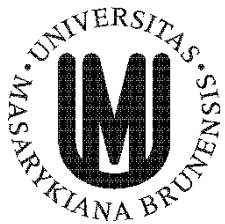# MASARYKOVA UNIVERZITA

PŘÍRODOVĚDECKÁ FAKULTA

ÚSTAV BOTANIKY A ZOOLOGIE

Diplomová práce

Brno 2016   **Ester Lajkepová**

MASARYKOVA UNIVERZITA
PŘÍRODOVĚDECKÁ FAKULTA
ÚSTAV BOTANIKY A ZOOLOGIE

GENETICKÁ DIVERZITA

A HYBRIDIZACE V RODU

CIRSIUM

Diplomová práce

Ester Lajkepová

Vedoucí práce: doc. RNDr. Petr Bureš Ph.D.   **Brno 2016**

Bibliografický záznam

Bibliographic Entry

Abstrakt

Hybridizací podmíněný mezidruhový genový tok je důležitým prekurzorem evolučních a speciačních procesů u rostlin, nelze jej však považovat za obecnou vlastnost veškerých fylogenetických linií. Nakolik může potenciální mezidruhový genový tok ovlivnit genetickou diverzitu druhu, však zatím nebylo studováno. Pro studium této problematiky byl použit modelový rod *Cirsium* (pcháč), který zahrnuje druhy s různou intenzitou přirozené hybridizace a tím odráží situaci, která je pozorovatelná napříč celou rostlinnou říší. Pro účel studie bylo ve třech eko-geograficky dobře vymezených regionech (Brněnsko, Bílé Karpaty, Žďárské vrchy) vzorkováno 10 druhů tohoto rodu, které se liší v ochotě tvořit mezidruhové hybridy. Celkem bylo pomocí AFLP analyzováno 523 morfologicky čistých jedinců a 71 vzorků hybridů. V rámci diplomové práce bylo molekulárně ověřeno 14 z 31 hybridních kombinací v rámci české flóry a objevit nového trihybrida, který ještě nebyl popsán. V rámci druhů byl zaznamenán pokles v genetické podobnosti mezi jedinci se vzrůstající geografickou vzdáleností. Bylo však prokázáno, že většina genetické variability druhu se nachází již mezi jedinci v rámci jednotlivých regionů, zatímco samotné regiony se od sebe významně geneticky neliší. Regionální rozdíly v genetické variabilitě jsou vyšší u druhů, které mají na území České republiky svoji areálovou hranici, zatímco u druhů, pro něž Česká republika leží uvnitř areálu, jsou tyto rozdíly mezi regiony nižší. Dále bylo prokázáno, že ochota druhu tvořit mezidruhové hybridy a mezidruhový genový tok nemají na genetickou diverzitu samotných druhů vliv. Naopak se zdá, že mnohem více vnitrodruhovou genetickou variabilitu ovlivňuje reprodukční strategie druhu. Druhy, které jsou nuceny k častějšímu samooplození (monokarpické druhy a druhy s nízkým počtem samic), měly signifikantně nižší genetickou diverzitu než druhy, které k samooplození nuceny nejsou. Mezidruhový genový tok byl tedy zaznamenán jen na recentní úrovni (F1 hybridi, zpětní kříženci), očekávané stopy dávné hybridizace u morfologicky čistých jedinců se při použitém postupu ani u častěji hybridizujících druhů zachytit nepodařilo.

Abstract

Hybridization and subsequent interspecific gene flow are important determinants of evolutionary a speciation processes, however, they cannot be assumed to be general characteristics of all phylogenetic lineages. To what extent may the potential gene flow influence genetic diversity of a species has never been studied. To study this problematic we used a model genus *Cirsium* (thistle), which includes species with various intensity of natural hybridization and thus reflect the situation observable across the whole plant kingdom. For the purpose of this study we sampled 10 species of this genus that are very variable in their ability to create hybrids. These species were sampled in three eco-geographically well delimited regions (Brno and its surroundings, White Carpathians and Žďárské vrchy Hills). Apart from morphologically pure individuals we also included hybrid plants. Using AFLP (amplified fragment length polymorphism) we analysed 523 morphologically pure individuals and 71 samples of hybrids. Within the diploma thesis we proved the existence of 14 out of total 31 hybrid combinations found in the Czech Republic so far. We also discovered a new trihybrid that has never been reported. Within respective species we detected a decrease in genetic similarity between the individuals as the geographic distance between them increased. We proved that most of the species genetic variability is found between the individuals of respective regions, whereas the regions themselves are not significantly genetically different. Regional differences in genetic variability are higher in species with range margin in the Czech Republic, whereas the species with range optimum in the Czech Republic showed lower interregional differences. Subsequently we proved that species promiscuity together with interspecific gene flow does not influence genetic diversity of the species. The interspecific genetic variability is probably much more influenced by species reproduction strategy. Species that are more vulnerable to self-fertilization (monocarpic species and species with low number of females) had significantly lower genetic diversity than outcrossing species. We were able to detect only recent interspecific gene flow (F1 hybrids and backcross hybrids). Based on our approach the expected signs of ancient hybridization in morphologically pure individuals were undetectable even in more frequently hybridizing individuals.

Masarykova univerzita

Přírodovědecká fakulta

ZADÁNÍ DIPLOMOVÉ PRÁCE

Student: **Bc. Ester Lajkepová**

Studijní program: **Ekologická a evoluční biologie**
Studijní obor: **Botanika**
Studijní směr: Biosystematika rostlin

Ředitel Ústavu botaniky a zoologie PřF MU Vám ve smyslu Studijního a zkušebního řádu MU určuje diplomovou práci s tématem:

Genetická diverzita a hybridizace v rodu *Cirsium*

Genetic diversity and hybridization in the genus *Cirsium*

Oficiální zadání (anglicky/česky): The genus *Cirsium* belongs to the most frequently hybridising species within the Czech flora. Particular species, however, are differing in their hybridisation intensity: apart from frequently hybridising species (*C. oleraceum, C. rivulare, C. palustre*), there are species with certain hybridizing constraints such as their geographical distribution or their tendency to hybridise with one species only (*C. heterophyllum, C. acaule, C. pannonicum, C. canum*) and finally there are extremely rarely or never hybridising species (*C. arvense, C. brachycephalum, C. eriophorum, C. vulgare*). Such characteristics make this genus a suitable model for hybridisation dependent interspecific gene flow studies.

The main aims of this diploma thesis is to detect (i) presence/absence of gene flow within morphologically pure individuals, (ii) F1, backcrossed and multiple interspecific hybrids; (iii) local gene flow among hybrid populations, (iv) genetic diversity within 9 observed species covering three model groups: (a) often hybridising and widely distributed (*C. oleraceum, C. palustre* and *C. rivulare*); (b) limitedly hybridising and rarer (*C. heterophyllum, C. acaule* and *C. canum*) and (c) very rarely hybridising or non-hybridising (*C. arvense, C. vulgare* and *C. eriophorum*). These species will be sampled in three different regions: White Carpathians, Brno and its surroundings and Žďárské vrchy Highlands. The detection will be based on AFLP method.

The elaboration of this thesis will include (i) a representative sampling of model species and their hybrids among populations with a varied geographical distance, which would, together with the hybridisation intensity, be another factor influencing the gene flow between the species, (ii) an analysis of "patterns" of genetic diversity among the species and populations with regard to interspecific hybridisation intensity.

Rod *Cirsium* patří mezi rostlinné rody české flóry u nichž se v přírodě často vyskytují mezidruhoví kříženci. Jednotlivé druhy se však intenzitou velmi výrazně liší: vedle často hybridizujících (*C. oleraceum, C. rivulare, C. palustre*), najdeme v tomto rodu také druhy s hybridizací v různých směrech omezenou (*C. heterophyllum, C. acaule, C. pannonicum, C. canum*) nebo druhy hybridizující jen velmi vzácně nebo nehybridizující vůbec: *C. arvense, C.*

brachycephalum, C. eriophorum nebo *C. vulgare*. Proto je tento rod vhodným modelem pro studium mezidruhového genového toku zprostředkovaného touto častou hybridizací.

Hlavním cílem práce by mělo být detekovat pomocí metody AFLP: (1) přítomnost/absence genového toku u morfologicky čistých jedinců; (2) F1, zpětné a vícenásobné mezidruhové; (3) lokální genový tok u hybridních populací; (4) genetickou diverzitu sledovaných 9 druhů, pokrývajících 3 modelové skupiny: (a) často hybridizující a hojně rozšířené (*C. oleraceum, C. palustre* a *C. rivulare*); (b) omezeně hybridizující a omezeně rozšířené (*C. heterophyllum, C. acaule* a *C. canum*); (c) velmi vzácně hybridizujících až nehybridizujících druhů (*C. arvense, C. vulgare, C. eriophorum*) ve vybraných územích: Bílých Karpat, okolí Brna a Žďárských vrchů.

Při zpracování je třeba provést (i) reprezentativní sběr modelových druhů a jejich hybridů v rámci populací s různou mírou geografické vzdálenosti, která by mohla být vedle samotné intenzity hybridizace dalším faktorem podstatně ovlivňujícím genový tok mezi druhy; podle možností (ii) analyzovat "patterny" genetické diverzity v rámci druhů a populací ve vztahu k intenzitě mezidruhové hybridizace.

Recommended literature/Doporučená literatura:

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BUREŠ, P., ŠMARDA P., ROTREKLOVÁ O., OBERREITER M., BUREŠOVÁ M., KONEČNÝ J., KNOLL A., FAJMON K., ŠMERDA J. (2010). Pollen viability and natural hybridization of Central European species of *Cirsium*. Preslia, roč. 82, č. 4, s. 391–422.
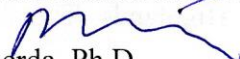
BUREŠ, P, WANG Y. F., HOROVÁ L., SUDA J. (2004). Genome size variation in Central European species of *Cirsium* (Compositae) and their natural hybrids. Annals of Botany, roč. 94, č. 3, s. 353–363.

SEGARRA-MORAGUES, J.G., VILLAR L., LÓPEZ J., PÉREZ-COLLAZOS E., CATALÁN P. (2007). A new Pyrenean hybrid *Cirsium* (Asteraceae) as revealed by morphological and molecular analyses. Botanical Journal of the Linnean Society, roč. 154, č. 3, s. 421–434.

JIMÉNEZ-MEJÍAS, P., LUCEÑO, M. KÅRE, A. L., BROCHMANN, C., GUSSAROVA, G. (2012). Genetically diverse but with surprisingly little geographical structure: the complex history of the widespread herb *Carex nigra* (Cyperaceae)Journal of Biogeography roč. 39, s. 2279–2291.

Jazyk závěrečné práce: angličtina
Vedoucí diplomové práce: doc. RNDr. Petr Bureš, Ph.D.
Podpis vedoucího práce:
Konzultant: Ing. Jakub Šmerda, Ph.D.
Datum zadání diplomové práce: 28. 11. 2013
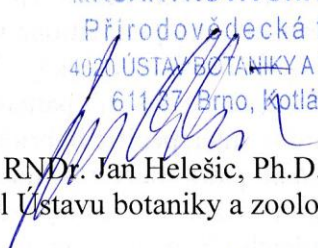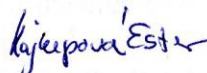
V Brně dne 30. 11. 2013

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Podpis studenta

Poděkování

Na tomto místě bych chtěla poděkovat všem, kteří mi jakýmkoli způsobem pomohli k tomu, aby diplomová práce byla zdárně dokončena. V prvé řadě musím poděkovat panu doc. RNDr. Burešovi Ph.D., že mi vybral tak zajímavé a podnětné téma, které mi umožnilo rozšířit si obzory na poli evoluční a molekulární biologie a hlavně naprosto podlehnout nekonečným možnostem, které pcháče jako modelový organismus nabízejí. Děkuji za uvedení do tématu, za vedení v terénu, za umožnění prezentování výsledků na doktorandských setkáních, za nekonečné hodiny konzultací (i za takové to občasné společné fantazírování, co by bylo skvělé ještě vyzkoumat) a především za velkou trpělivost.

Ing. Jakubu Šmerdovi Ph.D. děkuji za pomoc při veškerých laboratorních analýzách, konzultacích při vyhodnocování, vytvoření skriptu (viz práce) a za ochotu, s jakou mi při řešení nejrůznějších problémů a komplikací pomáhal. Hlavně děkuji za obrovskou motivaci v okamžicích, kdy jsem byla úplně zoufalá, že mi nic nevychází. Díky, Kubo, celá krabice čokolád značky Chateaux by byla za tvou pomoc málo.

Také musím poděkovat svým (hlavně) rkovým konzultantům, Mgr. Pavlovi Veselému Ph.D. (Pavle, nic si z toho nedělej, že jsme ten skript nakonec nevyužili, ale taky na neúspěchy nemusím být zvyklá jen já :-P) a Mgr. Vojtěchovi Zeiskovi (zejména za skvělý rkový kurz).

Další díky patří nesporně účastníkům botanického a biosystematického semináře a účastníkům doktorandských setkání za užitečné připomínky k práci a některým pak za tipy na lokality, či za pomoc sběru vzorků v terénu.

My special thanks go also to people from the University of Vienna for their remarks on my topic and most importantly for helping me to finally assess the AFLP scoring of my data successfully.

V neposlední řadě pak děkuji svojí rodině a blízkým za veškerou podporu v průběhu studia.

Prohlášení

Prohlašuji, že jsem svoji diplomovou práci vypracovala samostatně s využitím informačních zdrojů, které jsou v práci citovány.

Brno 2. května 2016 ………………………………

Jméno Příjmení

CONTENTS

A. Introduction

Although the classical conception of biological species (MAYR 1942) claims, that if a species cannot avoid hybridization with other species it must undergo genetic corrosion and may even perish (LAMONT et al. 2003, PARSONS & HERMANUTZ 2006)[1], hybrid origin has been detected in numerous plant species (RIESEBERG 1997) and interspecific hybridization is believed to be an important evolutionary mechanism in new species origin (ABBOT et al. 2013). Interspecific hybridization is, however, not occurring in all phylogenetic lineages with the same intensity. ELLSTRAND et al. (1996) claim, that only 6–16 % of genera of angiosperms are actually producing hybrids.

