

New information on the invasive to Europe aphid species *Brachycaudus divaricatae* Shaposhnikov, 1956 (Hemiptera: Aphididae)

Jekaterina BAŠILOVA¹, Jan HAVELKA², Rimantas RAKAUSKAS¹, Petr STARÝ²
 & Jurga TURČINAVIČIENĖ¹

¹Department of Zoology, Faculty of Natural Sciences, Vilnius University, M. K. Čiurlionio str. 21/27, LT 03101 Vilnius, Lithuania; e-mails: jekaterina.basilova@gf.vu.lt; rimantas.rakauskas@gf.vu.lt; jurga.turcinaviciene@gf.vu.lt

²Institute of Entomology, Biology Centre of the AS CR, Branišovská 1160/31, CZ-37005 České Budějovice, Czech Republic; e-mails: jhav@entu.cas.cz; stary@entu.cas.cz

Abstract: *Brachycaudus divaricatae* (Hemiptera: Aphididae), a recent invader to Europe, has already reached Czech Republic. Partial sequences of mitochondrial *COI* and nuclear *EF-1α* genes have been analysed across the sixteen Czech samples of *B. divaricatae*, together with morphometric analysis of the same samples based on eighteen morphological characters of the apterous viviparous females. For comparative studies, thirteen samples from the Eastern Baltic region of Europe (Latvia, Lithuania and Poland) were used. All sampled populations appeared similar in their genetic and morphological characters studied. One haplotype of mitochondrial *COI* gene was predominant; it was characteristic for all samples from Czech Republic and 8 out of 13 samples from Eastern Baltic region. Two other haplotypes were found in the Eastern Baltic region only. Four different haplotypes of *EF-1α* gene were detected. Most of the samples (except one sample from the Eastern Baltic region and two samples from Czech Republic) had the same haplotype. Out of remaining three haplotypes, one was unique for the Eastern Baltic region, whilst two were found in Czech Republic only. For the present, Moravia is the southernmost region in Europe, where *B. divaricatae* has been already reported. Presumably, this invasive aphid species has entered the Czech Republic from the north via the Moravian Gate, a natural pass formed by the depression between the Western Carpathians and Eastern Sudetes.

Key words: *Brachycaudus divaricatae*; morphometric analysis; mitochondrial DNA; *COI*; nuclear DNA; *EF-1α*; Europe

Introduction

Brachycaudus divaricatae Shaposhnikov, 1956 has been originally described from Turkmenia (Shaposhnikov 1956), and was earlier known from the Middle East (Turkmenia, Turkey, Iran) and Eastern Europe (Northern Caucasus, Crimea) only (Blackman & Eastop 2000, 2006). Starting from 2002, it appeared in the Eastern Baltic region of Europe, including also Belarus and North Ukraine, and is for the present the most common pest on cherry plum (*Prunus cerasifera*) in this area (Cichocka & Lubiarsz 2003; Rakauskas 2004; Zhuravlev 2005; Rakauskas & Buga 2010). Special search efforts in 2005–2011 showed recent invasion of *B. divaricatae* to Czech Republic in 2011.

This study used partial sequences of mitochondrial *COI* and nuclear *EF-1α* genes to investigate genetic diversity of *B. divaricatae* across the recently invaded areas throughout Czech Republic. Then genetic variability was compared with the morphometric variation of the same populations. Finally, to reveal the eventual origins of Czech populations their molecular and morphological features were compared with those of the Eastern Baltic region populations.

Material and methods

Special searches of *Brachycaudus* aphids were performed on regular basis in 2003–2011, covering six out of seven administrative regions of Czech Republic. *Brachycaudus divaricatae* was detected in 2011 only in Northern, Eastern and Southern Moravian regions of the Czech Republic (Fig. 1). Samples were collected both from the winter hosts (cherry plums mainly, also on other species of plums) and summer hosts (*Silene alba*, white campion). Seventeen *Brachycaudus* samples collected in Czech Republic in 2011 and one in 2005 were used in this study. For comparative studies, thirteen samples from Latvia, Lithuania and Poland were added (Table 1). Keys of Andreev (1990), Blackman & Eastop (2000, 2006), and Rakauskas & Turčinavičienė (2006) were used for the morphological identification of samples.

