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First record of *Aphidius ericaphidis* (Hymenoptera, Braconidae) in Europe: North American hitchhiker or overlooked Holarctic citizen?

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Abstract

Aphidius ericaphidis, an aphid parasitoid originally described from North America, is recorded in Europe for the first time, based on morphological and molecular analyses. The species is recorded in Serbia, the Netherlands, Sweden and the United Kingdom. It was formerly recorded as *Aphidius* sp./*Ericaphis latifrons*/*Vaccinium uliginosum* from the Alps (France). The possible origin of *A. ericaphidis*, as well as its likely distribution, is discussed in relation to its host. As it is a specific parasitoid of *Ericaphis* aphids, especially the invasive aphid *Ericaphis scammelli* on blueberries, its potential as a biocontrol agent is also discussed.

Keywords

Alien species, *Aphidius ericaphidis*, *Ericaphis scammelli*, blueberries, biological control

Introduction

Growing of highbush blueberry started with the experiments of Coville (1910) in the first decade of the 20th century in the United States. The first successful attempts to grow blueberries in Europe were made in Germany in the 1930s (Heermann 1932). They resulted in the first commercial plantation of 50 ha in 1951 (Pliszka 1997). World blueberry production has been significantly expanding in recent years, owing to the fruit's numerous health benefits. Between 1994 and 2014, the world area under commercial blueberries almost doubled, while production rose four times (FAOSTAT Database). With their high antioxidant capacity, long shelf life and minimal preparation prior to consumption, blueberries are considered a "superfruit" (Clarke 2016).

One of the economically most important blueberry pathogens is the blueberry scorch virus (BlScV), which was first observed in Washington, USA, in commercial blueberry fields (Martin and Bristow 1988). After that it was reported in British Columbia, Canada (Hudgins 2000) and has since spread to Europe (Ciuffo et al. 2005, Paduch-Cichal et al. 2011, Richert-Pöggeler et al. 2015, EPPO 2016). Most of the records are from the commercially grown highbush blueberry (*Vaccinium corymbosum* L.), and according to EPPO there are still no data on the susceptibility of native European *Vaccinium* species (EPPO 2005). Symptoms caused by BlScV differ depending on the cultivar of blueberry. In sensitive cultivars, infection can lead to complete necrosis (blighting) of flowers and young leaves and twig dieback followed by severe yield loss, while tolerant cultivars can show little or no visible symptoms of infection (Bristow et al. 2000, Martin et al. 2012).

The blueberry scorch virus is transmitted mainly by *Ericaphis fimbriata* (Richards) in a nonpersistent manner (Bristow et al. 2000). *Ericaphis fimbriata* is probably synonymous with *E. scammelli* (Mason), based on morphological and molecular analyses (Blackman and Eastop 1984, Foottit et al. 2008, G. Bosio – pers. comm. 2001, V. Eastop – pers. comm. 2002) and will be referred to as *E. scammelli* in this paper. The aphid is most likely native to North America and was probably introduced into Europe with plant material (Barbagallo et al. 1998), with the first record for Europe from the UK in 1964 (Cœur d'acier et al. 2010). In Britain it was described as *E. fimbriata* ssp. *pernettyae*, monoecious holocyclic on *Pernettya mucronata* but probably also holocyclic on *Vaccinium* species (Prior 1971), Italian populations are referred to as *E. scammelli* and are monoecious holocyclic on *Vaccinium* spp. (Blackman and Eastop 1984, Barbagallo et al. 1998, Pansa and Tavela 2008). Besides those two countries, it has also been recorded in the Netherlands, France (Nieto Nafria 2013) and Sweden (Nedstam 2008).

The braconid parasitoid complex of *E. scammelli* (=*E. fimbriata*) in North America consists of 10 species (Hymenoptera: Aphidiinae), among which the most common are *Praon unicum* Smith, 1944 and *Aphidius ericaphidis* Pike & Starý, 2011 (Raworth et al. 2008, Pike et al. 2011, Mathur et al. 2013). Here we present the first records of *A. ericaphidis* from Europe and discuss its potential as a biocontrol agent in European blueberry orchards.