In order to study hybridization and subsequent gene flow, a suitable model organism, that can reflect the situation throughout the whole plant kingdom, is needed. Such model could be the genus *Cirsium*. In the Czech Republic it is represented by 11 species that can form up to 31 different hybrid combinations (BUREŠ 2004), which makes it one of the most promiscuous genus within the Czech flora. Other often hybridizing genera are, e. g., *Verbascum* (27 hybrids/9 species)[2], *Ephilobium* (46 hybrids/18 species) or *Salix* (35 hybrids/27 species, in KUBÁT 2002). In *Cirsium*, the frequency of hybridization varies among respective species. There are species that hybridize any time they get into a contact with other species and on the other hand there are species that given the same conditions hybridize extremely rarely (BUREŠ et al. 2004, BUREŠ et al. 2010). Gene flow among the species is considerably influenced by prezygotic barriers, e. g., different biology, ecology and distribution, but postzygotic barriers, e. g., genetic compatibility, certainly play an important role too.

Hybridization and gene flow may be studied by various molecular methods. These methods, unlike the classical approaches (e. g., morphological comparison) do not only detect recent hybridization, but they also intercept ancient hybridization events in individuals that (according to morphology) look like pure species (ALLENDORF et al. 2001 and others). In preceding bachelor thesis (LAJKEPOVÁ 2013) we compared three molecular markers – haplotypes of cpDNA, microsatellites and AFLP. AFLP brought the best resolution and was therefore used in subsequent diploma thesis. Using AFLP we analysed 523 samples of morphologically pure individuals of species differing in their ability to create hybrids: non-hybridizing or extremely rarely hybridizing species (*C. arvense, C. eriophorum* a *C. vulgare*), often hybridizing species (*C. oleraceum, C. palustre* a *C. rivulare*) and restrictedly hybridizing species (*C. acaulon, C. canum* a *C. heterophyllum* – hybridizing with certain species exclusively or occurring only in certain areas). These species were sampled in three eco-geographically well defined regions (Brno and its surroundings, White Carpathians and Žďárské vrchy Hills) in order to detect potential role of local species absences and geographical isolation. Apart from morphologically pure individuals we also included 71 hybrid plants in order to prove their parents molecularly.

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[1] Such example from our flora is *Prunus spinosa*, which is endangered due to its hybridization with domestic *Prunus* taxa (CHRTEK 1992).

[2] These hybrids are, however, completely sterile.

Aims

The aims of the diploma thesis were:

- to prove molecularly (using AFLP) that our sampled individuals are not pure only morphologically, but also genetically (i. e., that there are no F2 or B2 hybrids in our dataset)
- to support molecularly the existence of common and rare hybrid combinations recognized according to their intermediate morphology (already reported within the Czech flora)
- to examine the influence of geographic isolation on genetic similarity; whether the regions are delimited not only geographically, but also genetically and whether we can observe genetic isolation by distance within the species
- to assess whether genetic diversity within respective species is related to species promiscuity[3] or whether it could be determined by other factors such as species life history, number of females in populations or genome size
- to detect gene flow in morphologically and genetically (proved within ther first aim) pure individuals based on local absences of species in respective regions

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[3] Genetic diversity within frequently hybridizing species may be either higher: putative gene flow would incorporate parts of DNA of another species into its genome; or lower: putative gene flow would result in higher uniformity.

B. Literature review

1. Hybridization and gene flow

Hybridization is defined as a reproduction between members of genetically distinct populations (BARTON & HEWITT 1985) or between individuals of different species (HARRISON 1993). The offspring of these individuals is called hybrids. ARNOLD (1997) defines natural hybridization as a successful reproduction taking place under natural conditions between individuals of two populations or groups of populations that are distinguishable in one or more heritable characteristics. The F1 offspring produced must be viable and at least to some extent fertile.

Hybridization by its definition usually involves two taxa, there are, however, rare reports of natural hybrids between three (or more) taxa (KAPLAN & FEHRER 2007). The interspecific gene flow is generated by hybridization and is defined as any movement of genes from one species to another (HIPP & WEBER 2008).

Unlike in animals (DOWLING & SECOR 1997) hybridization is a common phenomenon in plants. In angiosperms the number of species creating interspecific hybrids is around 27 500 (ARNOLD 1997, RIESBERG 1997) out of the total number of 352 000 species (PATON et al. 2008)[4]. ELLSTRAND et al. (1996), however, found that hybridization occurs only in certain families (16–34% of the families have at least one reported hybrid) and genera (6–16% of genera create hybrids). The hybridization frequency usually does not depend on family and genus size. In the Czech Republic, for instance, there are families with numerous species that create no or few hybrids (e. g., *Fabaceae* with only 4 hybrids per 142 species) and small families with numerous hybrids (e. g. *Scrophulariaceae* with 27 hybrids per 16 species;) and genera with numerous species that create no or few hybrids (e. g., *Trifolium* s. l. with 23 species and no hybrid) and small genera with many hybrids (e. g., *Mentha* with 8 hybrids per 5 species; BUREŠ ex KUBÁT et al. 2002).

The role of hybridization has always been one of the most controversial topics in the whole evolutionary research. DOBZHANSKY (1937) and MAYR (1942) considered hybrids to be "rare" or "exceptional," and they discussed hybridization as a negative selective agent that favoured the strengthening of discrimination to maintain species. The fact that there is an ongoing gene flow among populations that maintain their genetic integrity is certainly bizarre. On the other hand there are botanists and zoologists (ANDERSON & HUBRICHT 1938, ANDERSON 1949, ANDERSON & STEBBINS 1954, GRANT 1981, ARNOLD 1992, 1997, ALLENDORF et al. 2001, ABBOT et al. 2013) that claim that hybridization is not exceptional and it is an important generator of species diversity and an essential evolutionary stimulus.

Nowadays there is a general acceptance that interspecific hybridization is an important evolutionary determinant and may result in new species formation (RIESEBERG 1997,

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[4] Hybridization was, however, not found only within the species of the same genus, but also between closely related genera most commonly within families Poaceae, e. g., between *Triticum* and *Elythrigia* (MAHELKA et al. 2011), Asteraceae, e. g., between *Cirsium* and *Carduus* (BUREŠ 2004) and occasionally even between closely related families and Orchidaceae, e. g., between *Dactylorhiza* and *Gymnadenia* (JAGIELLO 1988).

ABBOT et al. 2013 and others) but also in current species extinction (CHRTEK 1992, LAMONT et al. 2003, PARSONS & HERMANUTZ 2006).

The hybrids get genotypic and phenotypic characteristics of both parents and they usually have an intermediate morphological appearance. Hybridization may be both harmful and beneficial and the hybrids may be handicapped but also favoured in environmental selection and competition with their parental species.

The hybrids may be disadvantaged because their fitness is often reduced, they may suffer genetic incompatibility[5] or they may even be completely sterile (BURKE & ARNOLD 2001). These hybrids can hardly persist in populations and their speciation is highly unlikely.

BAACK & RIESEBERG (2007) on the other hand report that hybrids may inherit profitable features of both parents. The replacement of harmful recessive alleles of one parent with beneficial alleles of another parent may result in larger and more robust individuals, environmental adaptation and higher viability[6]. These individuals may subsequently invade new environments or successfully compete with their parents and eventually evolve into new species.

The hybridization itself and the fact that hybridization tendency is restricted only to some taxa is very fascinating and induces various questions. Why is hybridization taking place only among certain taxa? Is the distribution across the plant phylogeny utterly random or is the fact that it is clustered only to certain phylogenetic lineages having some cause and consequence? (LAJKEPOVÁ 2013)

2. The genus *Cirsium* as a model organism for interspecific hybridization

The genus *Cirsium* has been attractive for botanists for its ability to create interspecific hybrid ever since the hybridization phenomenon was discovered by LINNEUS & HAARTMAN (1751). The thistles even engaged attention of J. G. Mendel, who chose *Cirsium arvense*, *C. oleraceum*, *C. rivulare*, *C. palustre* and *C. vulgare* for his hybridization experiments and claimed that thistles are *excellent experimental plants for studying various hybrids* (CORRENS 1905). They have also been used for interspecific hybridization experiments by CORRENS (1916). Nowadays the uneven promiscuity is used for studying various evolutionary processes, e. g., gain or loss in genome size (BUREŠ et al. 2004), species pollen viability (BUREŠ et al. 2010) and is also a hot topic in conservation biology, because *C. arvense* is a problematic invasive species in North America and the scientists are afraid of potential gene corrosion of the native species (BELL et al. 2013)[7].

In the Czech Republic there are 11 species of the genus (see **Fig. B1 - B11**). The list of species occurring in the Czech Republic (BUREŠ 2004) and their geographic distribution (BLIZŇÁKOVÁ 2010) is as follows:

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[5] The gametes are viable but they cannot fuse to create a zygote.
[6] The increased viability may be given by the fact that parental species may suffer inbreeding depression whereas the hybrids are predominantly heterozygous.
[7] Hybridization between *C. arvense* and native American species has, however, never been detected (BELL et al. 2013) and is extremelly rare even between the Czech species (BUREŠ 2004).

Cirsium acaulon (LINNAEUS) SCOPOLI Annus Hist.-Nat. 2:62, 1769.
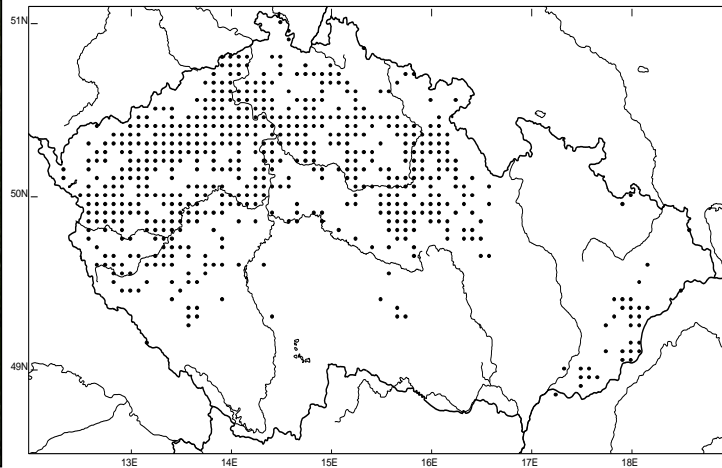
Fig. B1: *C. acaulon*

Cirsium arvense (LINNAEUS) SCOPOLI Fl. Carniol., ed. 2, 2:126, 1772.
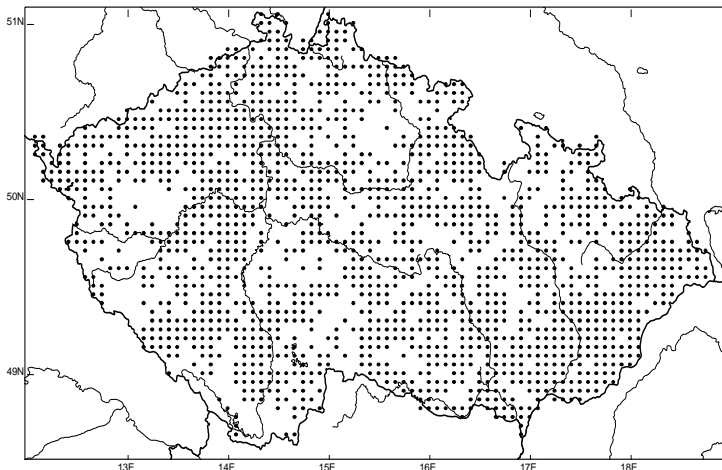
Fig. B2: *C. arvense*

Cirsium brachycephalum JURATZKA Verh. Zool.-Bot. Ges. Wien 7:99, 1857.
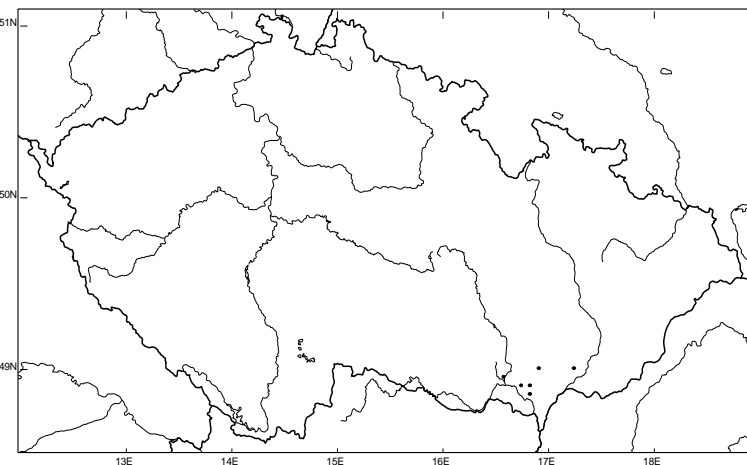
Fig. B3: *C. brachycephallum*

Cirsium canum (LINNAEUS) ALLIONI Fl. Pedem. 1:151, 1785.
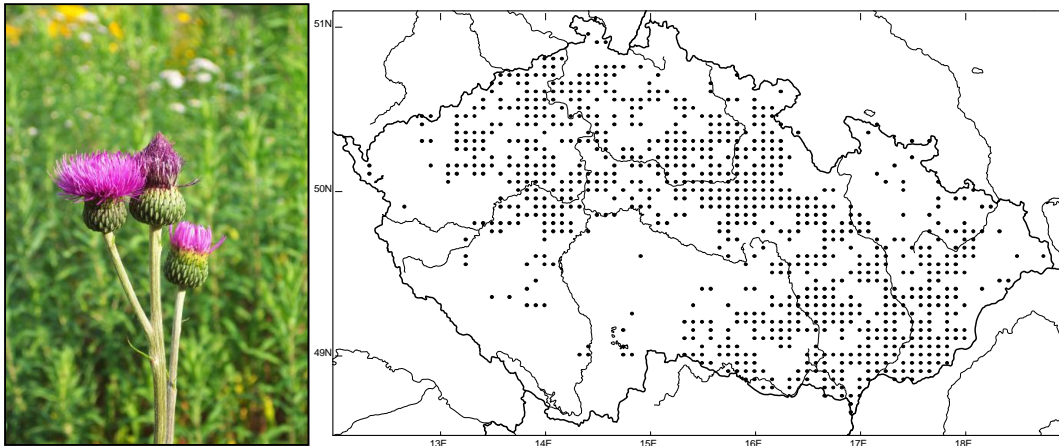
Fig. B4: *C. canum*