For molecular analysis, a single aphid individual from one sampled plant was considered as a unique sample. Total genomic DNA was extracted from a single individual using the DNeasy Blood & Tissue kit (Qiagen), which involved at least a 2 h digestion of tissue with proteinase K. Partial sequences of *COI* and *EF-1α* were PCR-amplified using previously published primers (Turčinavičienė et al. 2006). PCR amplification was carried out in a thermal cycler (Eppendorf) in 50 µl volumes containing 1–2 µl genomic DNA, 5 µl of each primer (10 µM), 5 µl of PCR-reaction buffer, 5 µl of dNTP mix (2 mM each), 4–8 µl of 25 mM MgCl₂

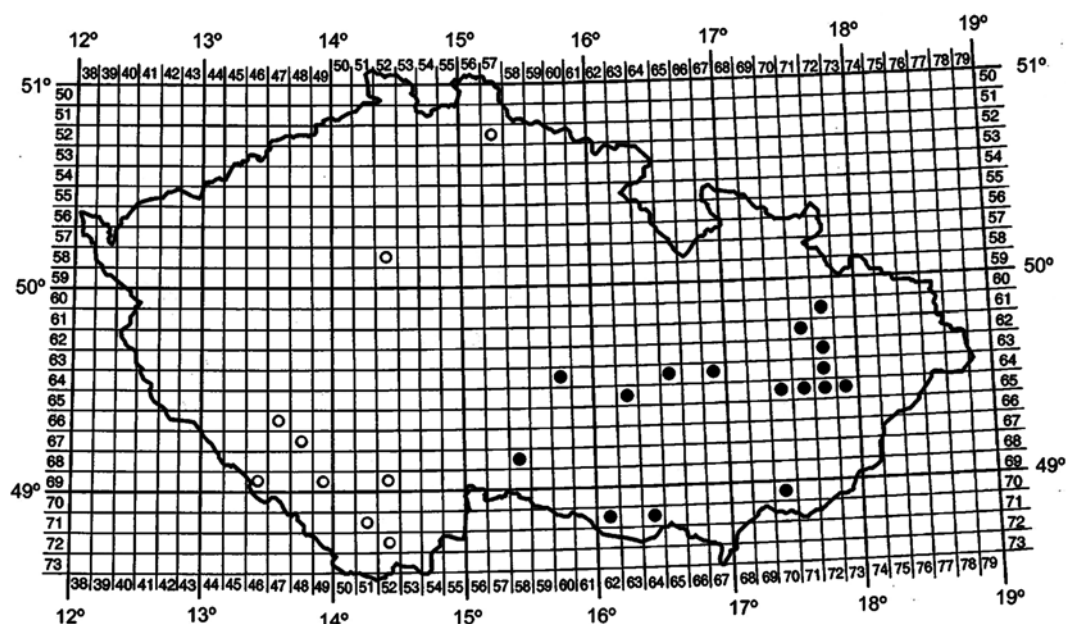


Fig. 1. Aphid material collection sites in Czech Republic (2003–2011) – black circles. White circles indicate aphid collection sites where *B. divaricatae* was not found.

Table 1. Samples of *Brachycaudus* used in the present study. Hosts plants were cherry plums (*Prunus cerasifera*), except if otherwise stated.

