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# Diversity of *Enterocytozoon bieneusi* genotypes among small rodents in southwestern Poland

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ABSTRACT

Diversity of *Enterocytozoon bieneusi* genotypes in wild small rodent populations still remains incomplete and only few molecular studies have been conducted among these hosts. Therefore, the aim of this study was to determine whether small rodents, i.e., *Apodemus agrarius*, *Apodemus flavicollis*, *Mus musculus* and *Myodes glareolus* act as hosts of *E. bieneusi* and can play an important role in spore spreading in the environment of south-western Poland. Molecular analyses were conducted to determine pathogen genotypes. A total of 191 fecal and 251 spleen samples collected from 311 rodent individuals were examined for the occurrence of *E. bieneusi* by PCR amplifying ITS gene. The overall prevalence of *E. bieneusi* in rodent samples was 38.9%. The nucleotide sequences of ITS region of *E. bieneusi* revealed the presence a total of 12 genotypes with two being already known, i.e., D and gorilla 1 genotypes. The remaining ten are novel genotypes (WR1–WR10) which segregated into three groups in a neighbor joining phylogeny. This study reports for the first time *E. bieneusi* occurrence in wild living rodents in Poland and shows extensive genetic diversity within *E. bieneusi* isolates of rodent origin.



## 1. Introduction

*Enterocytozoon bieneusi,* an intracellular eukaryotic parasite, infects intestinal enterocytes (Mathis et al., 2005) and causes chronic and life-threatening diarrhea in immunocompromised persons. *E. bieneusi* has also been found in different populations of immunocompetent humans where it is responsible for symptomatic or asymptomatic infections (Didier and Weiss, 2011; Matos et al., 2012). Since the first report of *E. bieneusi* presence in animals (pigs), many studies have focused on the reservoir role of other animal hosts in the epidemiology of this pathogen (Deplazes et al., 1996). *E. bieneusi* has been commonly identified in a wide range of domestic and wild animals, mostly mammals and some birds (Dengjel et al., 2001; Buckholt et al., 2002; Sulaiman et al., 2003; Haro, 2005; Santín et al., 2005; Sak et al., 2011; Santín and Fayer, 2011; Mori et al., 2013; Guo et al., 2014). Rodents which are frequently found in agricultural areas, have the opportunity to come into contact with other wild animals, livestock and humans. Given the biology and behavior of rodents, they may be considered an animal reservoir of various microsporidia species. *E. bieneusi* transmission routes in humans and domestic animals, its host specificity and the significance of its zoonotic potential are still difficult to evaluate (Santin and Fayer, 2011).

Sequence analysis of the internal transcribed spacer (ITS) of the ribosomal RNA gene has been used widely in molecular epidemiological studies of *E. bieneusi* in humans and animals. Based on the high degree of diversity of ITS – over 200 genotypes – some genotypes are host-adapted and have been found in a variety of domestic and wild animals while other genotypes have no host specificity. Several *E. bieneusi* genotypes have been found only in humans or only in animal hosts, whereas some have been observed in both, suggesting zoonotic potential (Sulaiman et al., 2003; Thellier and Breton, 2008; Widmer and Akiyoshi, 2010; Feng et al., 2011; Fayer and Santin-Duran, 2014; Karim et al., 2014; Karim et al., 2015).

Although the diversity of *E. bieneusi* genotypes within mammalian population has generally been intensively studied, the knowledge of the presence of this pathogen in wild small rodent populations remains incomplete and only few molecular studies have been conducted among these hosts, i.e., in house mice (*Mus musculus*), deer mice (*Peromyscus* spp.) and voles (*Myodes gapperi* and *Microtus pennsylvanicus*) (Sak et al., 2011; Guo et al., 2014). Furthermore, the majority of prevalence studies rely on the detec-

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tion of microsporidian DNA in stool samples of examined animals. Currently there are limited literature data on the dissemination of *E. bieneusi* and very uncommon cases concern pulmonary or bile duct infections (Didier, 2005). Therefore, the aim of this study was to determine whether small rodents belonging to subfamilies of Murinae (*Apodemus agrarius*, *Apodemus flavicollis* and *M. musculus*) and Arvicolinae (*Myodes glareolus*) act as hosts for *E. bieneusi* and if they can contribute in spore spreading in the environment. Additionally due to the availability of spleen samples of rodent origin, the possible pathogen dissemination in these hosts was analyzed. Molecular analyses were conducted to assess the occurrence and genotypes of *E. bieneusi* in small rodents.

## 2. Materials and methods

### 2.1. Study areas and collection of rodents

Surveys were performed in five locations in south-western Poland which comprised various environments. Three of these were suburban: Jerzmanowo district (J) (51°7′N; 16°52′E), water distribution area (MD) (51°4′N; 17°6′E) and irrigation fields (O) (51°9′N; 16°58′E) for Wroclaw agglomeration; the other two were typical rural locations: the ornithological reserve "The Milicz Ponds" (M) (51°31′N; 17°19′E) and recreation grounds surrounding the Ślęża Landscape Park (S) (50°50′N; 16°44′E).