Cirsium eriophorum (LINNAEUS) SCOPOLI Fl. Carniol., ed. 2, 2:130, 1772.
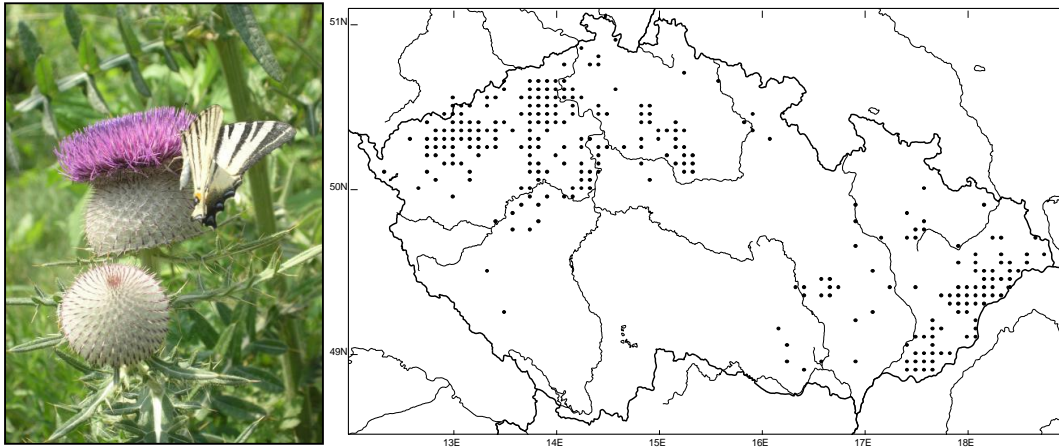
Fig. B5: *C. eriophorum*

Cirsium heterophyllum (LINNAEUS) HILL Hort. Kew. 64, 1768.
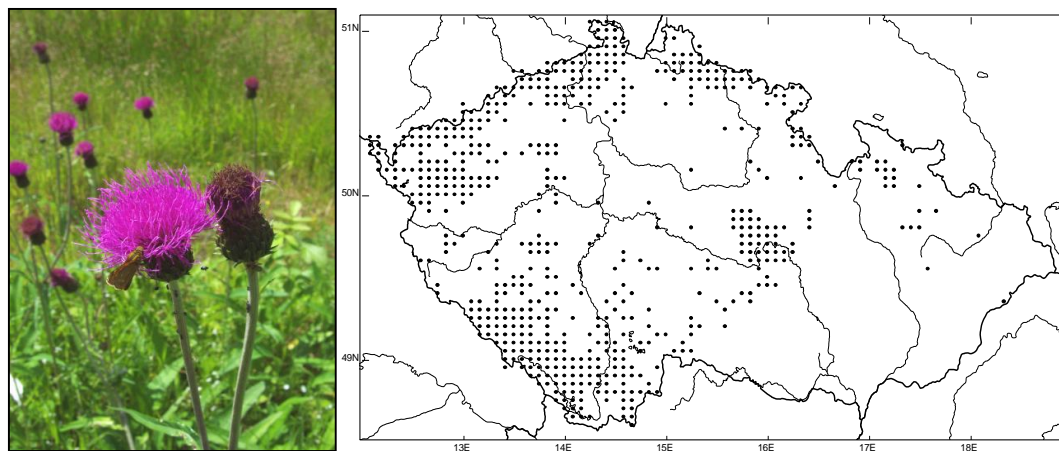
Fig. B6: *C. heterophyllum*

Cirsium oleraceum (LINNAEUS) SCOPOLI Annus Hist.-Nat. 2:61, 1769.
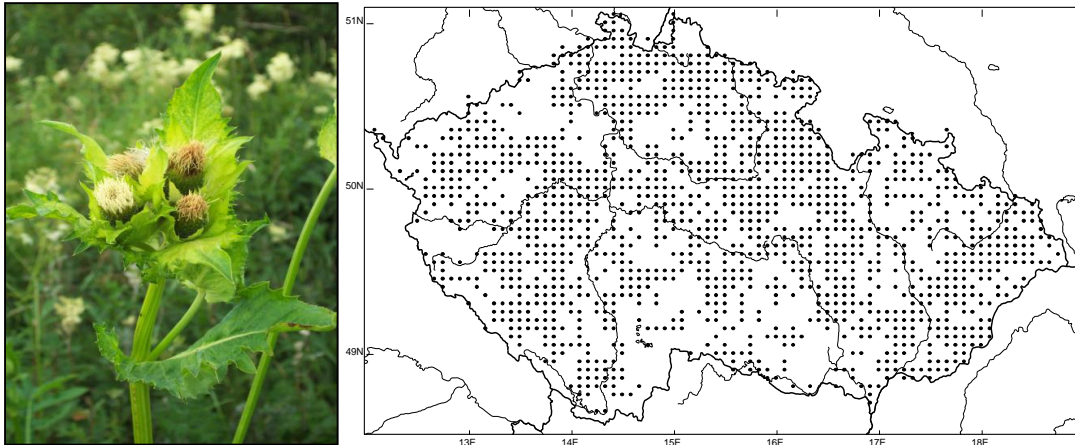
Fig. B7: *C. oleraceum*

Cirsium palustre (LINNAEUS) SCOPOLI Fl. Carniol., ed. 2, 2:128, 1772.
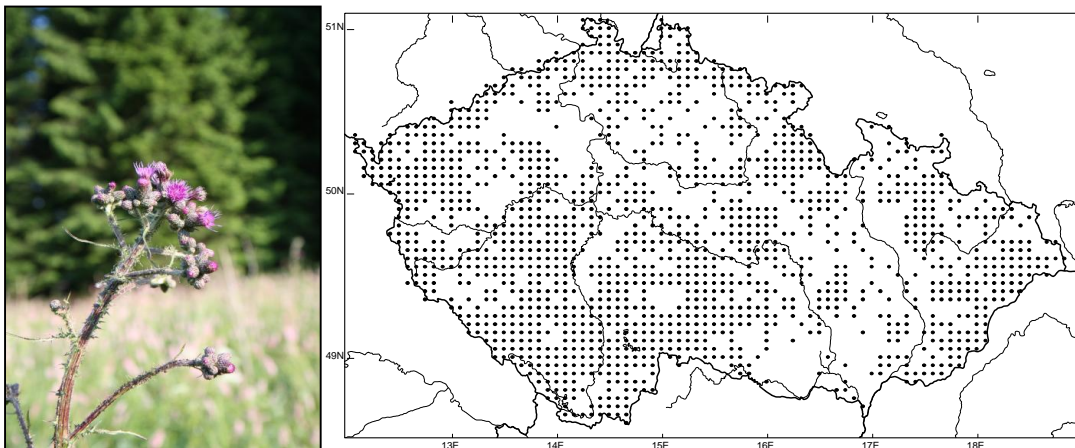
Fig. B8: *C. palustre*

Cirsium pannonicum (LINNAEUS fil.) LINK Enum. Horti Berol. Alt. 2:229, 1822.
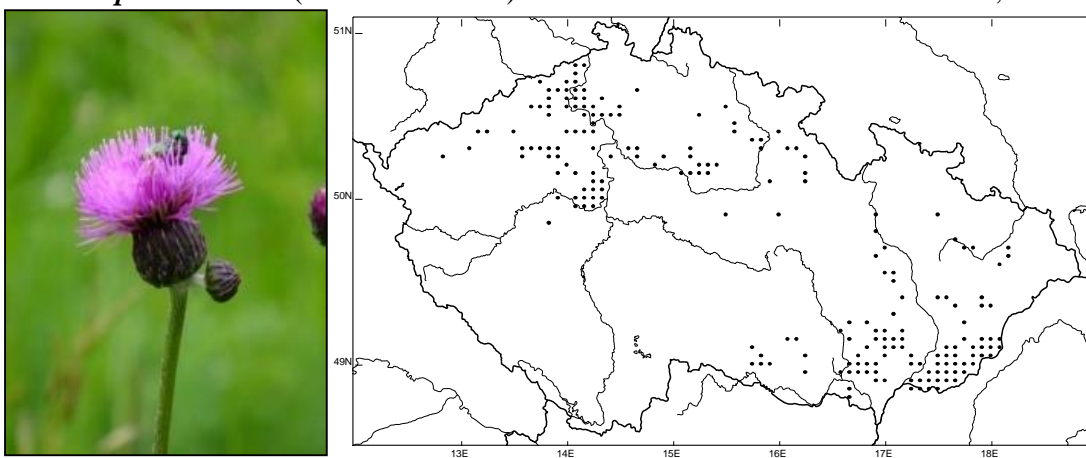
Fig. B9: *C. pannonicum*

Cirsium rivulare (JACQUIN) ALLIONI Auct. Fl. Pedem. 10, 1789.
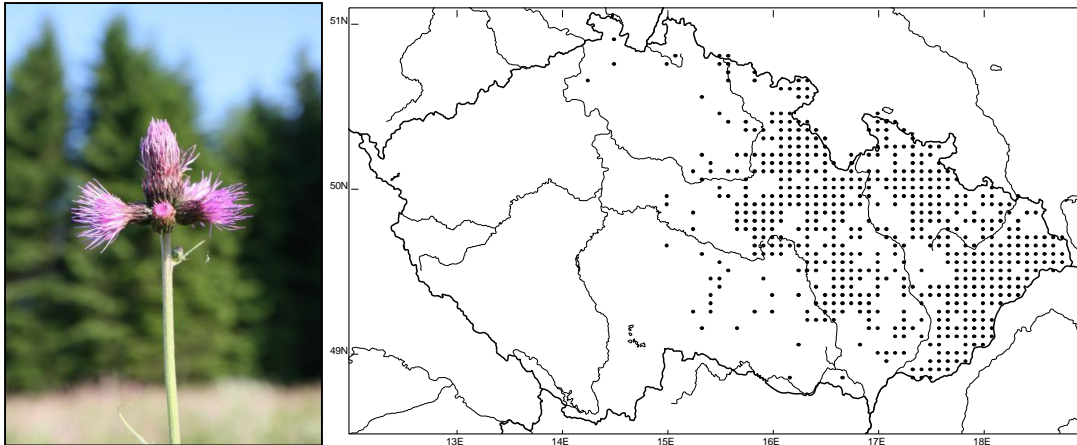
Fig. B10: *C. rivulare*

Cirsium vulgare (SAVI) TENORE Fl. Napol. 5:209, 1836.
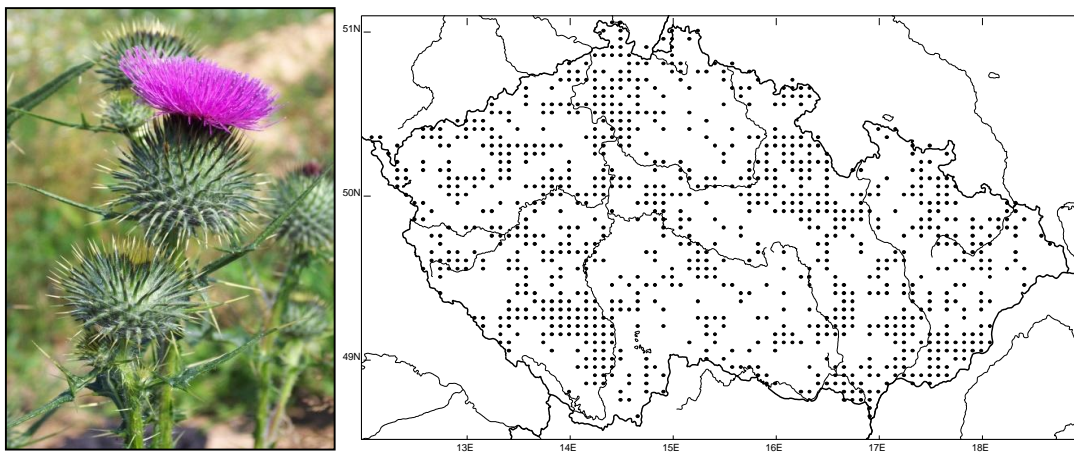
Fig. B11: *C. vulgare*

These species are able to create 31 different interspecific hybrids including several triple hybrids (BUREŠ ex KUBÁT et al. 2002). The hybrids are morphologically well distinguishable (unlike in many other genera), because the parental species are highly morphologically diverse (see **Fig. B1 - B11**) in the means of total size and robustness, leaf and stem morphology (some species have spiny leaves and stems while others have only very mild prickles on the leaves and the stem may have none), number of flower heads, flower colour (from deep purple to white) etc. There is also a difference in life history of various species. Some species are monocarpic: *C. eriophorum, C. brachycephalum, C. palustre* and *C. vulgare* whereas other species are polycarpic: *C. acaulon, C. arvense, C. canum, C. heterophyllum, C. oleraceum, C. pannonicum* and *C. rivulare*. The hybrids then usually have an intermediate phenotype of the parental species.