Place, date, collection No	GenBank Accession No	
	<i>COI</i>	<i>EF-1α</i>
Baltic region		
Carnikava, Latvia, 05.07.2008., 08–93	JN656449	JN656466
Ryga, Vagonu Parks, Latvia, 03.07. 2008, 08–76	JN656448	JN656465
Sigulda, Latvia, 06.07.2008, 08–98	JN656450	JN656467
Juodkrantė, Neringa, Lithuania, 11.07.2004, 04–162	JN656439	JN656456
Nida, Neringa, Lithuania, 08.07.2004, 04–108	JN904113	JN904131
Kaunas, Botanical Gardens, Lithuania, 12.07.2004, 04–165	JN656437	JN656454
Plateliai, Plungė distr., Lithuania, 16.08.2004, 04–170	JN656438	JN656455
Nolėnai, Utena reg., Lithuania, 19.05.2005, <i>Prunus</i> sp. cult. <i>Skoroplodnaya</i> , 05–110	JN656443	JN656460
Puvočiai, Varėna reg., Lithuania, 21.07.2005, 05–127	JN656444	JN656461
Kairėnai, Vilnius distr., Lithuania, <i>Prunus americana</i> , 25.09.2010, 10–08	JN656452	JN656469
Druskininkai, Lithuania, 29.09.2010, 10–12	JN656452	JN656469
Skirgiskės, Vilnius distr., Lithuania, 25.06.2004, C06.25	DQ779971	DQ779978
Augustów, Poland, 29.09.2009, 09–43	JN656451	JN656468
Czech Republic		
Telč, South Moravia, 30.05.2011, B11–10	JN904114	JN904132
Havraníky, South Moravia, 30.05.2011, B11–14	JN904115	JN904133
Hevlín, South Moravia, 30.05.2011, B11–15	JN904116	JN904134
Strážnice, South Moravia, 30.05.2011, B11–16	JN904117	JN904135
Bystřice pod Hostýnem – Loukov, East Moravia, 31.05.2011, B11–17	JN904118	JN904136
Slavkov u Opavy, North Moravia, 31.05.2011, B11–20	JN904119	JN904137
Mikolajice, North Moravia, 31.05.2011, B11–23	JN904120	JN904138
Odry, North Moravia, 31.05.2011, B11–24	JN904121	JN904139
Bělotín, North Moravia, 31.05.2011, B11–26	JN904122	JN904140
Rusava, East Moravia, 31.05.2011, B11–28	JN904123	JN904141
Přerov – Domaželice, North Moravia, 01.06.2011, B11–30	JN904124	JN904142
Tovačov, North Moravia, 01.06.2011, B11–31	JN904125	JN904143
Blansko – Malé Hradisko, North Moravia, 01.06.2011, B11–32	JN904126	JN904144
Boskovice, North Moravia, 01.06.2011, B11–33	JN904127	JN904145
Bystřice nad Pernštejnem – Lesoňovice, North Moravia, 01.06.2011, B11–34	JN904128	JN904146
Zdár nad Sázavou, North Moravia, 01.06.2011, B11–35	JN904129	JN904147
Nový Dvůr arboretum, North Moravia, 15.06.2005, <i>Silene alba</i> , 05–16	JN904130	JN904148
Telč, South Moravia, 30.05.2011, <i>Silene alba</i> , B11–09	JQ437442	JQ437443

Table 2. Characters used for the morphometric study of *Brachycaudus divaricatae*.

Acronym	Character
BODY	Body length (including cauda)
ANTEN	Antenna length
ANT3	Antennal segment III length
ANT4	Antennal segment IV length
ANT5	Antennal segment V length
BASE6	Basal part of the antennal segment VI length
PRT6	Processus terminalis of the antennal segment VI length
TARS2	Second segment of hind tarsus length
SIPHON	Siphunculus length
SIPHONBW	Basal width of siphunculus
CAUDA	Cauda length
CAUDABW	Basal width of cauda
URS	Ultimate rostral segment length
LHAN3	Longest hair on antennal segment III length
AWAN3	Articular width of antennal segment III
HANT3	Number of hairs on antennal segment III
HURS	Number of additional hairs on the ultimate rostral segment
HCAUDA	Number of hairs on cauda

and 1.25 U of *Taq* polymerase (recombinant) (5 U/ μ l) and ddH₂O to 50 μ l. The cycling parameters were as follows: denaturizing at 95°C for 10 min (1 cycle), denaturizing at 9 °C for 30 s, annealing at 49°C (for *COI*) and 57°C (for *EF-1 α*) for 30 s and extension at 72°C for 30 s (32 cycles in total), and a final extension for 5 min (1 cycle). PCR products were subjected to electrophoresis on 2% Top Vision LE GQ agarose (Fermentas, Lithuania), stained with ethidium bromide and sized against a Gene Ruler 100 bp DNA ladder (Fermentas, Lithuania) under UV light. PCR products were cleaned using the QIAquick PCR Purification Kit (Qiagen). Cyclic sequencing was performed at the Institute of Biotechnology (Lithuania) using a BigDye[®] Terminator v3.1 Cycle Sequencing Kit and products sequenced using a 3130xl Automated Sequencer (Applied Biosystems). The amplification primers were also used as sequencing primers. DNA sequences for each specimen were confirmed with both sense and anti-sense strands and aligned in the BioEdit Sequence Alignment Editor (Hall 1999). Sequences of *COI* gene were tested for stop codons and none were found. The sequence data for all species have been submitted to the GenBank, their Accession numbers are given in Table 1.

