirrels (Callosciurus erythraeus) from the Uto Peninsula, Kumamoto, Kyushu, Japan, exterminated as part of an eradication program in October, 2010, were surveyed for the present study. Only four nematode species were detected, including one dominant strongylid, Strongyloides callosciureus (prevalence: 49.1%; abundance: 2.13 ± 0.66), and three other fragmental species, Brevistriata callosciuri, Capillariidae gen. sp. and Rictularia cristata (prevalence: 0.18%–0.55%; intensity: 1–3). The ratio of the mean to variance and the k-value of the negative binomial distribution pattern on the abundance of S. callosciureus were 0.089 and 0.283 (0.387 and 0.278 in male and female hosts), respectively. Relationships between the abundance of the strongylid and relevant factors such as individual host traits (sex, age, and body size) were examined using a generalized linear model. Male hosts harbored significantly more worms than the females in the optimal model. These sexual differences are attributable to the behavioral features of the host.

Key words: alien parasite, Callosciurus erythraeus, gastrointestinal helminth fauna, Pallas’s squirrel, Strongyloides callosciureus

INTRODUCTION

The Pallas’s squirrel (Callosciurus erythraeus) is a medium-sized sciurid that occurs naturally in India, Myanmar, southeastern China-west of the Irrawaddy River, Thailand, Malaysia Peninsula, Indochina, southern China and Taiwan-east of the Irrawaddy River [1, 2]. Its introduced populations are naturalized on some areas of Japan, including Tokyo, Kanagawa, Shizuoka, Gifu, Osaka, Hyogo and Wakayama Prefectures on Honshu, Nagasaki, Kumamoto and Miyazaki Prefectures on Kyushu, and Izu-ohima, Tomo-gashima, Fukue, Iki and Takashima Islands [2-11]. Their naturalization presents various issues, such as damages to agriculture and forestry and competition with the native sciurids of Japan. In light of these problems, some control measurements are implemented in various areas [2-3, 8-13]. The Pallas’s squirrel also has the potential to carry the pathogens of some infectious diseases that can affect humans as well as domestic and native wild mammals [14]. We examined their gastrointestinal helminth fauna. Moreover, we conducted some ecological analyses regarding the abundance of Strongyloides callosciureus, which is a dominant nematode species of the gastrointestinal parasitic helminth fauna of this host species [14-17], to examine host-parasite ecological relationships between these sciurid host and nematode parasite species.
MATERIALS AND METHODS

Fifty-five Pallas’s squirrels (18 males and 37 females) were trapped and killed at October 2010 on the Uto Peninsula, in the western part of Kumamoto Prefecture, Japan as a part of an eradication program of this alien sciurid [5, 13]. The elevation of this peninsula varies from sea level to 478 m. A major part (84%) of the area is mountainous and covered with forests, mainly natural deciduous broad-leaved forests. This peninsula lacks any other wild sciurid species.

The carcasses of the squirrels were frozen until the necropsy conducted in University of Toyama. Their body weight, total length, tail length and the lengths of their front and hind legs (with and without nail length) were measured. Afterward, their stuff and skull specimens were prepared. Their stomachs and intestines were removed and stored in 70% ethanol for further examination. They were dissected under a binocular microscope to collect the parasitic helminths. All obtained nematodes were fixed in 10% formalin and cleared with glycerin-alcohol for microscopic observation (x100–x600). All measurements of the nematodes are presented in millimeters. The nematode specimens collected in this study were donated to the Meguro Parasitological Museum, Tokyo (Accession Number: MPM Collection Nos. 21179-21183).