Although thistles are one of the most promiscuous genera within the Czech flora, this cannot be assumed to be a general characteristics of all species. There are species that hybridize very frequently: *C. oleraceum, C. palustre* and *C. rivulare,* species that are to some extent restricted in their hybridization ability: *C. acaulon* hybridizes primarily with

C. oleraceum, C. canum mostly with *C. oleraceum, C. heterophyllum* with *C. oleraceum* and *C. palustre* and *C: pannonicum* hybridizes mainly with *C. acaulon*. There are also species that do not hybridize, or they hybridize extremely rarely: *C. arvense, C. eriophorum, C. vulgare* and *C. brachycephalum* (BUREŠ 2004, BUREŠ et al. 2004, BLIZŇÁKOVÁ et al. 2010, see **Fig. B12**). The hybridization intensity of the concrete species pairs may be given by various barriers of both prezygotic and postzygotic character.
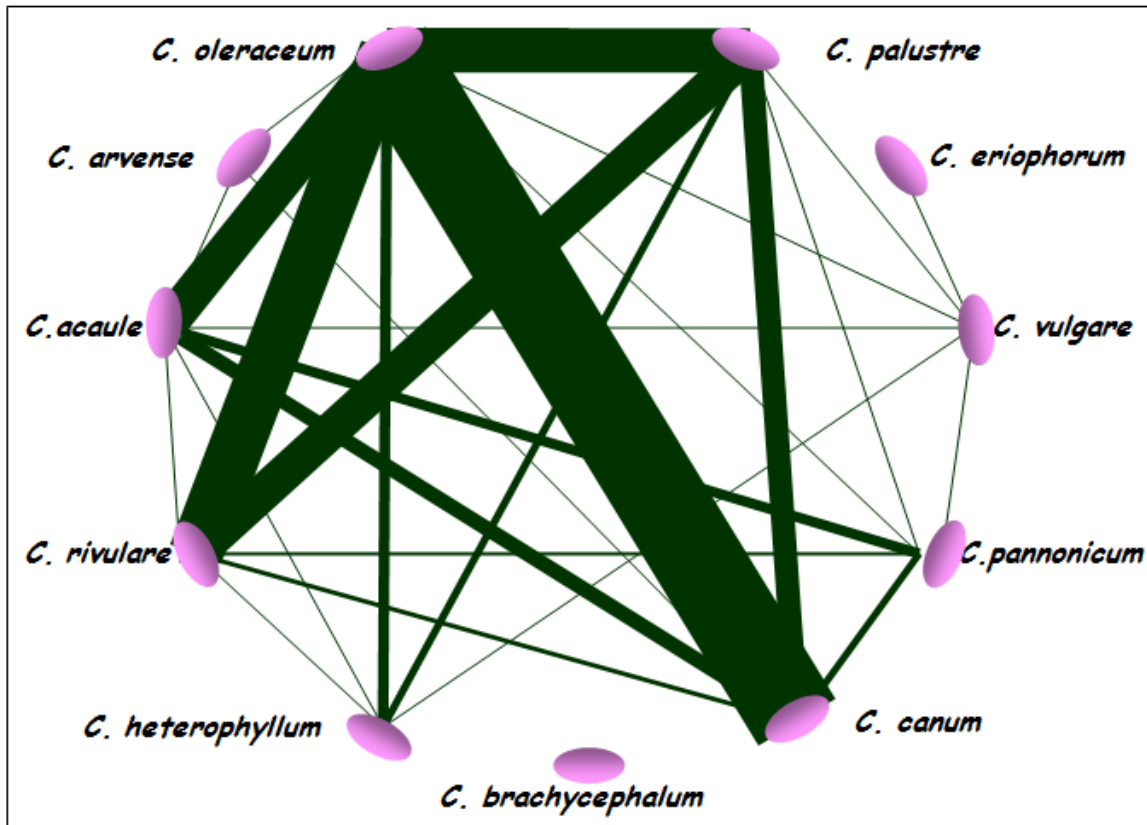
Fig. B12: Intensity of interspecific hybridization based on number of herbarium specimens of hybrids (BLIZŇÁKOVÁ 2010).

2.1. Biology

One of the prezygotic barriers might be the flowering period which is different in respective species. The first blossoming species is *C. rivulare* that creates first flowers by the end of May. The latest species, on the other hand is *C. acaulon*. There is also a difference in duration of flowering period. For most of the species it usually lasts three months, but *C. acaulon* and *C. heterophyllum* usually flower for only one month whereas *C. palustre* lasts even five months. The *Cirsium* species are predominantly allogamous, however, self-compatibility was also found, in *C. acaulon* (PIGOTT 1968), *C. oleraceum* (CORRENS 1916), *C. palustre* and *C. vulgare* (CORRENS 1916; MOGFORD 1974) and is probably widespread across the whole genus. Thistles are pollinated by a wide scale of generalist pollinators (PROCTOR et al. 1996), particularly by Hymenoptera (bees or bumblebees; PIGOTT 1968, see **Fig. B13A**), rarely by Lepidoptera (e.g. Grass Skippers see **Fig. B13B**), Diptera (namely by Syrphidae; see **Fig. B13C**) or by some other insect orders (THEIS 2006).

Fig. B13: Thistle pollinators

2.2. Ecology and distribution

Another prezygotic barrier is the actual co-occurrence of two species. The pollen transfer maintained by pollinators above usually does not exceed the distance of 10 m (DE VERE 2007) therefore if two species do not meet in proximity, they obviously cannot hybridize. The thistles can be found in various biotops and they often differ in their ecological preferences which disables them to co-occur sympatrically and create interspecific hybrids. Some species are euryvalent and can be found almost anywhere in the Czech Republic. Such species are ruderal species: *C. arvense* (occurring in ruderal and weedy vegetation classes *Galio-Urticetea, Artemisietea vulgaris, Epilobietea angustifolii* and *Stelarietea mediae* and also in ruderalized types of meadow vegetation *Molinio-Arrhenatheretea,* see **Fig. B2**) and *C. vulgare* (typically occurring in *Ephilobietea angustifolii, Artemisietea vulgaris* and *Galio-Urticetea,* see **Fig. B11**). Other euryvalent species *C. canum* (**Fig. B4**)*, C. oleraceum* (**Fig. B7**) and *C. palustre* (**Fig. B8**) occur mainly in wet meadows of class *Molinio-Arrhenatheretea* (they are diagnostic species of the order *Calthion palustris*), however, they are also frequently spreading across the wet road ditches which makes them to some extent ruderal species too. Another relatively common species is *C. rivulare* which, however, does not invade the road ditches and is restricted only to the natural vegetation of fen types of *Calthion palustris.* *C. rivulare* is having its western range margin in eastern Bohemia (see **Fig. B9**). The relatively abundant distribution of these species in the whole Czech Republic and their ecological preferences are allowing them to create hybrids almost anywhere.

On the other hand there are rare species that do not get into contact with other species and they are therefore restricted in their interspecific hybrid creation. The fact that two species meet may be given either by their geographical distribution or by different ecological preferences. These two factors usually correspond to one another.

Geographical distribution restricts *C. heterophyllum* (order *Calthion palustris*). *C. heterophyllum* is our most oreophytic species and can be found in higher altitudes that are constrained only to certain areas of the Czech Republic (see **Fig. B6**). This species is frequently hybridizing with wide range of species, but its specific ecological preferences and geographical distribution allow it to hybridize only with species that are growing nearby. *C. heterophyllum* and *C. rivulare* have different distribution within the Czech Republic and they can only meet at very few localities and hybridize there and for ecological

incompatibility there are no hybrids of *C. acaulon* × *C. heterophyllum* in the Czech Republic except of one report from Měděnec (BUREŠ 2004). *C. acaulon* is growing only in lowlands, where its indispensable calcareous substrate is present and therefore it cannot survive in higher altitudes where *C. heterophyllum* is located. In the Alps, however, the situation is different, because calcareous substrate reaches higher altitudes and allows these two species to meet and hybridize (**Fig. B14**).

Fig. B14: A hybrid of *C. acaulon* and *C. heterophyllum* from Passo Tonale. It is interesting that although *C. heterophyllum* is having white felt-like abaxial side of the leaves (and so are its hybrids), this hybrid does not show any such characteristic. The hybrid was proved molecularly (unpublished data).

C. acaulon (**Fig. B1**) and *C. pannonicum* (**Fig. B9**) are stenoecious species growing only in subxerophilic grasslands on calcareous substrate of vegetation class *Festuco-Brometea* (or *Molinio-Arrhenatherea*) and can only hybridize with one another. *C. acaulon* sometimes hybridizes also with *C. oleraceum.* The last two species – *C. brachycephalum* (**Fig. B3**) and *C. eriophorum* (**Fig. B4**) – are also rare. *C. brachycephalum* is extremely rare, growing only in a couple of localities in southern Moravia. Since it is a sub-halophilic species with unique ecology, it rarely gets into contact with other species. *C. eriophorum* has to some extent similar ecological preferences and geographic distribution as *C. acaulon* and *C. pannonicum.*

Sympatricity is certainly an important determinant of interspecific hybridization, nevertheless, it is not all explaining. If we compare the intensity of species promiscuity (**Fig. B12**) and similarity in geographic distribution which highly corresponds to ecological similarity (**Fig. B15**) we can see, that if the hybridization intensity was dependent only on species co-occurrence, it would be much higher in certain taxa than it is in reality. This indicates, that postzygotic barriers are possibly even more important than the prezygotic barriers when it comes to hybrid production.
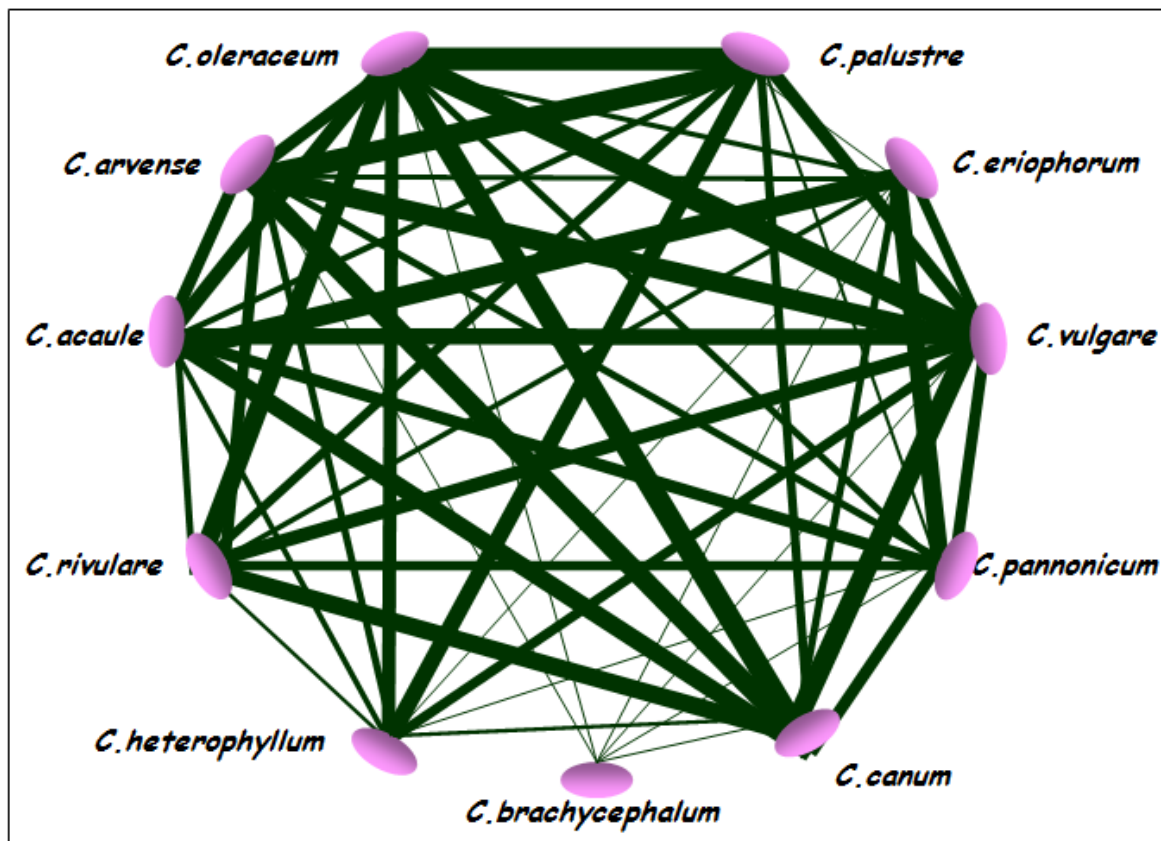
Fig. B15: Similarity in geographic distribution (BLIZŇÁKOVÁ 2010).

2.3. Potential consequences of interspecific gene flow

Hybridization and subsequent gene flow may have various consequences on species fitness (ARNOLD & MARTIN 2010) and genetic make-up (BAACK & RIESEBERG 2007). BUREŠ et al. (2010) discovered that hybrids have lower pollen viability than morphologically pure individuals and that the frequently hybridizing species had lower pollen viability than species that do not hybridize. The authors also remarked a pollen viability decrease in morphologically pure individuals that were sampled in populations where hybrids were present, which would indicate that these individuals probably underwent ancient hybridization and introgression.

BUREŠ et al. (2004) measured the genome size of all species occurring in the Czech flora and several hybrids. They discovered that the genome size of hybrids is not of the average size of parental species, but it is smaller. They also approved that non-hybridizing species are having larger genomes and on the other hand often hybridizing species have smaller genomes. Since the smaller genomes are being more successful under stressful conditions (ŠMARDA in ACTON 2012), the authors claim that hybrids with smaller genomes may be more successful in nature than those with larger genomes. The frequently hybridizing species therefore revenue the advantage of genome size reduction as a response to interpecific hybridization[8].

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[8] The question is whether this phenomenon that has been discovered in thistles is also applicable for other taxa. BAACK et al. (2005) examined several *Helianthus* hybrids and found that the hybrids have larger genomes than

Since the interspecific hybridization was proved to influence the genetic make-up of thistle species via genome size reduction (BUREŠ et al. 2004) the question is whether interspecific hybridization may also influence their genetic diversity and genetic variance.

3. The use of AFLP in hybridization and gene flow detection

3.1. The importance of molecular markers in gene flow detection

The endeavour to detect hybridization and understand its causes and consequences is a great challenge to all botanists ever since it has been discovered by Carl Linné (LINNEUS & HAARTMAN 1751). Although for a well experienced botanist should the hybrid identification itself not be problematic, detection of ancient interspecific gene flow and its role in populations is not so trivial and cannot be studied using morphological characteristics exclusively (RIESEBERG & ELLSTRAND 1993). Since a change in genetic information does not necessarily have to result in a change of morphological appearance, we need to study the hybridization events with the primary information source, the DNA (ALLENDORF et al. 2001).

