Coccidia of raptors: morphological and molecular phylogenetic studies of genus *Eumonospora*

Summary of Doctoral Thesis

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In recent years, importation of captive-breed raptors (Accipitriformes, Falconiformes, and Strigiformes) into Japan as exotic pets are increased due to the gained publicity of owls in novels and movies and the increase in the number of exotic animal cafes. According to the notification system for the importation of animals in Japan, the number of imported Accipitriformes (including Falconiformes by law) has been around 200 to 400, but the number of imported Strigiformes was 609 in 2006 and 1,741 in 2016 which had increased by 2.8 times. It is necessary to submit a notification document and a hygiene certificate to the Japanese quarantine station when importing raptors from overseas into Japan and the hygiene certificate requires proof of no clinical signs of West Nile fever and avian influenza, but other pathogens. In fact, detection of zoonotic pathogens, such as *Chlamydophila psittaci*, in imported companion birds was reported. As mentioned above, the number of imported raptors is increasing in Japan due to the social situation. It is assumed that the opportunities of those raptors visiting animal clinics would be increased with little knowledge of the pathogens they carry. In this study, morphological and molecular phylogenetic analyzation of the coccidia found in imported raptors was investigated. Moreover, review of its taxonomic position and comparison with known coccidia was also performed.

In chapter 1, feces of imported raptors from breeding facility in Tokyo and veterinary clinics in Tokyo and Osaka were collected. Ten species of raptors were positive with parasitological fecal examination with direct and floating methods. Eggs of *Ascaridia* sp. was collected from *Falco peregrinus peregrinus, F. cherrug,* and *Milvus milvus.* Eggs of trematode was collected from *Torgos tracheliotos.* On the other hand, two morphologically different coccidia oocysts were collected from five genus of Strigiformes and one genus of Falconiformes. The small oocysts collected from *Pulsatrix perspicillata* was identified as *Eimeria* sp. morphologically. The large oocysts with

characters of single sporocyst with eight sporozoites inside and the absence of Stieda body in the sporocyst, was identified as *Avispora* sp. This is the first report of genus *Avispora* in Japan and new host record for *Pulsatrix perspicillata*, *Ptilopsis leucotis* and *Falco columbarius.*

In chapter 2, for revealing the taxonomic position of genus *Avispora*, morphological and molecular phylogenetic analyzation were attempted. Morphological characters of the oocysts collected from five genus of Strigiformes in 2018 were compared with other Strigiformes-originated *Avispora* spp. and identified as *Avispora henryae*. However, the key features of genus *Avispora* Schuster et al. 2016, single sporocyst in its oocyst and absence of Stieda body in its sporocyst, were already mentioned in the key features of genus *Eumonospora* in 1933. Thus, we resurrected and redescribed genus *Eumonospora* since we found that genus *Avispora* was junior synonym of genus *Eumonospora*. Furthermore, the genetic sequences of nuclear 18S rRNA (18S), nuclear 28S rRNA (28S), and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) of *E. henryae* were also determined. The oocysts collected from *Falco columbarius* in 2019 was identical to genus *Eumonospora* and morphologically identified as *Eumonospora kutzeri*. Nevertheless, genetic comparison of 18S, 28S, and *cox1* showed 100% identity to *E. henryae* originated from Strigiformes and we concluded that *E. henryae* to be the final identification. In this chapter, we found that *E. henryae* can infect both Strigiformes and Falconiformes which indicated the host specify of genus *Eumonospora* could be various with species nor than genus specific as theory.

In chapter 3, the genetic sequences of *E. henryae* were used for molecular phylogenetic study to investigate the taxonomic position of genus *Eumonospora*. The monosporocystic and octasporozoic coccidia, *Caryospora* and *Eumonospora*, were classified into family Eimeriidae until some studies revealed close relationship between genus *Eumonospora*

and family Sarcocystidae. Hence, the phylogenetic position of genus *Eumonospora* in suborder Eimeriorina was first investigated by performing 18S dataset with maximum likelihood (ML) method. In result, genus *Eumonospora* was clustered in family Sarcocystidae with clade of Toxoplasmatinae, Cystoisosporinae, genus *Hyaloklossia*, and *Nephroisospora eptesici.* The phylogenetic analysis of 18S, 28S, *cox1*, and concatenated datasets performed with ML and Bayesian inference, which *Sarcocystis rileyi* was used as outgroup, revealed that genus *Eumonospor*a and Toxoplasmatinae were sister groups. Moreover, the phylogram of parasite was highly congruous with that of the phylogram of host birds which might indicate the occurrence of cospeciation of between parasite and host, while molecular identification of *E. henryae* from different avian order boundaries might indicate host switching occurred in this species. On the other hand, family Sarcocystidae can be differentiated into Toxoplasmatinae, Cystoisosporinae, and Sarcocystinae based on their biological features. In this study, a new subfamily, Eumonosporinae, within family Sarcocystidae was proposed based on the unique morphological feature of monosporocystic and octasporozoic which can be differentiated from other subfamilies within family Sarcocystidae. In the end, defining the family Sarcocystidae based on criteria of oocyst morphology (disporocystic and tetrasporozoic) should be modified.

In this study, I resurrected the avian-parasitizing coccidia, genus *Eumonospora*, and proposed a new subfamily, Eumonosporinae. Furthermore, successive detection of *E. henryae* from imported raptors in Japan was also reported. Coccidia possesses high environmental and chemical resistant and it is difficult to eradicate once it has spread to the environment. The genus *Eumonospora* is pathogenic to avian species, especially raptors, and may cause severe illness and death in native birds in Japan if these protozoa

distributed to the environment. The animal quarantine of should be re-considered and tightened to prevent the introduction of hidden foreign pathogens.

The results of this research will be the basis for the taxonomic features of the genus *Eumonospora* and molecular phylogenetic findings in family Sarcocystidae. Further studies such as, regular parasitic surveys of native and imported birds in Japan, host specificity of the genus *Eumonospora*, identification of infection sites and life cycle pattern, discovery of intermediate or paratenic hosts, and cyst formation ability should be performed to determine the biological characters of this coccidia. However, duet to the high expense of raptors, the main definitive host, and the difficulty of acquiring SPF individuals, it is unlikely to prove the pathogenicity and pathophysiology via experimental infection. Hence, continuous collecting of host information, clinical symptoms, and histopathological examination are expected.