Fifteen metric and three discrete quantitative characters were studied (Table 2). Measurements were performed by means of interactive measurement system Micro-Image (Olympus Optical Co. GmbH). STATISTICA 8 version software (Statsoft 2007) was exploited when calculating basic statistics and performing canonical variates analysis (CVA) of samples representing different regions as well as different *COI* and *EF-1 α* haplotypes. The morphological interrelationships among samples from different geographic locations were examined using hierarchical cluster analysis based on squared Mahalanobis distances. Characters having strong ($r \geq 0.5$) and statistically significant correlation with the length of the body were removed from the analysis. These were ANTEN ($r = 0.62$), ANT3 ($r = 0.71$), ANT4 ($r = 0.61$), AWAN3 ($r = 0.55$), ANT5 ($r = 0.48$) and TARS2 ($r = 0.47$).

Results

All *Brachycaudus divaricatae* samples in Czech Republic were collected from the only host, cherry plum, which has been repeatedly reported to be the primary host plant of this aphid species (Shaposhnikov 1962, 1964; Rakauskas & Turčinavičienė 2006). Samples from the summer host, white campion, appeared to be of closely related species, *B. lychnidis* (L., 1758), both by their key morphological characters and analyzed partial sequences of the *COI* and *EF-1 α* genes (Andreev 1990; Rakauskas & Turčinavičienė 2006; Blackman & Eastop 2006).

The alignment of *COI* fragment contained 580 bp, three variable sites among them. Three different haplotypes were detected. One haplotype was predominant, and it was characteristic for all samples from Czech Republic and 8 out of 13 samples from Eastern Baltic region. Two other haplotypes were found in the Eastern Baltic region only. The analyzed region of *EF-1 α* consisted of two parts of three exons and two introns, which were not removed before the further analysis. The alignment of this fragment contained 452 bp, with 4 variable sites. Four different haplotypes were detected. Most of the samples (except one sample from the Eastern Baltic region and two samples from Czech Republic) had the same haplotype. Out of remaining three haplotypes, one was unique for the Eastern Baltic region, whilst two were found in the Czech Republic only.

Canonical variates analysis based on 11 morphological characters for apterous viviparous females of *B. divaricatae* across the Eastern Baltic region and Czech Republic resulted in a following canonical function with standardized coefficients: $x = \text{HCAUDA} \times 0.753 + \text{HANT3} \times 0.301 + \text{LHAN3} \times 0.297 - \text{SIPHONBW} \times 0.233 + \text{PRT6} \times 0.215$ (eigenvalue = 0.198, cumulative variance = 100%, Wilks' Lambda = 0.835, Chi-square = 52.428, df = 5, P -level = 0.000). The results of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership indicate moderate percentage of individuals being reclassified correctly into their original groups (55.7% in samples from the Eastern Baltic region and 77.9% in samples from Czech Republic). Scatter plot of mean canonical scores for each sample is shown in Fig. 2.

The dendrogram of hierarchical cluster analysis based on squared Mahalanobis distances using unweighted pair-group average linkage among 29 samples of *B. divaricatae* representing Eastern Baltic region and Czech Republic is shown in Fig. 3. Three main branches emerge: two of them include samples from eight localities of the Eastern Baltic region (Nida, Kaunas, Skirgiškės, Plateliai and Nolėnai, Augustow, Kairėnai, Puvočiai, respectively), whilst the third one combines the remaining samples both from the Eastern Baltic region and Czech Republic sharing the same *COI* haplotype.

