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Molecular phylogenetic characterization of *Collyriclum faba* with reference to its three host-specific ecotypes

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ABSTRACT

Collyriclum faba (Bremser in Schmalz, 1831) is a monostome digenetic trematode with unknown life cycle. On its definitive bird host, *C. faba* occurs in pairs within a subcutaneous cyst, the location of which on the host body is the base for differentiating three ecotypes of *C. faba*. Here we examined the hypothesis connecting strong host-specificity of the ecotypes with the possible existence of cryptic speciation among *C. faba*. Analysis of conserved nuclear ribosomal DNA regions rejected the cryptic speciation hypothesis. Analyses of the variable ITS1 and ITS2 regions revealed that, despite some differentiation between the ecotypes, several large-scale indels occur in multiple ecotypes. Individuals from multiple cysts affecting each host individual differed in their ITS1 sequences, suggesting the individuals infecting a single host did not have common parents. Since we were the first to sequence *C. faba*, we attempted to verify its position in the current taxonomic system (Plagiorchiida: Gorgoderoidea). We found that *C. faba* segregates with the superfamily Microphalloidea (in order of maximum likelihood phylogeny: Prosthogonimidae, Pleurogenidae and Microphallidae) instead of any species of the superfamily Gorgoderoidea, as proposed in the past. The results necessitate reclassification of the family Collyriclidae as a member of Microphalloidea.

1. Introduction

Uncovering cryptic biodiversity is essential for understanding evolutionary processes and patterns of ecosystem functioning, as well as for nature conservation. Recent estimates as to the distribution of cryptic diversity are contradictory and are only weakly supported by empirical data [1]. Even though the level of unrecognized diversity is expected to be low in temperate regions such as Europe (due to lower species richness and more intense taxonomic research compared to the tropical areas), multicellular parasites nevertheless comprise a group of organisms about which knowledge is very limited regarding their diversity, distribution and conservation status. In particular, cryptic biodiversity may account for yet unrecognized host-specificity and explain the potential for each parasite to serve as a vector for intra- and interspecific transfer of various diseases. Even though most of the recent gains in the number of reported cryptic species are due to studies based on DNA barcoding [2], integrative morphological, ecological and molecular approaches are more informative inasmuch as they provide more stable phylogenetic data [3,4].

In this report, we focus on genetic analysis of ecotypes of the cutaneous monostome trematode *Collyriclum faba* (Bremser in Schmalz, 1831). On its definitive bird host, *C. faba* occurs in pairs within a subcutaneous cyst, predominantly at three host-species specific locations: in the femoral or tibial regions (leg ecotype, Fig. 1A), in the area of the host's vent or in the abdominal area (vent ecotype, Fig. 1B), or above the coccygeal gland (rump ecotype, Fig. 1C). Considerably less frequently the cysts may occur also in the thoracic, sternal and orbital regions. While the differences in cyst location are highly host-species specific (Table 1), the reasons for these differences remain unknown. An individual host species usually is affected by only one ecotype of *C. faba*, and this suggests that genetic diversification may exist between the individual ecotypes and/or between the individuals parasitizing certain bird species. Confirmation of this hypothesis would argue in favor of the host specialization-induced changes in genetic diversity known from numerous other parasitic organisms [5–7]. Contrariwise, the syntopic occurrence of all three major ecotypes of *C. faba* suggests an alternative hypothesis: There may be no genetic distance between these ecotypes and that the existence of different ecotypes was caused rather by some yet unknown host-derived factor, such as a signaling molecule (cf. [8–12]).

Monotypic genus *Collyriclum* was established in 1911 by Kossack [13] for *Monostomum faba*, being initially included in Troglotrematidae (together with *Troglotrema*, *Pholeter*, *Renicola* and *Paragonimus*). In 1917, Ward [14] proposed the existence of Collyriclidae, but the existence of this family was not generally accepted until late 1930s. More recently, Vaucher [15] described *Collyricloides massanae*, the

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Fig. 1. Three major ecotypes of *Collyriclum faba* in Europe. (A) Leg ecotype (*Erithacus rubecula*). (B) Vent ecotype (*Cinclus cinclus*). (C) Rump ecotype (*Regulus regulus*). Photo by I. Literák, Velká Fatra Moutains, Slovakia, August 2000.

Table 1
Cyst ecotypes of *Collyriclum faba* differ in their locations on the hosts's bodies. [A]Cysts collected from these birds were subjected to the genetic analysis in this study. [B]Number of cysts (not birds) is provided. [C]The only bird reported by Rivolta and Delprato [34] had cysts in the abdominal area, on the legs, on the chest and on the neck. [D]Specimens from the U.S.A. (near Boston, MA). [E]Specimens from southern Europe (Postojna, Slovenia). [F]The only bird reported by Govoni et al. [35] had cysts both in the abdominal area and on the legs. [G]Specimen from western Europe (Delémont, Jura, Switzerland).

Cyst location Species	Leg	Vent or abdominal area	Rump (above coccygeal gland)	Other	Reference
Typically on the leg					
Periparus ater	1				[18]
Riparia riparia	7				[19]
Sylvia communis	1				[36]
Erithacus rubecula	219[A]	3	2	17	[17][B], [36,37]
Phoenicurus phoenicurus	1				[17]
Phoenicurus ochruros	4	2			[17][B]
Saxicola rubetra	1[A]				[18]
Fringilla coelebs	13	1		2	[17][B]
Emberiza citrinella	1				[17]
Typically near the vent or in the abdominal area					
Anas sp.		1			[38]
Molothrus ater		1			[39]
Corvus brachyrhynchos		9			[40–42]
Cyanocitta stelleri		1			[43]
Hirundo rustica		3			[19,44]
Petrochelidon pyrrhonota		4			[45]
Sylvia atricapilla		88[A]	5		[17][B]
Sylvia borin		1			[36]
Acrocephalus arundinaceus		1			[36]
Sturnus vulgaris		1			[46]
Cinclus cinclus		1			[17]
Toxostoma rufum		1			[47]
Turdus merula		1			[17]
Turdus philomelos		1			[36]
Turdus migratorius		1			[39]
Ixoreus naevius		1			[48]
Luscinia megarhynchos		3			[36]
Prunella modularis		1			[36]
Motacilla cinerea		5			[17]
Typically on the rump (above the coccygeal gland)					
Regulus regulus			25[A]		[17,36,49–51]
Typically on other parts of the body					
Tyranniscus vilissimus				4	[52]
Lipaugus unirufus				1	[52]
Lepidocolaptes affinis				1	[52]
Certhia familiaris				1	[36]
Catharus frantzii				1	[52]
Oporornis philadelphia				1	[52]
Basileuterus culicivorus				1	[53]
Thraupis episcopus				1	[54]
Rhodinocichla rosea				1	[52]
Piranga rubra				1	[52]
Cyanocompsa cyanea				1	[52]
Zonotrichia capensis				1	[52]
Atlapetes gutturalis				3	[52]
Atlapetes brunneinucha				1	[52]
Pselliophorus tibialis				2	[52]
No prevalent ecotype					
Numenius tenuirostris[C]	1	1		1	[34]
Passer domesticus[D]		3			[55]
Passer domesticus[E]	>2				[56]
Passer domesticus[F]	1	1			[35]
Passer domesticus[G]		1			[49]