Methods

During the last several years, the Aphidiinae fauna on different fruit species was investigated throughout Europe. Samples of *Vaccinium* spp. leaves with aphids and mummies were collected. The samples were kept under laboratory conditions until parasitoid emergence. After emergence, parasitoids were immersed in 96% ethanol and preserved for later examination. External morphology of the specimens was studied using a ZEISS Discovery V8 stereomicroscope. Scanning electron micrographs were obtained using a JEOL JSM-6390 scanning electron microscope. All specimens are deposited in the collection of the Institute of Zoology, Faculty of Biology, University of Belgrade and the collection of P. Starý, České Budějovice, Czech Republic.

Molecular analysis

Three *A. ericaphidis* specimens from Scotland were used for molecular analysis. DNA was extracted from individual adult wasps using the Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The barcoding region of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994).

DNA amplification was performed in a final volume of 20 µl containing 1 µl of DNA, 11.8 µl of H_2O, 2 µl of High Yield Reaction Buffer A with 1 x Mg, 1.8 µl of $MgCl_2$ (2.25 mM), 1.2 µl of dNTP (0.6 mM), 1 µl of each primer (0.5 µM) and 0.2 µl of KAPATaq DNA polymerase (0,05U/µl) (Kapa Biosystems Inc., USA). PCR was conducted in an Eppendorf Mastercycler® (Hamburg, Germany) using the following thermal profile: initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 60 s, 54°C for 60 s, 72°C for 90 s and a final extension step at 72°C for 7 min. Purification of PCR products and DNA sequencing in both directions was performed by Macrogen Inc. (Seoul, Korea).

Sequences of *A. ericaphidis* specimens from North America and other *Aphidius* species were obtained from GenBank and used in phylogenetic analysis. Those *Aphidius* species were chosen because of their morphological similarity to *A. ericaphidis* or because they parasitize *E. scammelli*. An *Ephedrus* sp. sequence from GenBank (acc. number KR787408) was used as an outgroup taxon. Sampling data for specimens used in this study are given in Table 1.

Sequences were edited using FinchTV ver 1.4.0 (http://www.geospiza.com). Alignment was conducted using CLUSTAL W integrated in MEGA 5 software (Tamura et al. 2011). Sequences were trimmed to a length of 611 bp. The sequences of analysed *A. ericaphidis* specimens were deposited in GenBank under accession numbers KY513289–KY513291. Calculation of average genetic distances between sequences was performed using Kimura's two-parameter method (K2P) of base substitution (Kimura 1980).

A phylogenetic tree was constructed using the MEGA 5 software (Tamura et al. 2011) and the Maximum likelihood method with 1000 bootstrap replicates.

Table 1. Sampling data for Aphidiinae specimens used in the molecular analysis.

Parasitoid	Code	Geographic origin	Aphid host	Plant	Accession number
Aphidius ericaphidis	IM50	Scotland	*Ericaphis scammelli*	*Vaccinium corymbosum*	KY513289
Aphidius ericaphidis	IM51	Scotland	*Ericaphis scammelli*	*Vaccinium corymbosum*	KY513290
Aphidius ericaphidis	IM52	Scotland	*Ericaphis scammelli*	*Vaccinium corymbosum*	KY513291
Aphidius ericaphidis		Canada/USA	*Ericaphis fimbriata*	*Vaccinium corymbosum*	KC211024
Aphidius ericaphidis		Canada/USA	*Ericaphis fimbriata*	*Vaccinium corymbosum*	EU574902
Aphidius avenaphis		USA	*Sitobion avenae*	*Triticum aestivum*	JN164784
Aphidius matricariae		Canada			KR888554
Aphidius urticae		UK			JX507436
Aphidius ervi		Canada/USA			KC211026
Ephedrus sp.					KR787408

Results

Aphids infesting *Vaccinium corymbosum* in Serbia, Sweden and Scotland were identified as *E. scammelli*. Rearing parasitoids from *E. scammelli* resulted in finding the species *A. ericaphidis* for the first time in Europe. *Aphidius ericaphidis* is recorded in Serbia, Sweden and the United Kingdom (Scotland). Additional re-examination of collections (P. Starý) led to identification of *A. ericaphidis* in France and the Netherlands as well, the re-examined specimens from both countries having been previously identified as *Aphidius* sp. (Starý et al. 1971, P. Starý unpubl.). In total 24 females and 19 males were found.