RESULTS

No platyhelminths and acanthocephalans were obtained, and only four species of parasitic nematodes were obtained. Their principle statistical values are shown in Table 1. The most dominant species was *S. callosciureus*, of which the prevalence and abundance attained ca. 50% and 2.13 ± 0.66, respectively. This species mainly parasitized the small intestines and fragmentally in stomachs with a few occurrences in the large intestine of the hosts. The ratio of the mean to variance and the k-value of the negative binomial distribution pattern could be calculated for this nematode species (0.089 and 0.283, respectively). This calculation revealed a negative binomial distribution pattern. The k-value was 0.387 and 0.278 in male and female hosts, respectively. This indicated that this nematode species has a higher degree of clumped distribution in female hosts than in male hosts. The other three species were few in number.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of positive hosts (prevalence in parenthesis, %)</th>
<th>Abundance (mean ± SE)</th>
<th>Intensity (mean ± SE or real numbers)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strongyloides callosciureus</strong></td>
<td>S**, SI, C 25 (49.1) 2.13 ± 0.66 4.68 ± 1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brevistriata callosciuri</strong></td>
<td>SI 1 (1.8) 0.05 ± 0.05 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillariidae gen. sp.</td>
<td>S 1 (1.8) 0.04 ± 0.04 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rictularia cristata</td>
<td>SI, LI 3 (5.5) 0.07 ± 0.04 2, 1, 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*S: stomach, SI: small intestine, LI: large intestine, C: caecum; **fragmental

The log-transformed abundance values of *S. callosciureus* were used as an objective variable. The ‘glm.nb’ function was used because this variable showed a negative binomial distribution pattern (see “Results”). We used the following data as explanatory variables: (1) host sex (0–1 data), (2) host age (0–4 data), (3) host body size (length of hind foot, mm). Each external measurement of the host animals was used in preliminary analyses tentatively, in which the optimal model with the lowest AIC (see below) was obtained when the length of hind foot was used. All main effects of the three explanatory variables and the interactive effects of all three combinations of two variables were tested. The optimal model was selected using the ‘step’ function and Akaike’s Information Criterion (AIC) [18, 20].
Helminths of Pallas's squirrel in Kyushu

GZLM analysis obtained for the abundance of S. callosciureus, and only the former was significant, as it had positive estimate value (Table 2). Thus, the male Pallas’s squirrels harbored significantly more S. callosciureus than the females. The abundance (mean ± standard error) of this nematode species was 4.06 ± 1.72 and 1.19 ± 0.43 in the male and female hosts, respectively.

**DISCUSSION**

Of the four species of parasitic nematodes, S. callosciureus and Brevistriata callosciuri were the primary parasites of the Pallas’s squirrel. They occur in the indigenous and artificial distribution areas of this host species [17, 21-24]. These two species were likely introduced in the present area with the hosts. Rictularia cristata primarily parasitizes the intestines of wood mice (Apodemus spp.) in Japan (Reviewed in Asakawa [25], and additional studies [26-34]) and its neighboring areas [35-38]. Some capillariid nematodes (Aonchotheca, Eucoleus and Pseudocapillaria) have been found in the alimentary tracts of various murids (mainly Apodemus spp.) present in Japan (Reviewed in Asakawa [25], and additional studies [26-33, 39, 40]) and its neighboring areas [35, 37, 38]. The present R. cristata and Capillariidae gen. sp. are assumed to be acquired by the host species from the habitats where it has been introduced. They include the present study area or the intermediate areas from native areas to the present study area. Gozzi et al. [41] found that parasitic helminth fauna of alien Pallas’ squirrels in Argentine lacks specialized species introduced with the hosts, and attributed this fact to difficulty of acquisition of such species with some ecological characteristics of this host species (e.g. an arboreal habit and a mostly vegetarian diet mainly based on fruits and seeds).