Nowadays, there are various molecular markers that are being used for gene flow detection. The methods are based on[9]:

- DNA sequence polymorphism, e. g., analysis of haplotypes of chloroplast DNA (TABERLET et al. 1991)
- polymorphism of repetitive segments of genome, e. g., microsatellite markers (JARNE & LAGODA 1996), that are based on existence of various alleles differing in their length
- analyses of restriction sites within the whole genome, e. g., AFLP (amplified fragment length polymorphism, VOS et al. 1995), where the information is given by either presence or absence of restriction sites that are recognised by specific restriction endonucleases.
- Recently, there is a remarkable increase in application of methods using next generation sequencing approach, e. g., RAD sequencing (restriction site associated DNA markers, PETERSON et al. 2012) which combines both analysis of restriction sites and DNA sequence polymorphism.

3.2. AFLP method

AFLP (amplified fragment length polymorphism, VOS et al. 1995) is one of the most popular markers used in molecular botany. This method is based on selective PCR amplification (MULLIS et al. 1984) of fragments, whose restriction sites are located across the whole genome. These fragments are then separated according to their length. The output of the method is a pattern of fragments differing in presences (or absences) of fragments of

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their parents which is a contradiction of BUREŠ et al. (2004). The authors claim that the gain in genome size in hybrids was caused by transposable elements proliferation.

[9] There are of course numerous other molecular approaches such as isozymes, FISH, GISH, flow cytometry, RFLP, RAPD etc. that can also be applied in hybridization or gene flow detection. Their resolution is, however, much more limited than resolution of the markers mentioned above.

respective lengths. The differences in fragment allocations presences are used for subsequent analyses.
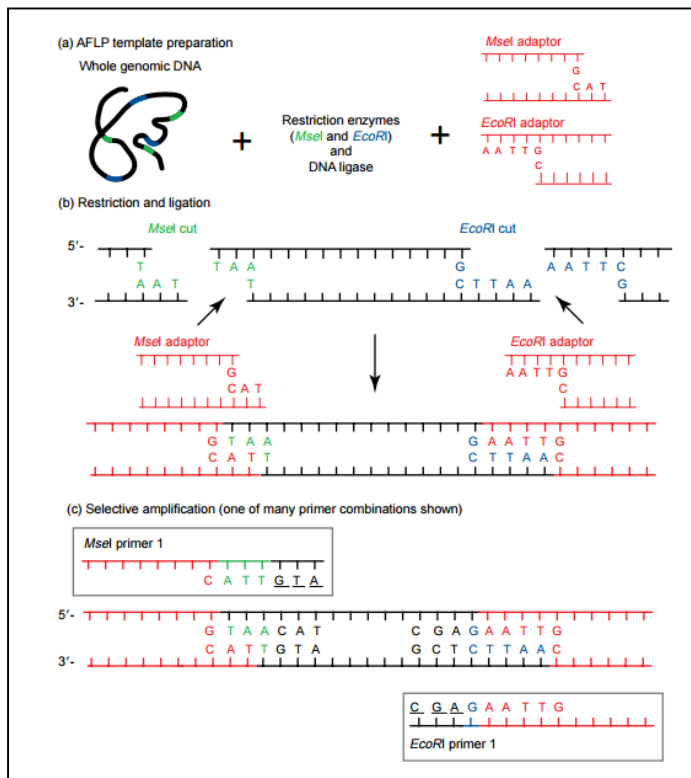
Fig. B16: A scheme of AFLP (MUELLER & WOLFENBAREG 1999)

The method consists of five steps (see **Fig. B16**). In the first step (i) the whole genome DNA is fragmented by two restriction endonucleases. This fragmentation is very specific, because the endonucleases are recognising particular sites in DNA sequence. Widely used restriction endonucleases that were also used for the purpose of this diploma thesis, are enzymes *Eco*RI (fragmenting the sequence at the sites of GAATTC) and *Mse*I (its target is a sequence at the site of TTAA). The next step is (ii) ligation during which adaptors are ligated to the ends of fragments that have GGAATTC sequence on one side and TTAA sequence on the other side. Depending on genome size, restriction-ligation generates thousands of adapted fragments. To reduce the number of fragments, a selective amplification needs to be performed. For this purpose the primers are extended of one, two or three bases reducing the number of amplified fragments by factors of 4, 16 and 64, respectively. To minimize possible artefacts, most protocols incorporate two amplifications – pre-selection and selection. Pre-selection (iii) is performed with a single base pair extension. Subsequent selection (iv) is more selective and primers have up to a 3 base pairs extension (MUELLER & WOLFENBAREG 1999). The primers used in selection amplification also contain a fluorescent dye. Because of their high selectivity, primers differing by only a single base pair amplify a different subset of fragments. By using combinations of primers with different primer sequences, a series of AFLP amplifications can thus screen a representative fraction of the genome. The last step is (iv) fragment analysis using the capillary electrophoresis which separates respective fragments according to their size. The product is then visualised in automatic sequencer MUELLER & WOLFENBAREG 1999).

The output of fragment analysis can be resolved in several software. A popular software which was also used for the purpose of this diploma thesis is a commercial software

GeneMarker 2.4.0 (SoftGenetics, USA). GeneMarker converts the pattern of bands (representing fragments) visualised in gel image of fragmentation analysis (see **Fig. B17**) into a pattern of peaks (see **Fig. B18**). The pattern of peaks is then converted to binary matrix where "1" represents fragment presence and "0" represents fragment absence. The scoring is done automatically by the software, but manual correction needs to be done. The binary matrix is then used for further analyses. Most commonly it is converted into a distance matrix representing distances between respective samples in a multidimensional space.
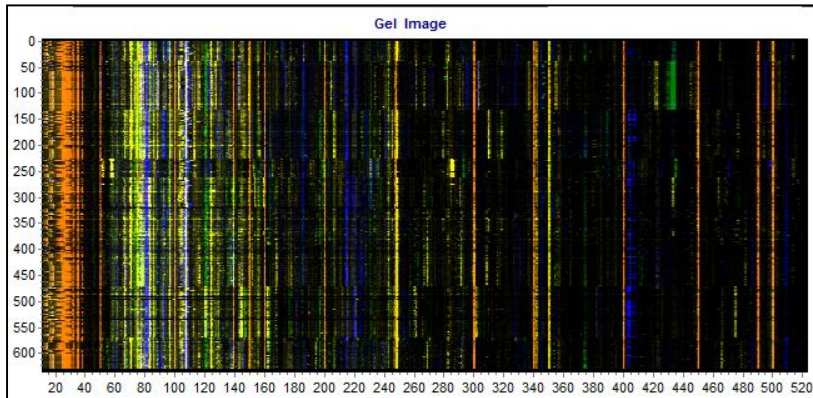
Fig. B17: Gel image of fragment analysis visualized in GeneMarker, horizontal axis shows the length of fragment in base pairs
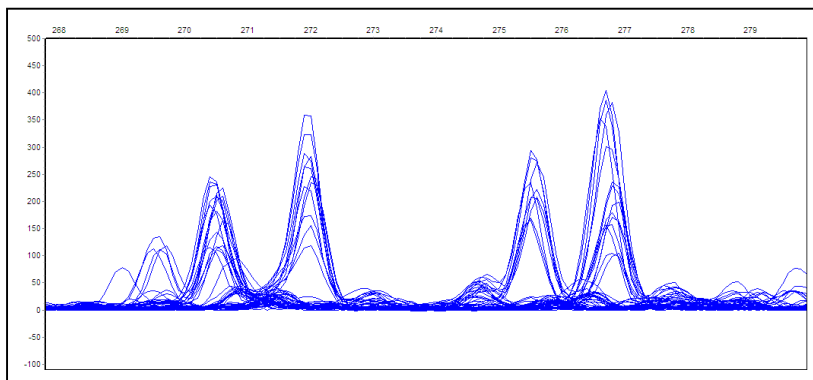
Fig. B18: Gel image of fragment analysis converted into a pattern of peaks in GeneMarker, each line represents one sample, horizontal axis shows the length of fragment in base pairs, there are 5 scorable (the presence or absence of a fragment (peak) is well distinguishable) loci present.

3.3. AFLP – advantages and limitations

Although AFLP method is over 20 years old (VOS et al. 1995) it is still widely used in many different fields of botany. Most recent studies include, e. g. phylogeny (TAKAHASHI et al. 2016), hybridization and gene flow detection (MARCZEWSKI et al. 2015), species diversity (YANG et al. 2016) and population genetics (SORKHEH et al. 2016). Very recently a new approach of AFLP scoring was published by COVARRUBIAS-PAZARAN et al. (2016). This indicates that AFLP is having an undoubted importance even nowadays.

Researchers in the field of molecular ecology and evolution require versatile and low-cost genetic typing methods and the AFLP shows many features that fulfil these requirements (BENSCH & AKESSON 2005). AFLP (unlike cpDNA sequencing or microsatellites) is a multilocus marker. The restriction sites are found across the whole genome and therefore it is possible to analyse a large number of independent loci at once (MUELLER &

WOLFENBAREG 1999). Many authors refer to AFLP as to genomic approach, because it is not restricted to variability only one part of genome, but represents the variability of the whole genome (MUELLER & WOLFENBAREG 1999, BACON et al. 2001).

Another benefit is that AFLP is a very universal marker and may be applied on any organism without any prior knowledge of its genetic structure. It is widely used in bacteria (LA ROSA et al. 2006 claim that AFLP is a powerful, highly reproducible and discriminatory tool for revealing genetic relationships in bacterial populations), plants and fungi (MUELLER et al. 1996). Although AFLP is well applicable also for animals it has always been much underused at the expense of microsatellites and DNA sequencing (BENSCH & AKESSON 2005).

AFLP is also popular because of its price. Since it is a universal marker there is no need to develop specific primers (like there is in the case of microsatellites or DNA sequencing) that are often expensive to develop (PROVAN et al. 1999). AFLP is almost a guarantee that the markers are going to capture the genetic variability among samples and that the dataset will be robust enough to build the analyses on.

AFLP, however, suffers various methodical limits. The gravest limitation when applied for gene flow detection is the fact, that AFLP is a dominant marker (MUELLER & WOLFENBAREG 1999). We can only detect presence (or absence) a fragment of certain length, but we do not know, whether the individual is a dominant homozygote or a heterozygote. Heterozygous states that would carry the crucial information about gene flow would therefore never be detected. The use of AFLP may be also problematic, because of possible false homologies, i. e., fragments of same size do not necessarily have to be homologous and their inner sequence may differ (ROBINSON & HARRIS 1999). The bias can, however, be much reduced if we use closely related species, which is the case of *Cirsium,* because the species should be closely genetically related (TRACEY et al. 2005). The marker is also anonymous and we do not know from which part of genome are the fragments actually coming from. We only know their length. There are also no mutation models like there are in case of DNA sequencing and microsatellites.

As it has been mentioned, the presence and absence of peaks is decided manually which is very subjective. Nevertheless if the person scoring the dataset is well experienced, the mistakes are negligible and the method is highly reproducible (BONIN et al. 2004). Manual scoring is also very time consuming, because many loci need to be analysed.

Despite the above mentioned limitations, AFLP has been successfully applied for various studies in numerous taxa including the genus *Cirsium* (SMULDERS et al. 2000, DURKA et al. 2004, SEGARRA-MORAGUES et al. 2007, BUREŠ et al. 2010 and GUGGISBERG et al. 2012) and was also used for this diploma thesis.

C. Material and methods

1. Sampling strategy

In order to capture most of the promiscuity tendency pattern within thistles, sampling strategy had to be designed carefully. To have a representative dataset we had to do a convenient choice of studied species and also take into account the potential geographic pattern. Additionally we sampled some interspecific hybrids to support the morphological observation molecularly and approve the evidence that hybridization and therefore interspecific gene flow do occur within the genus *Cirsium*.

1.1 Sampled species

In our sampling we included 10 species occurring within the Czech flora[10]. The only species that was not included in our study was *C. brachycephalum*. These species would form three notional groups in the means of their hybridization intensity (**Tab. C1**).

Tab. C1: List of analysed species and their notional group membership

Group	Species	Number of samples	Abbreviation
Non - hybridizing	*C. arvense*	75	ARV
	C. eriophorum	21	ERIO
	C. vulgare	55	VUL
Hybridizing	*C. oleraceum*	77	OLE
	C. palustre	64	PAL
	C. rivulare	54	RIV
Restrictedly hybridizing	*C. acaulon*	31	ACA
	C. canum	71	CAN
	C. heterophyllum	45	HET
	C. pannonicum	30	PAN
Total		523	

In the first group there are species that are generally assumed to be non - hybridizing.[11] *C. arvense* and *C. vulgare* are euryvalent species and they are abundantly distributed everywhere in the Czech Republic and they are frequently sharing their habitats with other thistle species. Even though they would have many opportunities to hybridize, their actual intensity of hybridization (comparing to the other species) is negligible. *C. eriophorum* on the other hand is much rarer, but its tendency to hybridize is similar to previously mentioned species.

The other group – frequently hybridizing species – was in our sampling represented by most "promiscuous" species within the Czech flora. *C. oleraceum, C. rivulare* and

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[10] In the bachelor thesis (LAJKEPOVÁ 2013) only nine species were studied.
[11] Although hybrids can be found even within these species. BUREŠ et al. 2004 found herbarium specimens of 12 hybrid combinations including *C. arvense,* 3 combinations including *C. eriophorum* and 14 combinations including *C. vulgare*.

C. palustre, are frequently creating hybrids not only within this notional group, but also with their less frequently hybridizing species. Their relatively abundant distribution in the whole Czech Republic is allowing them to create hybrids almost anywhere.

The last group is represented by species that are somehow restricted in their ability to create hybrids. They either differ in their distribution (*C. acaulon* and *C. heterophyllum*) are occurring in only certain regions. In the case of *C. canum* this hybridizing restriction is mainly given by the fact that it hybridizes very often with *C. oleraceum*[12], but rarely with the other species. Comparing to bachelor thesis (LAJKEPOVÁ 2013), this group was enhanced with a fourth species, *C. pannonicum.* Like in the case of *C. acaulon* its distribution is limited by calcareous substrate where it can potentially hybridize and cause interspecific gene flow. In order not to miss out this important information it was decided to include this species into the dataset.

Based on these findings it can be assumed that interspecific gene flow would have the lowest intensity within the first group. In the second group the gene flow is going to be the strongest and in the third group it is going to be limited and only within certain species.