Canonical variates analysis based on 11 morphological characters for apterous females of *B. divaricatae* was also performed across the individuals represent-

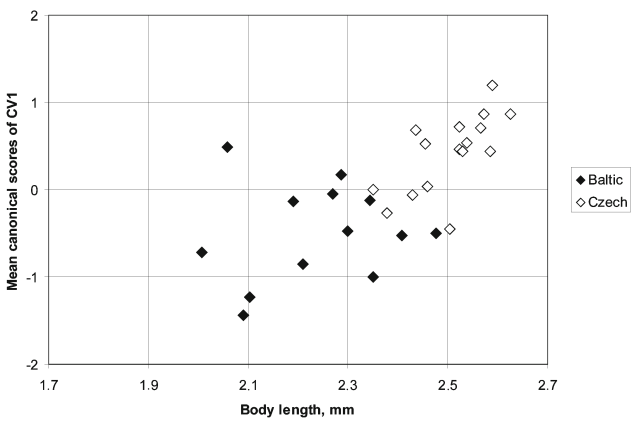


Fig. 2. Scatter-plot of the mean canonical scores of the canonical variate for *B. divaricatae* from the Eastern Baltic region and Czech Republic.

ing three different *COI* haplotypes. The summary of canonical functions received is given in Table 3. Table 4 shows the standardized coefficients of canonical variables that determine the relative discriminative impor-

tance of particular characters. The characters BASE6, CAUDABW and PRT6 show the highest magnitudes at CV1 whereas the characters HCAUDA, SIPHON and SIPHONBW at CV2. The results of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership indicate that 96.7% of individuals having haplotype A were reclassified correctly into their original group. The percentage of correctly reclassified individuals for haplotypes B and C was lower, 25.8% and 39.3% respectively. The graphical presentation of the two canonical variates is shown in Fig. 4, suggesting certain morphological distances between samples representing different *COI* haplotypes.

Canonical variates analysis based on 11 morphological characters for apterous females of *B. divaricatae* was also performed across the individuals representing four different *EF-1 α* haplotypes. The summary of canonical discrimination functions received is given in Table 5. Table 6 shows the standardized coefficients of canonical variables that determine the relative discriminative importance of particular characters. The characters LHAN3, HCAUDA and PRT6 show the highest

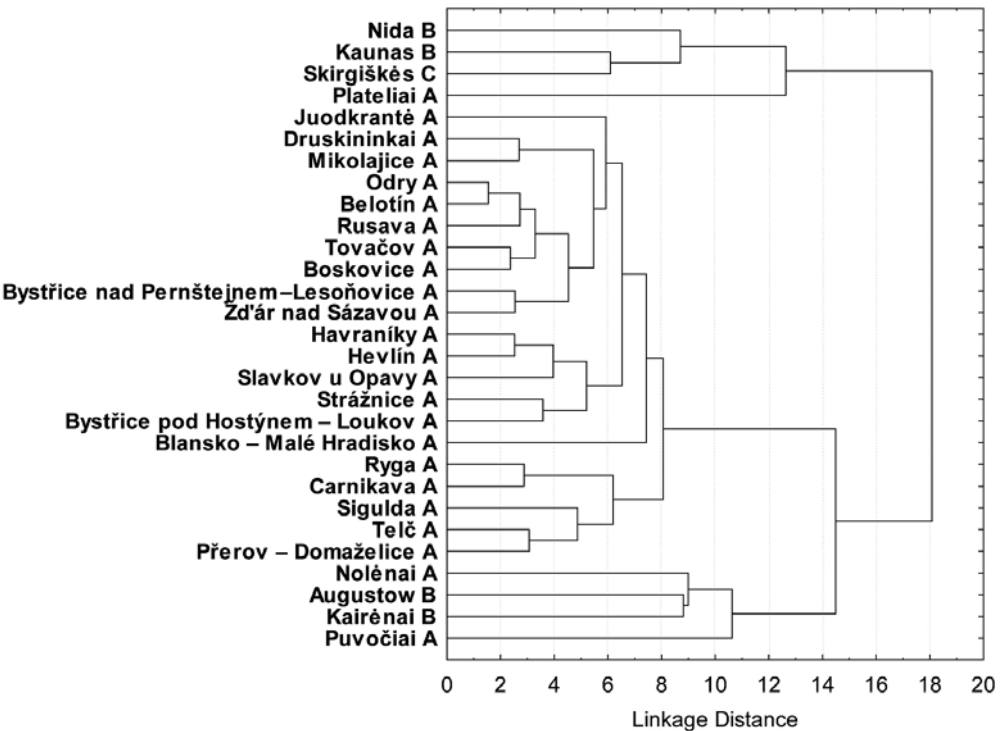


Fig. 3. Dendrogram of hierarchical cluster analysis based on squared Mahalanobis distances using unweighted pair-group average linkage among 29 samples of *B. divaricatae* representing Eastern Baltic region and the Czech Republic. *COI* haplotypes are indicated next to the name of sampling site.