In this study, female hosts displayed a higher degree of clumped distribution pattern of S. callosciureus than the males. Male hosts had more worms of this nematode species than females, though the number of male hosts investigated in this study was less than that of females. Koizumi et al. [42] showed that two parasitic nematode species of the lesser Japanese mole (Mogera imaizumii) presented highly clumped distribution with remarkable lower k-values (0.106 and 0.265) than other various terrestrial vertebrate host-parasite relationships (reviewed in Yokohata [18]). They attributed the highly clumped distribution of parasitic nematodes of the mole mainly to the exclusive territorial habit of the host. In the case of the mole, the sexual difference of the spatial utilization pattern is absent except for during the mating season (e.g. Yokohata [43]). In contrast to the moles, home ranges of every Pallas’ squirrels frequently overlap with each other with less exclusiveness than moles, and the degree of overlapping is higher in male squirrels than in females [44]. Areas of the home ranges of males (ca. 4 ha) are much larger than those of the females (ca. 0.7 ha) [44]. The present higher degree of clumped distribution and lower abundance of this nematode species in the female hosts would be the result of limited opportunities for transmission of the nematode in smaller and less-overlapped territories of the female hosts. On the other hand, the highly clumped distribution of the nematodes of moles was also attributable to frequent multiple infections, which occur in latrines near the mole nests [42]. However, this is not the case of the Pallas’s squirrel, as there are no latrines in its arboreal habit. Other factors, such as physiological sexual difference, may affect the features of the S. callosciureus in its parasitizing ecology (also see Zuk and McKeen [45]). However, the characteristics of the spatial utility pattern and social behavior of the hosts should be ubiquitously important factors when considering the ecological interests of many parasitic organisms.

**ACKNOWLEDGMENTS**

We thank all hunters and research members for the control program of the Pallas’s squirrel on the Uto Peninsula for their assistance in collecting the materials. We also acknowledge

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**Table 2** The optimal model of the analysis of relationships between the abundance of *Helminthoides callosciureus* and some relevant factors using a generalized linear model

<table>
<thead>
<tr>
<th>Explanation variables</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>z-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>20.8751</td>
<td>11.1407</td>
<td>1.874</td>
<td>0.06096</td>
</tr>
<tr>
<td>Sex (Male to female)</td>
<td>1.3735</td>
<td>0.5331</td>
<td>2.577</td>
<td>0.00998*</td>
</tr>
<tr>
<td>Length of hind foot</td>
<td>-0.4065</td>
<td>0.2177</td>
<td>-1.867</td>
<td>0.06190</td>
</tr>
</tbody>
</table>

(AIC: 189.85; *P < 0.01)
Dr. N. Hayashi of the Tama Forest Science Garden, Forestry and Forest Products Research Institute, Japan, for providing information on the Pallas’s squirrel and some other sciurids.

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熊本県宇土半島産クリハラリス（Callosciurus erythraeus）の胃および腸管内寄生蠕虫相と外来糞線虫 Strongyloides callosciureus の感染数の分析

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[2016年1月10日受領, 2016年2月10日採択]

要約

熊本県宇土半島で外来種駆除事業の一環として2010年10月に捕殺された55頭のクリハラリス（Callosciurus erythraeus）の消化管内寄生蠕虫相を調査した。4種の線虫のみが見出され、そのうち優占的な糞線虫類、Strongyloides callosciureus は陽性率が49.1%、寄生虫体数が2.13 ± 0.66 であり、他の3種の線虫はBrevistriata callosciuri, Capillariidae gen. sp., Rictularia cristata で、これらの陽性率は0.18-0.55%、虫体数は3以下であった。S. callosciureus の寄生虫体数の平均/分散比および負の2項分布様式における k 値はそれぞれ0.089および0.283であり、集中分布を示しており、後者は雄宿主では0.387、雌宿主では0.278であった。この線虫種の寄生虫体数と宿主個体の特性（性、齢、体サイズ）の関係を一般化線形モデルを用いて調べたところ、雄の宿主が雌よりも多くの線虫を擁していた。これらの雌雄差は宿主の行動学的な差異によるものとみることができた。

キーワード：Callosciurus erythraeus, 外来寄生虫, 寄生虫体数, クリハラリス, Strongyloides callosciureus

——日本野生動物医学会誌 21(2): 29-34, 2016

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