1.2. Study areas and potential hybridization within them

To do the sampling, three regions were chosen: Brno and its surroundings, White Carpathians and Žďárské vrchy Hills. These regions are well delimited and there is a relatively significant geographical distance between them. Another very important criterion is an eminent ecological variability between the regions. This variability is creating a suitable environment for different composition of studied species.

Brno and its surroundings is the poorest region considering the number of studied species. Most common species in this region are euryvalent taxa such as *C. arvense, C. oleraceum* and *C. vulgare. C. canum, C. palustre* and *C. rivulare* can also be quite frequently found. *C. eriophorum* has only one isolated locality at Vilémovice, where it is probably not indigenous and the seeds were brought there in fur of introduced sheep (VANĚČKOVÁ et al. 1997). In steppe south east part of this region we can also find sparse localities of *C. pannonicum.* Potential hybridization can take place between frequently hybridizing species (*C. oleraceum, C. palustre* and *C. rivulare*) and these species (mostly *C. oleraceum*) and *C. canum. C. pannonicum* would most probably hybridize with *C. canum,* but since is very rare in this region it is going to have very few opportunities.

The mosaic of steppes t and thermophilous forests together with sandstone and clay bedrock of White Carpathians is suitable for most studied species. As well as in Brno, most common there are *C. arvense, C. vulgare* and *C. oleraceum.* Other very frequent species are *C. canum* and *C. rivulare.* In steppe regions there are numerous localities with *C. pannonicum* whereas *C. acaulon* is quite rare. White Carpathians are Moravian centre of *C. eriophorum* distribution and it is the only region where this species could be sampled from more localities. The environmental conditions are not suitable for oreophytic species *C. palustre* and *C. heterophyllum. C. palustre that* requires higher precipitation and lower temperature is much rarer in this region then in the other two. *C. heterophyllum* is the only species that is

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[12] BUREŠ et al. 2004 found 331 herbarium specimens of *C. canum × C. oleraceum* hybrids. This was the largest number of hybrids followed by 200 herbarium specimens of *C. oleraceum × C. rivulare.*

absent from this region[13]. Most frequent hybrids can be expected between *C. oleraceum* and *C. canum* followed by hybrids between the three often hybridizing species (*C. oleraceum, C. palustre* and *C. rivulare*). Since *C. palustre* is rarer in this region, it is going to have fewer opportunities to hybridize than in the other two regions. There are also going to be hybridization events between *C. acaulon* and *C. oleraceum,* or *C. acaulon* and *C. pannonicum.* Rarer hybridization between *C. canum* and *C. pannonicum* might also take place.

Žďárské vrchy Hills is the coldest and most oreophytic region. The bedrock is formed by crystalline rocks making suitable environment for acidophilic species. Most common species are again *C. arvense, C. vulgare* and *C. oleraceum;* however, *C. palustre* is also widely distributed here. Žďárské vrchy Hills is the only region where there is *C. heterophyllum.* It is also one of few areas in the Czech Republic, where *C. heterophyllum* is getting into a contact with *C. rivulare.* On dry claystone slopes there are sparse occurrences of *C. acaulon. C. canum* – being a lowland species – is relatively rare here. *C. eriophorum* and *C. pannonicum* are completely absent from this region. Potential hybridization is going to happen between *C. oleraceum, C. palustre* and *C. rivulare.* These three species may also frequently hybridize with *C. heterophyllum.* Since *C. canum* is rare, it is not going to hybridize with *C. oleraceum* as often as in the other two regions. We may also expect some hybridization events between *C. acaulon* and *C. oleraceum.*

1. 3. Sampled hybrids

We also included several samples of hybrids in our dataset. These hybrids were sampled in 22 populations - 2 were in Brno, 10 in White Carpathians and 10 in Žďárské vrchy Hills (see **Fig. C3** and Supplements, **Tab. H19 - H32** and Discussion, **Fig. E3 – E16**). The hybrids were sampled when discovered on the locality with their parental species[14]. We did not go to search for them on special localities. We found 74 hybrid individuals of 14 different hybrid combinations (see **Fig. C1**).

1. 4. Sampling strategy

The sampling was performed in three years. The first and the most extensive sampling took place in 2011. Second sampling in 2013 and third sampling in 2014 covered up the under sampled species and added *C. pannonicum* individuals and hybrids into the dataset. The period of sampling was split between the end of May and the end of July. This period of year is most suitable for thistles sampling, because the plants are young, vital, they contain little secondary metabolites and are less damaged by herbivores. The species are also mature enough to be well recognisable.

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[13] There is one small locality of *C. heterophyllum* at Velké Kralovice in Javorníky, but our sampling was not preformed in proximity of this locality.
[14] Although we were trying to sample flowering and morphologically well recognisable individuals, we were not always sure whether the sampled individual is really F1 hybrid of certain two species. We also included potential triple hybrids in our dataset e. g., "triple hybrid" of *C. oleraceum* and *C. rivulare* that could possibly bear some morphological characteristics of a third species *C. heterophyllum*. The following molecular analyses, however approved these individuals to be only hybrids of two species *C. oleraceum* and *C. rivulare.*

Samples of morphological "pure" individuals (i.e. with no morphological indications of potential hybridization) were sampled in 99 populations - 25 were in Brno, 31 in White Carpathians and 43 in Žďárské vrchy Hills (see **Fig. C2** and Supplements, **Tab. H8 - H18** and **Fig. H1 - H10**). The localities where the samples were collected were selected in advance and we had a list of species, their locations and GPS coordinates.
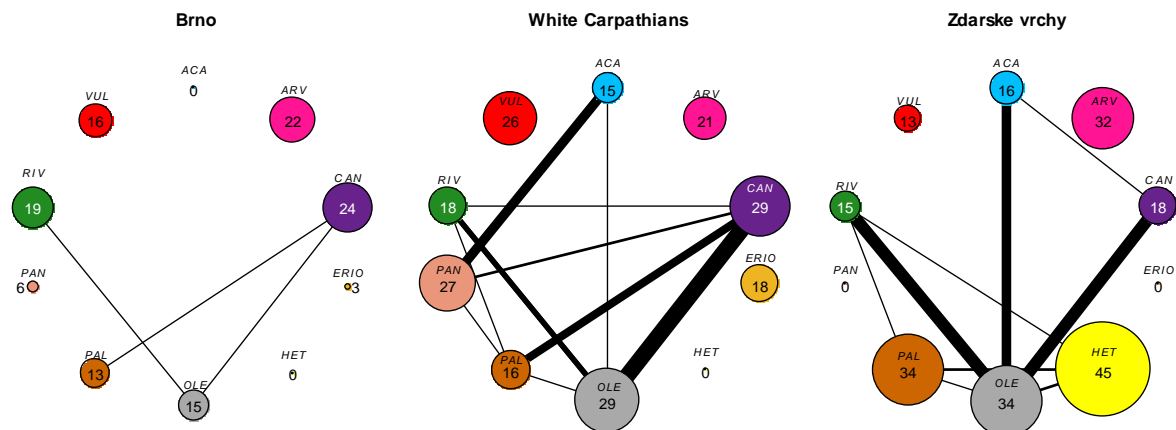
Fig. C1: Visualisation of sampling (circle size is proportional to number of samples analysed for different species, line thickness is proportional to number of hybrids between species)

In every locality we sampled three individuals of each species, which was occurring there.[15] When sampling clonal species (*C. acaulon, C. canum, C. heterophyllum, C. oleraceum* and *C. rivulare*), plants growing at least 15 m from one another were taken in order to minimise the risk of sampling the same genet. In the case of *C. arvense,* species with extremely long plagiotropic roots, the distance between sampled individuals was even longer. There was no such risk in monocarpic species, but more distant individuals were favoured in order to prevent their relatedness.

Sampled individuals had to be clearly identifiable as morphologically pure species. When sampling non-hybridizing species this criterion did not create any problem and plant material could be sampled from juvenile, non flowering plants. When sampling potentially hybridizing species we minimised the risk of collecting a hybrid by sampling them in flowering stage and with clear, species distinctive features.

During the actual sampling we picked at least three young leafs. It was necessary to make sure the leaves were undamaged and not invaded by some phytophagous insects or mould or contaminated by other plants. Since the markers used in further molecular analyses are non specific and any contamination could get amplified together with the sample, it was necessary to pay increased attention to it.

Fresh leafs were placed in a zipbag with a specific code defining the individual. The code is including species epithet (first three letters), locality (see Code in locality tables in Supplements) and sample order (a, b or c). This code was used during any work with samples and also specifies individuals in results.

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[15] There was an attempt to apply the same criteria for hybrid sampling, but since the hybrids are usually rare, we hardly found three individuals per locality.

During the field sampling the samples were stored in a polystyrene box with freezer packs to prevent postmortal DNA damage Fresh samples were then moved to deep freezer MDF-792 (SANYO, USA) and stored at temperature – 80 °C.
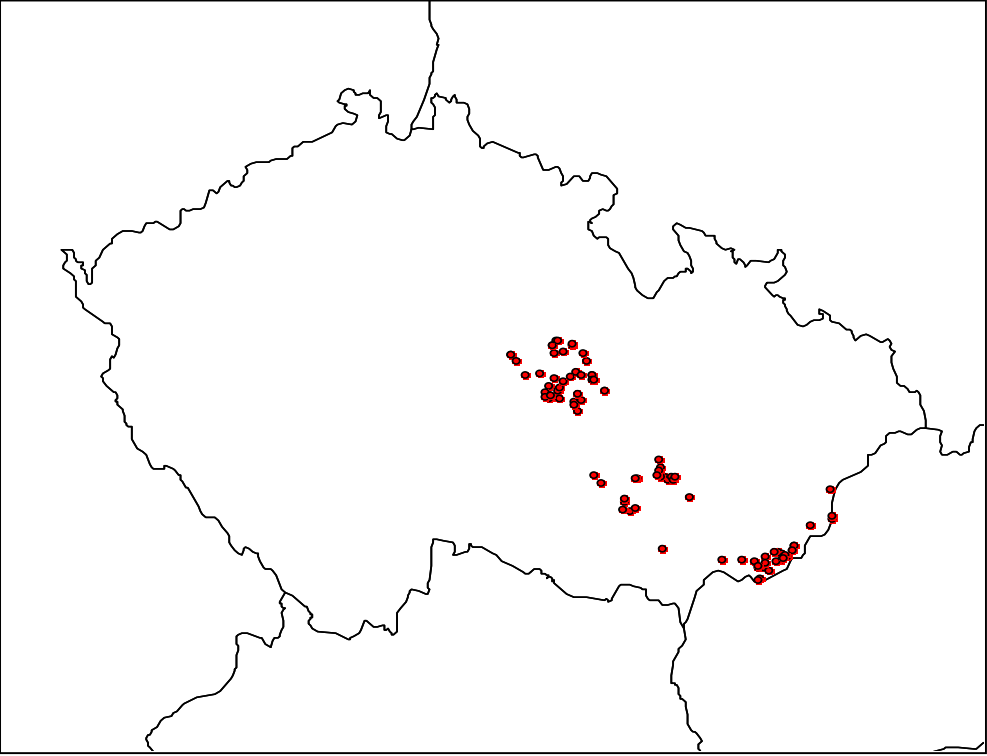
Fig. C2: Map of all localities of non-hybrid samples
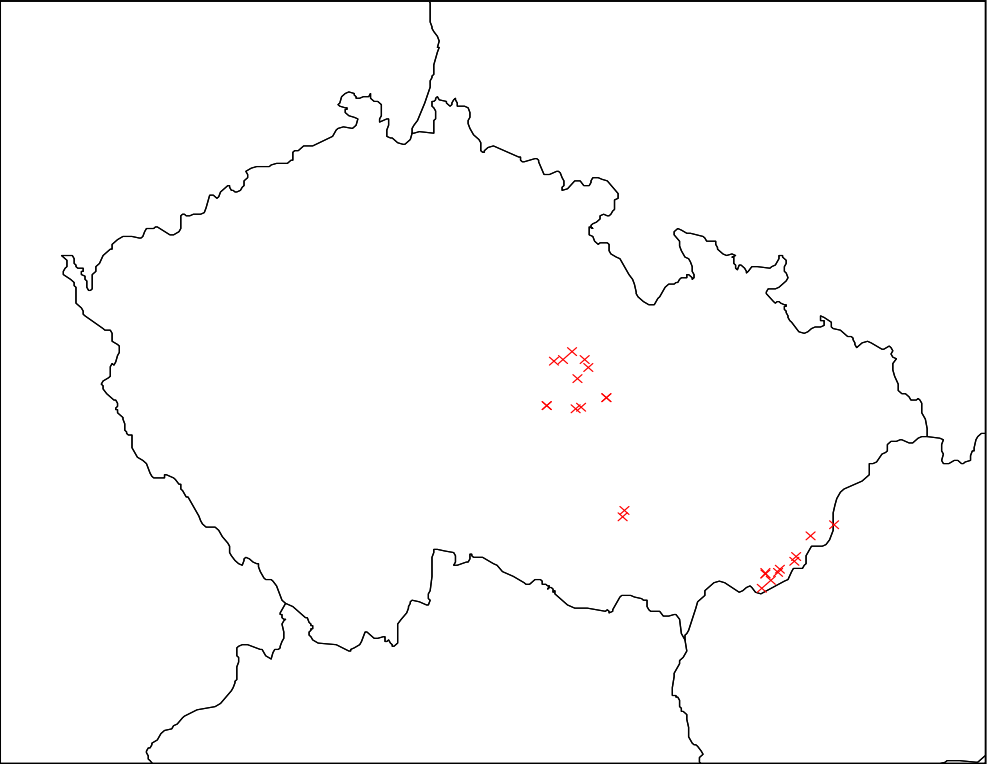
Fig. C3: Map of all localities of hybrids

2. Data analyses

2.1. Choice of suitable marker for molecular analysis

To accomplish the DNA analysis, AFLP (amplified fragment length polymorphism) (VOS et al. 1995) was used. Originally two more molecular approaches were tested – cpDNA sequencing and microsatellites (LAJKEPOVÁ 2013). Sequencing analysis was aiming to find potentially introgressed cpDNA haplotypes of one species in another species indicating ancient hybridization. This method would also allow us to detect the gene flow direction (PETIT et al. 1997, SEGARRA-MORAGUES 2007) since cpDNA is exclusively maternally inherited (PETIT et al. 1997, SANG et al. 1997)[16]. Out of 18 originally tested primer pairs most variability was found in *rpo*C1 (designed by GRUENSTAEUDL 2009) region. The variability within this region was, however, not sufficient to distinguish different species according to their haplotypes, so cpDNA haplotyping was dismissed. The second approach we tried was the use of microsatellites. We used 10 microsatellite primer pairs designed for *Cirsium* by JUMP et al. 2002. Since microsatellites are co-dominant markers and allow us to detect heterozygotes (OUBORG et al. 1999), they would be very suitable for gene flow detection (BALLOUX et al. 2002). The pilot analysis, however, showed that there was a large incidence of null alleles in tested samples and for this reason the analysis could not be continued. The analysis of cpDNA and microsatellites showed to be inapplicable for our dataset and for our studied problem (LAJKEPOVÁ 2013).