Table 3. Canonical functions for morphological separation between *B. divaricatae* samples having different *COI* haplotypes.

Function	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Wilks' Lambda	Chi-square	df	P-level
1	0.253	56.8	56.8	0.669	115.575	18	0.000
2	0.192	43.2	100	0.839	50.608	8	0.000

Table 4. Standardized coefficients of canonical variables for *B. divaricatae* samples having different *COI* haplotypes.

Characters	Function	
	1	2
CAUDABW	0.599	-0.269
HCAUDA	-0.182	-0.664
BASE6	0.660	-0.013
PRT6	-0.593	0.330
SIPHON	-0.038	-0.569
SIPHONBW	-0.104	0.488
URS	0.089	0.391
HURS	0.197	-0.210
LHAN3	0.272	-0.075

magnitudes at CV1 whereas the characters SIPHON, URS, and HCAUDA at CV2. The results of reclassification of individuals according to the original discriminant functions, derived with *a priori* specified group membership indicate that 99.7% of individuals having haplotype D were reclassified correctly into their original group. The percentage of correctly reclassified individuals for haplotypes E, F and G were 0.0%, 0.0%, and 25.0%, respectively. The graphical presentation of the first two canonical variates is shown in Fig. 5.

Discussion

When applying definition of invasive species as "... a set of individuals that has been introduced into a new area,

Table 6. Standardized coefficients of canonical variables for *B. divaricatae* samples having different *EF-1 α* haplotypes.

Characters	Function		
	1	2	3
LHAN3	0.567	0.223	0.186
HCAUDA	0.547	-0.341	-0.163
SIPHON	0.109	0.674	-0.621
PRT6	-0.494	-0.204	0.081
CAUDA	0.388	-0.283	-0.307
URS	0.262	0.615	0.303
SIPHONBW	0.188	-0.157	0.708
BASE6	-0.314	-0.043	0.244

in which these individuals have established themselves, increased in numbers and spread geographically" (Estoup & Guillemaud 2010), *B. divaricatae* appears to be a good example. High genetic variability was usually expected to favour adaptation in remote territories (Reznick et al. 1997; Lee 2002). This is not the case for *B. divaricatae*, which appeared to be unexpectedly monomorphic in its partial sequences of mitochondrial *COI* and nuclear *EF-1 α* fragments analyzed. Twenty six out of twenty nine analyzed samples from the Eastern Baltic region and Czech Republic had the same haplotype of nuclear *EF-1 α* gene fragment (abbreviated in this paper as haplotype D), and three other haplotypes (E, F and G) are represented only by one sample. Similarly, twenty four out of twenty nine samples had the

Table 5. Canonical functions for morphological separation between *B. divaricatae* samples having different *EF-1 α* haplotypes.

Function	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)	Wilks' Lambda	Chi-square	df	P-level
1	0.161	63.3	63.3	0.787	69.040	24	0.000
2	0.068	26.8	90.1	0.913	26.120	14	0.025
3	0.025	9.9	100	0.975	7.175	6	0.305

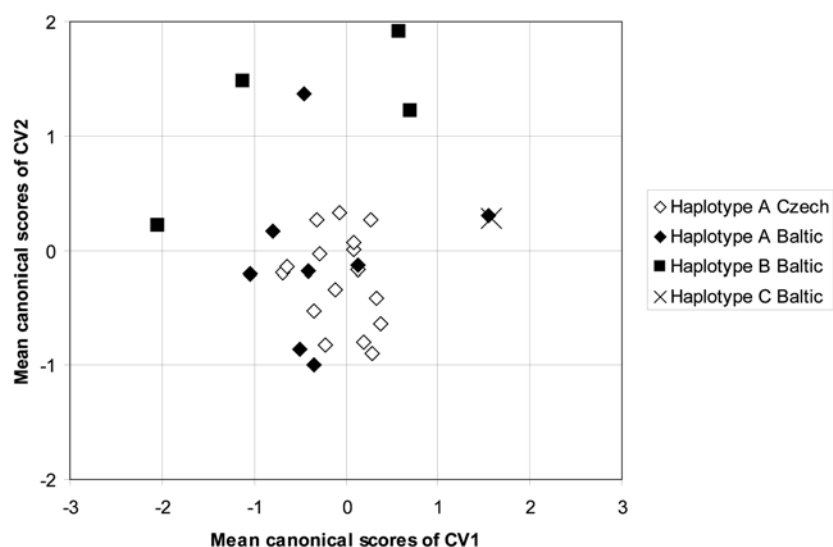


Fig. 4. Scatter-plot of the mean canonical scores of the first two canonical variates for *B. divaricatae* samples having different *COI* haplotypes. Geographic affiliation of samples is also shown.