As the original differential diagnosis of *A. ericaphidis* referred to North American congeners (Pike et al. 2011), it is advisable to relate it also to those in Europe as follows: *Aphidius ericaphidis* (Fig. 1) is most similar to *Aphidius matricariae* Haliday 1834. It can be easily distinguished from the latter by the number of maxillary and labial palpomeres [*A. matricariae* has 3-segmented maxillary palps and 2-segmented labial palps vs. 4-segmented maxillary palps and 3-segmented labial palps in *A. ericaphidis*] and by pterostigma length / R1 forewing vein ratio [*A. matricariae* = 1.1 (range 1–1.2) vs. *A. ericaphidis* = 2.1 (range 1.7–2.7)].

Aphidius ericaphidis

Fig. 1

Serbia, Mladenovac, 10 VI 2015, 7 females and 4 males reared from *Ericaphis scammelli* on *Vaccinium corymbosum*; 23 VI 2015, 3 males reared from *Ericaphis scammelli* on *Vaccinium corymbosum.* United Kingdom, Scotland, 19 VI 2014, 9 females and 5 males reared from *Ericaphis scammelli* on *Vaccinium corymbosum*. Sweden,

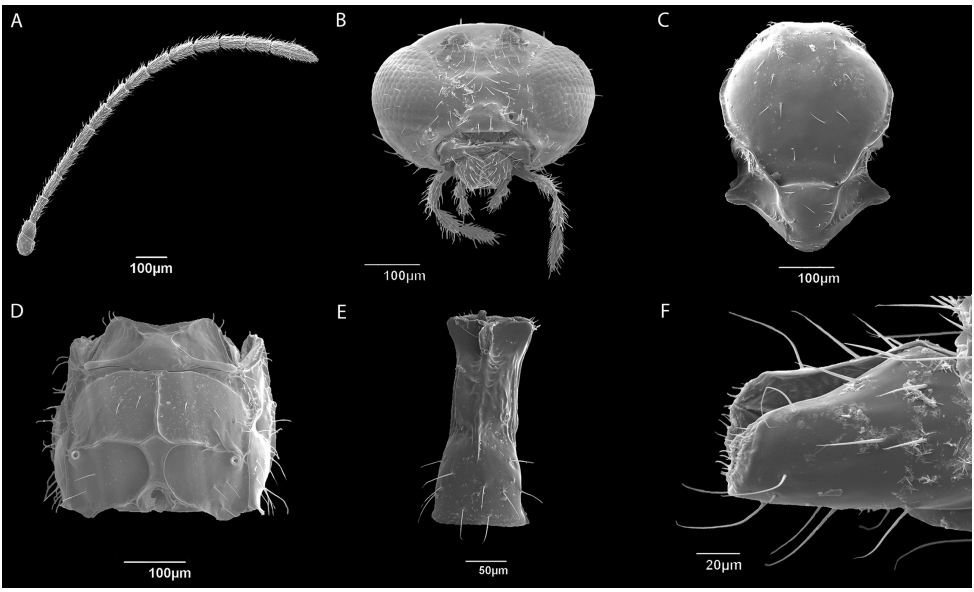

Figure 1. *Aphidius ericaphidis* female: **A** antenna **B** head **C** mesonotum – dorsal aspect **D** propodeum – dorsal aspect **E** petiole – dorsal aspect **F** ovipositor – lateral aspect.

Glemmingebro, Scania, 24 VI 2008, 1 female and 1 male from *Ericaphis scammelli* on *Vaccinium corymbosum*; 30 VI 2008, 5 females and 5 males from *Ericaphis scammelli* on *Vaccinium corymbosum*, greenhouse. Netherlands, Kootwijk, 8 VI 1965, 1 female from *Ericaphis latifrons* on *Empetrum nigrum.* France, Lognan (Hte-Savoie), 12 VIII 1968, 1 female and 1 male from *Ericaphis latifrons* on *Vaccinium uliginosum*.

Molecular analysis of *Aphidius ericaphidis*

Three barcoding sequences of *A. ericaphidis* originating from Scotland were compared with two sequences of *A. ericaphidis* from the USA and were determined to be identical, with no variable sites detected.