2.2. Molecular data processing

In order to extract DNA from samples we used isolation kit NucleoSpin®Plant II (MACHEREY-NAGEL, Germany) according to the manufacturer's instructions with some modifications. The kit is allowing two methods based on different types of extraction buffers. Widely used CTAB method (DOYLE & DOYLE, 1987) could not be used, because if applied for thistles, the DNA quality is worse and it makes further analyses very complicated (ŠMERDA, in verb). For this reason we used SDS method, because the DNA yields are better and further analyses are less complicated (see Supplements, **1. 1. DNA isolation**).

AFLP reaction was done using protocol AFLPᴛᴍ plant mapping kit (APPLIED BIOSYSTEMS, USA) – see Supplements, **1. 2. AFLP**) according to the manufacturer's instructions. The analysis was done for all sampled species and three combinations of primer pairs (proved to be useful for thistles by BUREŠ et al. 2004) were applied. In order to calculate error rate, we randomly selected 66 samples of which duplicates were made. These individuals were analysed twice. We repeated in total 12.4% of all samples which has made a decent dataset for error rate analysis, since the number of repeated samples is advised to be around 10% (BONIN et al. 2004).

During restriction-ligation reaction (RLR) whole genomic DNA was fragmented by restriction enzymes *Eco*RI and *Mse*I. Thus produced sticky ends were then ligated by ligase to *Eco*RI and *Mse*I adaptors (see Supplements, **Tab. H1**). In preselection reaction the diluted product of RLR was amplified using preselective primers *Eco*RI-C and *Mse*I-A (see

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[16] Although there are studies suggesting that cpDNA is not strictly maternally inherited as proved, e. g., in *Silene vulgaris* (McCAULEY et al. 2007).

Supplements, **Tab. H3**). Yields of preselection reaction were checked using gel electrophoresis and unsuccessfully amplified samples were excluded from the analysis. In selection reaction the diluted product of preselection reaction was amplified using three primer combinations (see Supplements, **Tab. H5** and **H6**). Fragmentation analysis of final product was done at Department of morphology, physiology and Animal Genetics (Mendel University, Brno).

2. 3. AFLP data scoring

Sizing and scoring (distinguishing between presence or absence of a locus) of the data was performed with GeneMarker 2.4.0 (SoftGenetics, USA). Size standard used was GS500_1_old. The result of fragmentation analyses was at first automatically pre-analysed using default settings of the software. In this pilot study 2,145 loci were detected using peaks ranging from 36 bp to 499 bp. When visualised as PCO projection (distance matrix based on Jaccard´s dissimilarity; see **Fig. C4)**, the pilot study already showed some data pattern and samples forming clusters according to species. Non-hybridizing species formed remote clusters. The other species formed one big cluster within which, however, the species could already be decently distinguishable. Although all clusters were rather scattered or in some cases they were even complicated to tell apart, the pilot study approved that the AFLP method is suitable for our data.
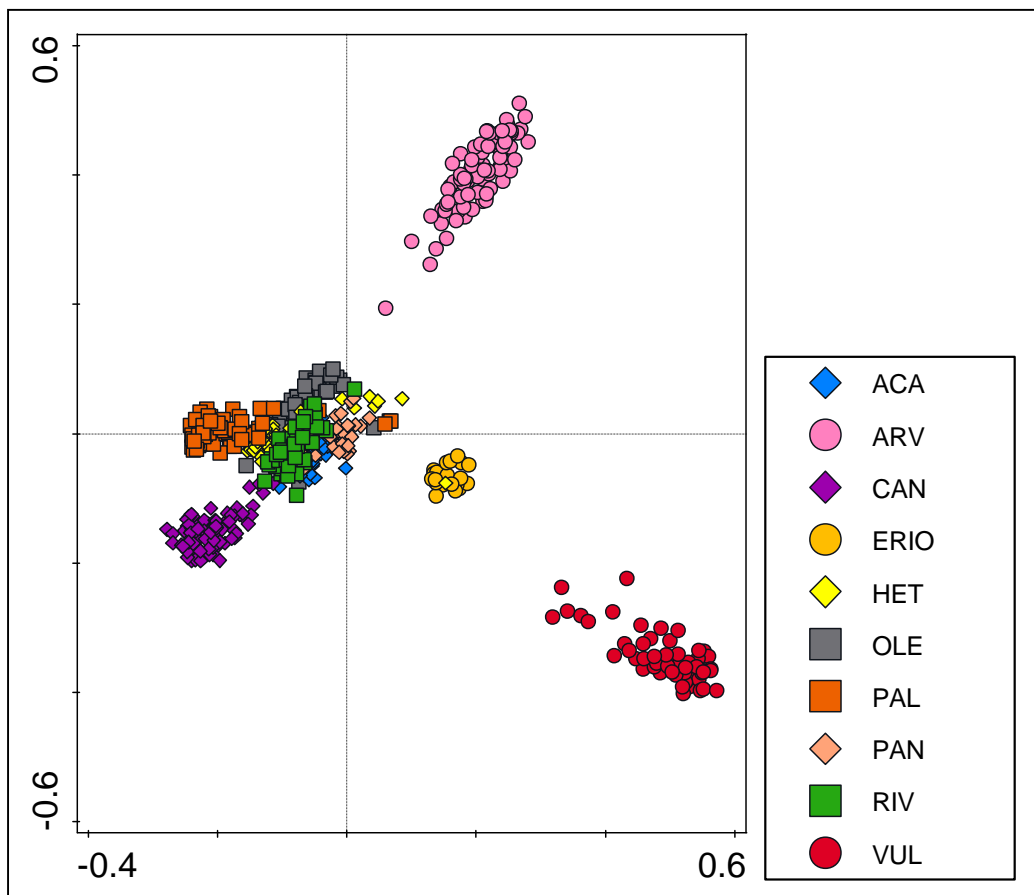
Fig. C4: PCO analysis based on raw data, eigenvalues: 1st axis: 0.468, 2nd axis: 0.41; (Canoco5)

This automatic scoring however does not establish and recognise peaks correctly (HOLLAND et al. 2008, TURNER et al. 2013) – this is why the result of the pilot study was not optimal – and manual control is necessary (BONIN et al. 2004, TURNER et al. 2013). A panel of scorable peaks for each primer combination had to be created manually. Since we were dealing with numerous samples of 10 species, creation of this panel was complicated. Different samples and species were overlapping and typical binary distribution was missing (see **Fig. C5**). Another problem was, that the software was unable to deal with so many samples. When we uploaded all samples at once, it was working very slowly.
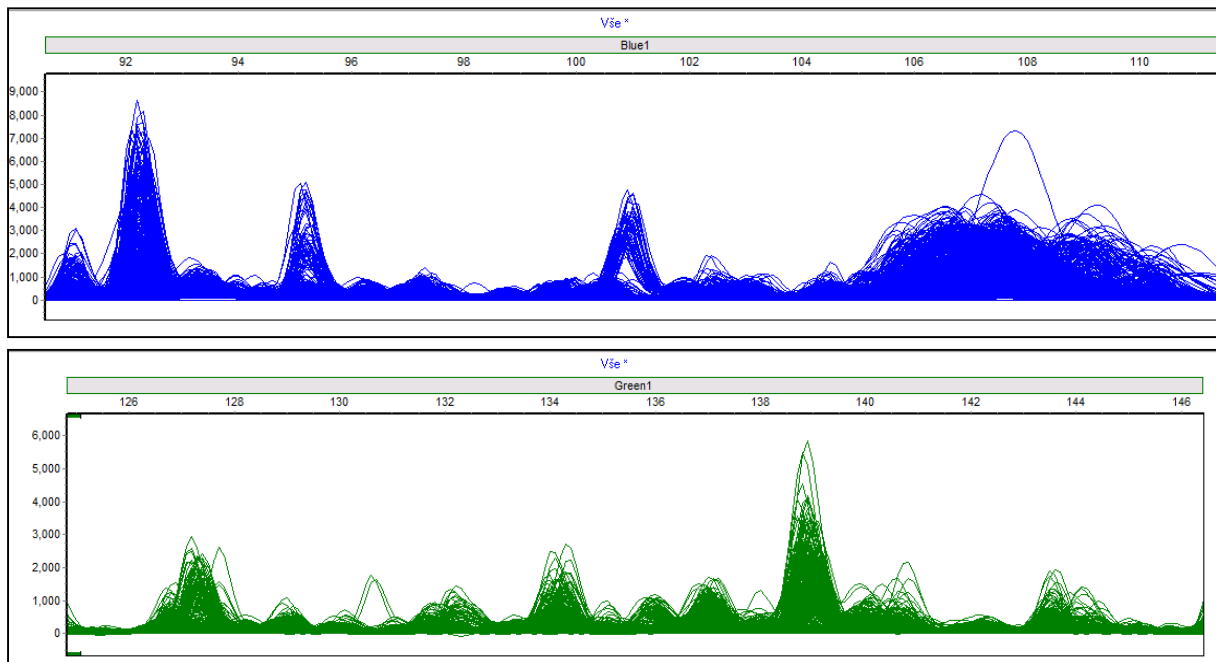
Fig. C5: Panel editor visualising parts of fragmentation analysis for blue and green colour, binary pattern is hardly distinctible (GeneMarker 2.4.0)

In order to get rid of these problems, the analysis had to be divided into several steps. To start the panel creation we chose only 15 samples per species that were representing allelic diversity of the whole species. Fragments from 73 to 540bp were scored. While creating a panel based on 150 samples only, the binary pattern was well distinguishable and thus created panel was decently accurate. In order not to miss out the rest of allelic diversity existing within the samples that were not chosen for primary panel creation, we projected all samples of one species into the panel and added characteristic loci of this species into the panel[17]. Every time the panel was modified by adding loci of certain species, the original dataset of 15 samples per species was projected into this modified panel and if newly added loci were confronting and overlapping the original ones, they were removed. This way we created a panel for each colour respectively. The blue panel had 329 loci, the green panel had 273 loci and the yellow had 173 loci.

Scoring of all samples including hybrids according to these panels was done automatically[18] and was manually checked and corrected. There were numerous errors

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[17] Hybrids were not included in panel defining since their loci should be identical to the parent species.
[18] The relative fluorescent unit (RFU) threshold was set at 50.

associated with shifted and stuttered peaks and atypical peak shapes[19]. There were often problems with peaks smaller than 150 RFU, because the software did not consider them as peaks and they had to be added in manually.

Manual scoring is very subjective and although if the person scoring the dataset is well experienced, the mistakes are negligible and the method is highly reproducible (BONIN et al. 2004) mistakes are sometimes made (see **Fig. C6**). There may also be some molecular analysis artefacts that also contribute to genotyping errors (see **Fig. C7**). For this reason error rate estimation (estimation of problematic loci) needs to be done. We used 66 duplicates to check in which loci does the original sample differ from its duplicate (where in one sample there is the peak marked as present in the other one as absent). Since the problematic loci were different for every species error rate estimation was done for each species respectively according to BONIN et al. 2004[20] (see **Tab. C2**).
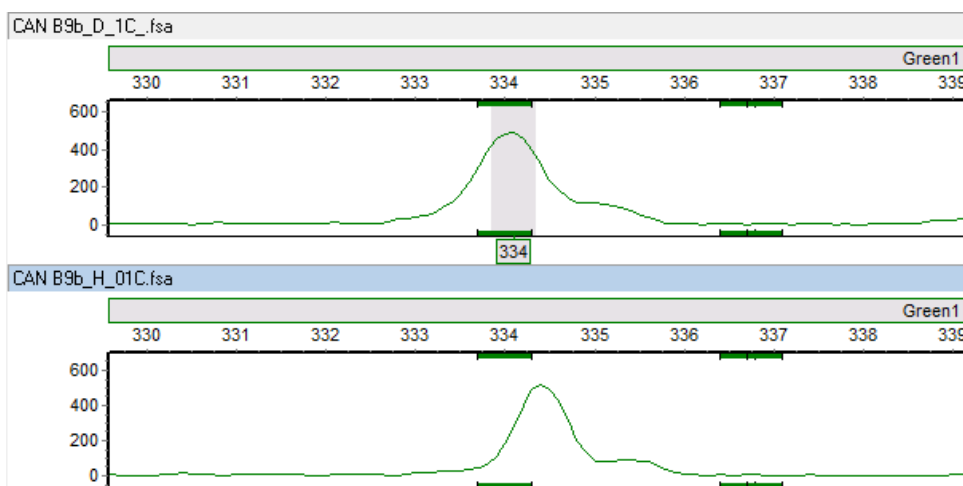
Fig. C6: Manual scoring error (second duplicate is slightly shifted and should have been edited manually); (GeneMarker)
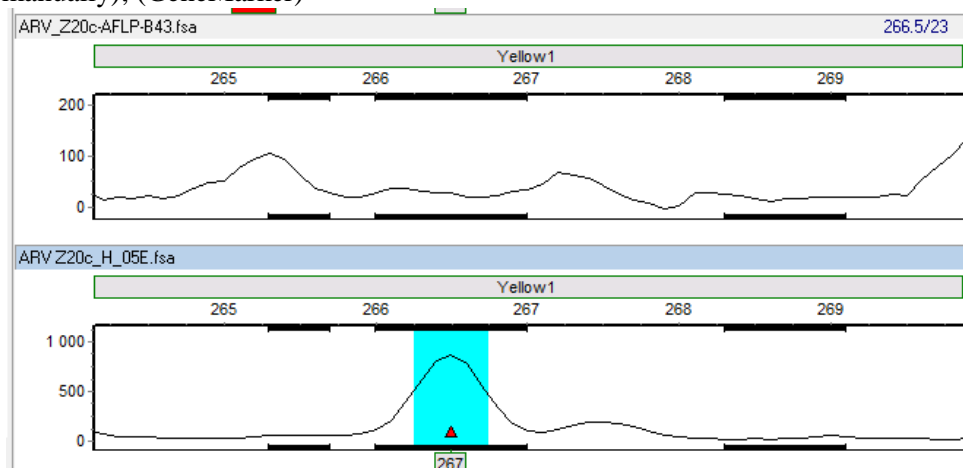
Fig. C7: Molecular analysis error (two duplicates differing in peak presence on position 267); (GeneMarker)

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[19] Molecular analysis artefact

[20] Number of loci with at least one difference within the locus/number of all loci within the dataset

Tab C2: Error rate estimation

Species	ACA	ARV	CAN	ERIO	HET	PAL	PAN	RIV	VUL	Average
% of duplicates	12.5	10.52	49.29	40.9	13.33	18.18	6.06	9.25	3.63	
Error rate (%)	1.29	13.55**	4.51	3.35	4.39	3.61	0.52	7.23*	1.16	2.97

One species − *C. arvense* − exceeded the critical level of 10% (marked **). In case of *C. rivulare* the error rate was higher, but still bellow the critical level (marked *). The error rate estimation for the rest of species was optimal and the geometric average of all species error rates of was 2.97 indicating that the dataset is acceptable.