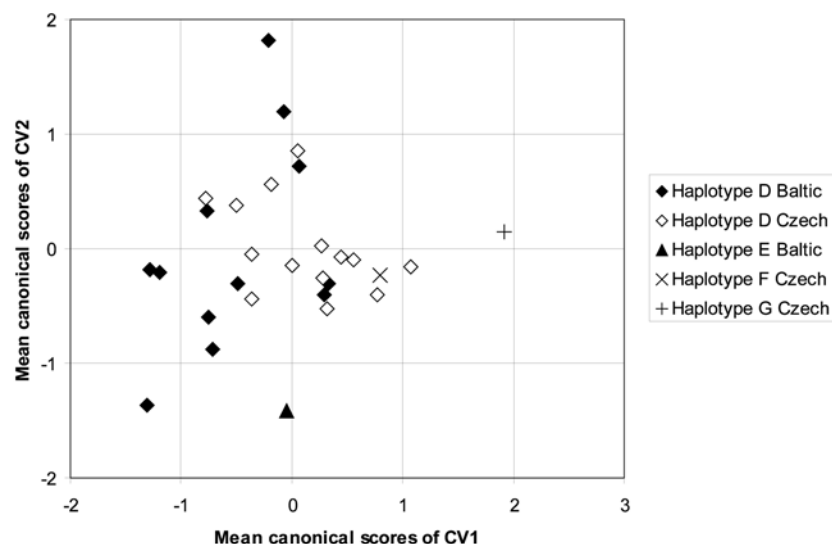


Fig. 5. Scatter-plot of the mean canonical scores of the first two canonical variates for *B. divaricatae* samples having different *EF-1α* haplotypes. Geographic affiliation of samples is also shown.

same haplotype of mitochondrial *COI* gene fragment (abbreviated in this paper as haplotype A), four samples had another haplotype (B), and one sample had the third one (C). All sampled populations also appeared rather monomorphic in their morphological characters analysed. Although canonical function based on 11 morphological characters for apterous viviparous females of *B. divaricatae* from Eastern Baltic region and Czech Republic was statistically significant, its discriminatory power could be evaluated as low (Wilks' Lambda = 0.835) (Fig. 2). Cluster analysis did not confirm the existence of any geographically based linkage between populations either (Fig. 3). Our data indicate certain linkage between the morphological characters and haplotypes of mitochondrial *COI* gene (Fig. 4). The present data suggest the Eastern Baltic region and Czech Republic being invaded by the closely related *B. divaricatae* propagules. All *Brachycaudus* aphids collected from white campion in Czech Republic were identified as *B. lychnidis*. This was already reported from the Eastern Baltic region (Rakauskas & Turčinavičienė 2006). The present data confirm that the European populations of *B. divaricatae* tend to the monoecy on cherry plum, avoiding migration to the summer host plant, white campion.

Eastern Baltic region seems to be invaded some ten years earlier when compared to Czech Republic (Cichocka & Lubiarsz 2003; Rakauskas 2004). Starting from 2003, *B. divaricatae* has been repeatedly reported from the Upper Silesia region of Poland (Rakauskas & Turčinavičienė 2006). For the present, Moravia is the only region of Czech Republic, and the southernmost region in Europe, where *B. divaricatae* has been already reported.

Geomorphological relief and predominant direction of wind are the key factors for the passive distribution of aphids. The probable path of the aphid invaders to the territory of the Czech Republic is the Moravian Gate – a natural pass between the Eastern Sudetes and the

Western Carpathians, the drainage divide between the upper Oder River and the Baltic Sea in the north and the Bečva River of the Danube basin in the south. The above facts suggest that invasive aphid species *B. divaricatae* has entered Czech Republic from the north via the Moravian Gate.

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