Topology of the phylogenetic tree shows clear separation of *A. ericaphidis* from other *Aphidius* species used in the analysis (Fig. 2). Though morphologically more similar to it than to the other *Aphidius* species, *A. ericaphidis* did not cluster with *A. matricariae*, and the mean K2P distance between the two species was 8.1% (Table 2). Divergence rates in relation to other species morphologically similar to *A. ericaphidis* or parasitizing *E. scammelli* were as follows: *A. urticae* – 8.9%, *A. avenaphis* – 10.4% and *A. ervi* – 8.3% (Table 2). Those distances are greater than what is considered to be enough for the separation of Aphidiinae species (Derocles et al. 2012, Tomanović et al. 2014). Thus, after morphological description, we here support the status of this taxon with molecular analysis.

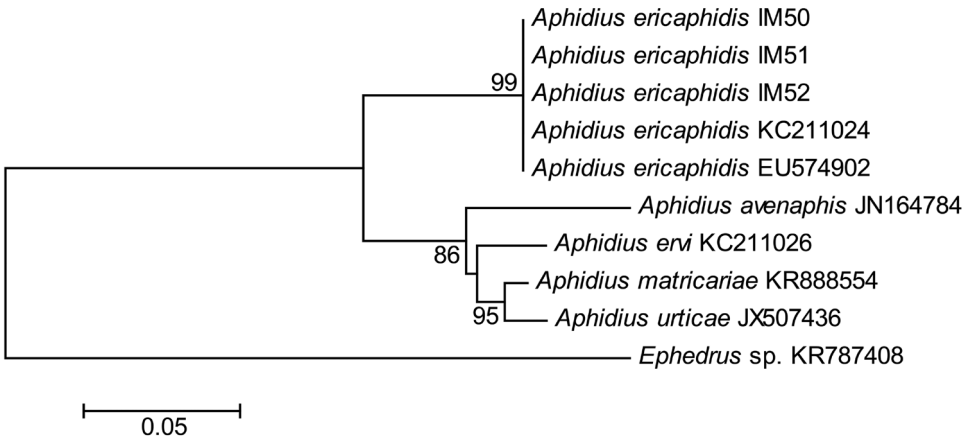

Figure 2. Phylogenetic tree based on COI sequences inferred by Maximum Likelihood (ML) method. Bootstrap values over 80% are shown.

Table 2. Mean K2P distances between COI sequences of *Aphidius* species used in this study.

	A. ericaphidis	***A. matricariae***	***A. urticae***	***A. avenaphis***
A. ericaphidis				
A. matricariae	0.081			
A. urticae	0.089	0.018		
A. avenaphis	0.104	0.058	0.063	
A. ervi	0.083	0.034	0.037	0.065

Discussion

Highbush blueberry production has been on the rise in the world, and as a consequence of increased international trade of planting material, plant pathogens and pests are also being spread to new areas. In the last 15 years, two exotic *Vaccinium* pathogens with North American origin (*Monilinia vaccinii-corymbosi* and BlScV) were detected in Europe, and it is assumed that both were imported with plant material (Gosch 2003, Ciuffo et al. 2005, Munda 2011, Paduch-Cichal et al. 2011, Richert-Pöggeler et al. 2015). The situation is the same with at least two pest species, blueberry gall midge *Dasineura oxycoccana* and the aphid *Ericaphis scammelli*, which most likely also have a North American origin (Bosio et al. 1998, Barbagallo et al. 1998). Although *E. scammelli* (under different names) has been present in Europe for over half a century (Cœur d'acier et al. 2010), *A. ericaphidis* is its first parasitoid detected in Europe (in Serbia, Sweden, the Netherlands, France and the United Kingdom). While most alien Aphidiinae species reported in Europe were introduced intentionally as biocontrol agents (Roy et al. 2011, Petrović et al. 2013), for *A. ericaphidis* this is not the case. Some *A. ericaphidis* populations were most likely founded by specimens introduced accidentally with *Vaccinium* plant material "hitchhiking" in *E. scammelli*. This is the