Rodents, 311 individuals represented by striped field mouse *A. agrarius* ($n = 184$), yellow-necked mouse *A. flavicollis* ($n = 60$), bank vole *M. glareolus* ($n = 46$) and house mouse *M. musculus* ($n = 21$) were captured in Sherman live traps during 2010–2012. Only *M. musculus* individuals were captured in Jerzmanowo district (Table 1). After identification of species and sex, the trapped rodents were euthanized and anatomized. In the laboratory individual fecal ($n = 191$) and spleen ($n = 251$) samples were collected from rodents and used for further examination. The differences in the numbers of examined samples can be explained by the availability of samples. All animal procedures were approved by the Local Ethical Committee (No. 46/2008).

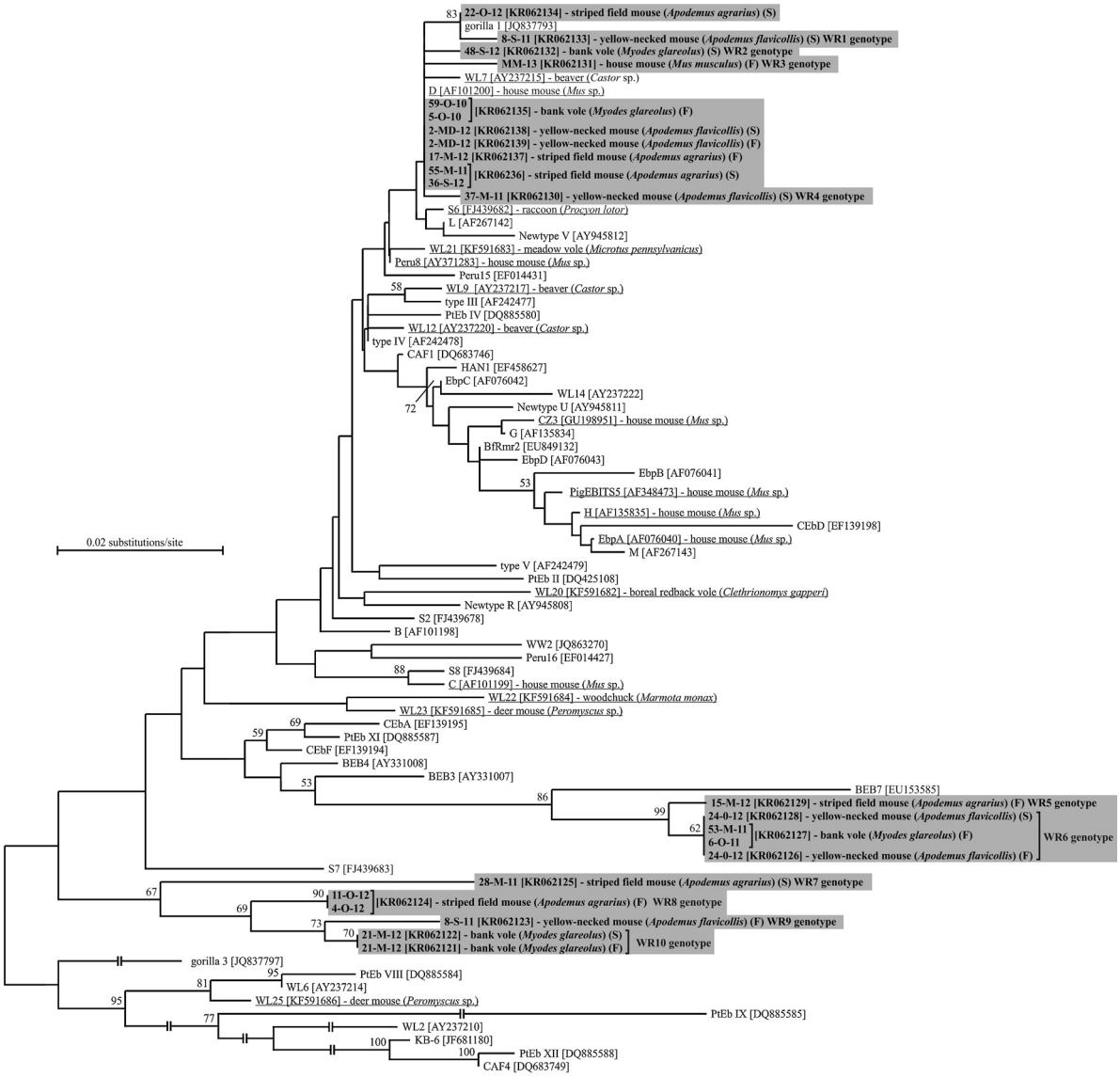


**Fig. 1.** Phylogenetic relationships among isolates of *Enterocytozoon bieneusi* genotypes detected in the present study (highlighted in grey) and others as inferred by a neighbor joining analysis of the internal transcribed spacer 1 (ITS). Isolates previously found in rodents are underlined. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). Numbers at the nodes represent bootstrap values for the nodes gaining more than 50% support. Scale bar is included. Interrupted branches have been shortened twenty-fold. Sample origin: spleen (S) and feces (F).