most obvious scenario for the records from Serbia and Sweden. Intensive research of Aphidiinae in Serbia has been ongoing for more than 20 years, and one of the main focuses has been on the Aphidiinae fauna of high mountain plants (including native *Vaccinium* species). Until recently (2015), there was no evidence of either *E. scammelli* or *A. ericaphidis* (Kavallieratos et al. 2004, Žikić et al. 2012). Both the aphid and the parasitoid were detected for the first time on a commercial *V. corymbosum* plantation with material imported from the Netherlands (probably originating from North America). *Aphidius ericaphidis* from *E. scammelli*/*V. corymbosum* in a greenhouse in Sweden might also be a result of an accidental introduction from North America via blueberry nurseries in Germany. The same year when *A. ericaphidis* was recorded (2008), a detailed survey of aphids on blueberries (native and highbush plantations) was conducted, and the parasitoid was found only in one greenhouse situated about 30 km from the nearest forest woodland and surrounded by farmland (B. Nedstam unpubl.).

However, our revision of material from earlier collections from France and the Netherlands showed that the parasitoid has been present in Europe at least as long as *E. scammelli* (if not longer). At the time of the records, blueberry production in Europe wasn't as extensive as it is today, and the import of plants from North America was limited to a few countries. The record from France suggests that those populations of *A. ericaphidis* had enough time to spread and establish, especially since the record is from a native high mountain ecosystem (*Vaccinium uliginosum*/*Ericaphis latifrons*/*Aphidius ericaphidis*).

The lack of any genetic differences shows that analysed European and North American populations of *A. ericaphidis* are very closely related. This can suggest that the analysed specimens were from a recent introduction or that they represent a species with no genetic differentiations based on the COI gene, as has been recorded before for some other *Aphidius* species (*A. uzbekistanicus* and *A. avenaphis*) (Tomanović et al. 2013).

Since molecular and morphological analyses of the target parasitoid populations revealed no significant differences, it might be concluded that *A. ericaphidis* is a member of the Holarctic forest tundra faunistic complex (Starý 1970) associated with different *Ericaphis* aphids in both Europe and North America. Although it is a very common parasitoid of *Ericaphis* in North America (at least in the Pacific Northwest) (Pike et al. 2011), in Europe it is quite rare, with only two records prior to 2008 (Starý et al. 1971, P. Starý unpubl.). There are two main factors contributing to the spread of *A. ericaphidis* in Europe during the last decade. The first one involves probable multiple introductions from North America with planting material and *E. scammelli*; the second one consists of a possible new adaptation of European populations of *A. ericaphidis* to this introduced aphid.

The current known host range of European populations of *A. ericaphidis* is similar to that of North American populations, with the vast majority of records reported from *Ericaphis* aphids (Pike et al. 2011). Although three out of 10 parasitoid species (*Aphidius ervi*, *Aphidius matricariae* and *Lysiphlebus testaceipes*) that parasitize *E. scammelli* in North America also occur in Europe, *A. ericaphidis* is the only species recorded that successfully parasitizes *E. scammelli* (Suppl. material 1: Table S1.). In this respect, there is potential

in biological control of these aphids, which can serve as vectors of the blueberry scorch virus if populations are left uncontrolled. Since the virus has spread to Europe recently, the possibility of using *A. ericaphidis* as a biocontrol agent should be investigated thoroughly. It can then be added to the list of already tested European Aphidiinae species for which the ability to control *E. scammelli* in field conditions has been determined (Dassonville et al. 2013). Of course, this requires very careful additional testing, since there are several parasitoid species that were introduced as biocontrol agents and then became widespread (Roy et al. 2011) and broadened their host range in non-native areas (Mitrović et al. 2013, Petrović et al. 2013). On the other hand, Pike et al. (2011) report rare occurrences of parasitization of *Macrosiphum parvifolii* Richards by *A. ericaphidis*, which implies its potential to parasitize other species.

The current distribution of *A. ericaphidis* and that of its host *Ericaphis scammelli* in Europe are most likely much broader than those recorded so far, and field surveys should therefore be conducted in all *Vaccinium* growing areas to monitor the spread of *A. ericaphidis* in Europe and possible changes of its host range.

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Supplementary material 1

Table S1. Parasitoids of *Ericaphis* aphids from North America and Europe

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