The result of allele scoring was then exported as a binary matrix where 1 stands for locus presence and 0 for locus absence. All three primer-combinations were combined in a single matrix. The matrix was consisting of all analysed samples (in rows) and all 775 loci of the three panels (in columns). Number of occupied loci (present at least once within the samples) and average number of loci per individual was different for every species (see **Tab. C3**), but each species had enough loci to build further analyses on.

Tab. C3: Number of occupied loci per species and average number of alleles per individual

Species	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
Average number of alleles per individual	163	153	140	156	164	164	157	111	162	194
Number of occupied loci per species	305	363	267	254	331	422	272	198	385	250

2. 4. Molecular approval of samples

First of all an initial analysis was done in order to exclude obviously failed samples and genetically impure (potential F2 or B2 hybrids) individuals. This analysis was done using PCO in Canoco5 (TEER BRAAK & ŠMILAUER 1998). We used Jaccard´s dissimilarity coefficient for distance matrix (representing distances for each pair of samples) calculation. First we projected all samples and removed the most obviously failed individuals (incorrect amplification). In next step we projected all species respectively and obvious outliers were also excluded from the analysis.

To visualise samples membership identity and see whether the samples are genetically pure individuals belonging to certain species, several analyses were performed. The first analysis we used PCO analysis (see Results, **Fig. D1**). The second approach was agglomerative clustering analysis - neighbour-joining (NJ) based on Jaccard´s dissimilarity coefficient (see Results, **Fig. D2**). Since this analysis was not focused on thistles phylogeny, we did not have any outgroup and we used unrooted tree. To test the tree quality, we examined cophenetic correlation of original distance in matrix and reconstructed distance used for NJ construction (See Results, **Fig. D3**). The analysis was performed in R 3.2.3, distance matrix was calculated in r package vegan (OKSANEN et al. 2016) and NJ tree was calculated in r package ape (PARADIS et al. 2004) and constructed in r package phangorn (SCHLIEP 2011); see Supplements, **2.2. NJ construction**).

Another approach was to quantify to what extent a sample belongs to certain species/cluster. For this purpose, two different methods were used.

First method was Bayesian analysis performed in STRUCTURE 2.3.4 software (FALUSH et al. 2003). This method is widely used for species resolving based on AFLP data (MEUDT et al. 2009, TURNER et al. 2013 and others). Unlike in these mentioned articles, we have already had a prior knowledge of membership of samples to species. We have used this method to test to what extent is this method suitable for our data and whether other analyses (such as hybrid recognition) can be performed using STRUCTRUE software.

The dataset uploaded to STRUCTURE was the binary matrix of all morphologically pure individuals and hybrids and all loci. We used admixture model and independent allele frequencies. We ran STRUCTURE for K = 10 (since we had 10 species, we assumed 10 "populations") for 20 times. Each run had 100000 iterations with 100000 burn in.

The runs were checked and only the runs that recognised all studied species correctly (11 runs out of total 20) were included in further analyses. The incorrect 9 runs most often miss-joined a species with less individuals present in the dataset (e. g., *C. acaulon, C. eriophorum, C. pannonicum*) to species with more individuals (often *C. oleraceum* and *C. rivulare*). The incorrect cluster – after joining these two species together – was most often represented by *C. canum × C. oleraceum,* because this hybrid it had most individuals in dataset and the software assumed it to be a species.

From the correct 11 runs we calculated the mean value within every cluster (representing species). Every sample had ten values representing species identity given by mean of all 11 runs (see Supplements**, Tab. H33**). To visualise the results, we plotted all results into a line chart[21]. Every line represented one individual. This line chart was plotted for each species respectively (see Results, **Fig. D4 - D13**) in R 3.2.3 software (see Supplements, **2.3. Sample membership identification - species identity identification**).

The second approach we used was our own script written in MS Excel[22]. Our script uses the binary matrix of individuals and loci and is based on probability that the examined individual gained certain locus from certain species. The script does not make a difference between presence (1) and absence (0) of a locus in order to reduce phylogenetic background.

The first step of this script is to calculate the percentage of samples within one species that have a present locus in certain position. This number may vary from 0% (no individual of this species is bearing this locus) to 100% (all individuals of this species are bearing this locus). This calculation is done for all loci and all species resulting in a table of percentages (see **Fig. C8** - Allele frequencies).

The next step is asking what is the probability that the examined individual gained certain locus from certain species. E. g., we have got an individual with "1" on the first locus position. We go to the percentages table and see that all species are having 0 % on this position, only *C. vulgare* it is present from 100 % and *C. eriophorum* from 25 %. This means, that this "1" could be gained only from these two species. The sum of percentages of all species is always equal to 100% together. Species other than *C. vulgare* and *C. eriophorum* would contribute with 0% to this sum of percentages and the probability that this locus was gained from *C. vulgare* and *C. eriophorum* is divided between them two. In this hypothetical

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[21] Normally these results from STRUCTURE analysis are visualised as bar plot where every bar represents an individual. For our purpose, however, we used a line chart to visualise the results, because we wanted to compare the output of both methods we used.

[22] This script was written by Ing. Jakub Šmerda Ph.D.

case we would assume that the individual got this locus with 80% probability from *C. vulgare* and with 20% probability from *C. eriophorum* (see **Fig. C8** - Matrix for allele 1, first column). If the examined individual had "0" instead of "1" in this locus, the result would look as seen in **Fig. C8** - Matrix for allele 0, first column. After calculating the probability for each locus, we count the average of all loci. The outcome of this analysis is a table of values showing what is the probability (in %) that the loci of the individual were gained from individual species (see Supplements, **Tab. H35**). The largest percentage probability – which is much higher than the rest of more or less comparable probabilities – is then representing the species to which the individual belongs. If there was a steep increase in total probability percentage also for another species, it would mean that this individual is not a pure species, but a hybrid. To visualise the results and see the steepness of probability percentage increases, we plotted all results into a line chart. Every line represented one individual. This line chart was plotted for each species respectively (see Results, **Fig. D4 - D13**) in R 3.2.3 as in the case of STRUCTURE results. The MS Excel sheet containing the script is available on the attached CD.

Allele frequences	locus	1	2	3	4
	ACA	0	100	0	89,47368
	ARV	0	100	37,5	100
	CAN	0	100	5,882353	17,64706
	ERIO	25	100	0	85,71429
	HET	0	100	33,33333	100
	OLE	0	100	12	92
	PAL	0	100	13,63636	95,45455
	PAN	0	100	0	83,33333
	RIV	0	100	16,66667	100
	VUL	100	90	0	0

Matrix for allele 1	locus	1	2	3	4
	ACA	0	10,10101	0	11,717
	ARV	0	10,10101	31,50765	13,09547
	CAN	0	10,10101	4,942376	2,310965
	ERIO	20	10,10101	0	11,22469
	HET	0	10,10101	28,0068	13,09547
	OLE	0	10,10101	10,08245	12,04783
	PAL	0	10,10101	11,45733	12,50022
	PAN	0	10,10101	0	10,91289
	RIV	0	10,10101	14,0034	13,09547
	VUL	80	9,090909	0	0
	suma [%]	100	100	100	100

Matrix for allele 0	locus	1	2	3	4
	ACA	11,42857	0	11,35098	4,453188
	ARV	11,42857	0	7,094362	0
	CAN	11,42857	0	10,68327	34,83965
	ERIO	8,571429	0	11,35098	6,043612
	HET	11,42857	0	7,567319	0
	OLE	11,42857	0	9,988861	3,384423
	PAL	11,42857	0	9,803118	1,922967
	PAN	11,42857	0	11,35098	7,050881
	RIV	11,42857	0	9,459149	0
	VUL	0	100	11,35098	42,30528
	suma [%]	100	100	100	100

Fig. 8: Scheme of our script; MS Excel

2. 5. Hybrids – identification of parental species

Our aim was to identify parents of sampled hybrids with no former knowledge about what their parental species could be. If for instance we projected the hybrids and species that we (according to morphological appearance) believe are parental species as PCO, we would have two remote clusters of parental species and their hybrids in the middle. This is however not a prove that these putative parents really are parental species. This seemingly intermodal position of hybrids between their parental species could be given by phylogenetic background. Some species – e. g., *C. oleraceum* and *C. rivulare* – are closely related (BUREŠ et al. 2010). If we did a PCO projection of an individual that we believe to be a hybrid of *C. oleraceum*, but in reality it was a hybrid of *C. rivulare*, we wouldn´t be able to recognise that until we also projected *C. rivulare* and saw that the hybrid was closer to *C. rivulare* cluster.

For this reason we also had to exclude the use of NewHybrids software, because it is able to compute hybrids if only two parental species are taken into account (ANDERSON & THOMPSON 2002). The analysis - although it is more accurate than a simple PCO projection – suffers from the same problem. Without previous preconditioning of parental species we are not able to successfully distinguish the hybrids.

To get rid of this problem, two different approaches were used. Bayesian clustering in STRUCTURE and our own script (as described above). Using Bayesian clustering the data for hybrids were obtained from the same dataset as for morphologically pure individuals. To visualise the results, we plotted all putative hybrids into a line chart. Every line represented one individual. This line chart was plotted for each hybrid combination respectively (see Results, **Fig. D14 - D28**) in R 3.2.3 software as it was done in the case of "pure" individual. As expected, hybrid charts had more than one steeply increased peak since the AFLP patterns of hybrid individuals are actually a combination of more than one species.

Secondly we used our script explained in chapter above. Since the script calculates the probability that the examined individual gained certain locus from certain species, we do not need any previous knowledge about potential parental species.

The method was performed same way as for pure species. The results were plotted as line chart for each hybrid combination respectively. Every line represents one individual (see Results, **Fig. D14 - D28**).

2. 6. Geographical analysis of genetic variance

Since our sampling was done in three geographically separated regions, we wanted to see whether the geographic pattern is also portrayed in genetic information. To visualise putative geographic pattern of each species, we used 3D PCO plot with three different colours symbolizing three different regions (See, Results **Fig. D29 - D38**). Distance matrix was again based on Jaccard´s dissimilarity coefficient. The analysis was done in R 3.2.3. Distance matrix and PCO was calculated in r package vegan (OKSANEN et al. 2016) and 3D plot was projected using r package scatterplot3d (LIGGES & MÄCHLER 2003); (See Supplements, **2.4. Geography pattern identification - 3D PCO**) To visualise not only the regional differences within species, but also seeing whether samples sampled on same localities are clustered together, we created unrooted NJ dendrograms. This was done in R-r package

phangorn (SCHLIEP 2011); (see Supplements, **2.2. NJ construction**[23]). Other, more accurate approach was AMOVA (analysis of molecular variance) that is often being used for population resolution when analysing AFLP data (e. g., TRAVIS et al. 1996, BAHULIKAR et al 2004, BREINHOLT 2009, TURNER et al. 2013, BUSCONI et al. 2015). This method calculates molecular variance within populations (in our case within Brno, White Carpathians and Žďárské vrchy Hills respectively) and among populations (among all studied regions). Molecular variance within and among populations is given in % and ΦPT estimates whether there is complete panmixis among populations ΦPT = 0 (in our case it would mean that there is no genetic difference between our studied regions), or whether the populations are completely isolated ΦPT = 1 (in our case the regions would be completely genetically distinctive) (HOLSINGER & WEIR 2009).

AMOVA was calculated in FAMD125 (SCHÜLTER & HARRIS 2006) and it was done only for some species. *C. heterophyllum* could not be included in this analysis, because it was sampled only in Žďárské vrchy Hills and *C. eriophorum* from Brno is represented only by three samples, which is too little to carry the analysis on. All other species were uploaded to the FAMD software respectively and distance matrix (based on Jaccard´s dissimilarity coefficient) was calculated. After the dataset was divided into groups symbolizing different regions, AMOVA was performed. The results for every species were put in a table. (see Results, **Tab. D1**).

To show the effect of increasing distance on genetic similarity between samples we used two matrices – matrix of geographical distances and matrix of genetic distances between samples. Matrix of geographical distances was calculated from GPS coordinates we measured for each locality. If there were more samples per locality[24], we set the distance between them as 0 although the distances between the samples were over 15 meters in order to avoid clonality (see chapter **Sampling methods**). In final matrix we had distances of all samples. We calculated genetic distance matrix using Jaccard´s dissimilarity coefficient in FAMD. This matrix of genetic dissimilarities was then converted to Jaccard´s similarities[25].
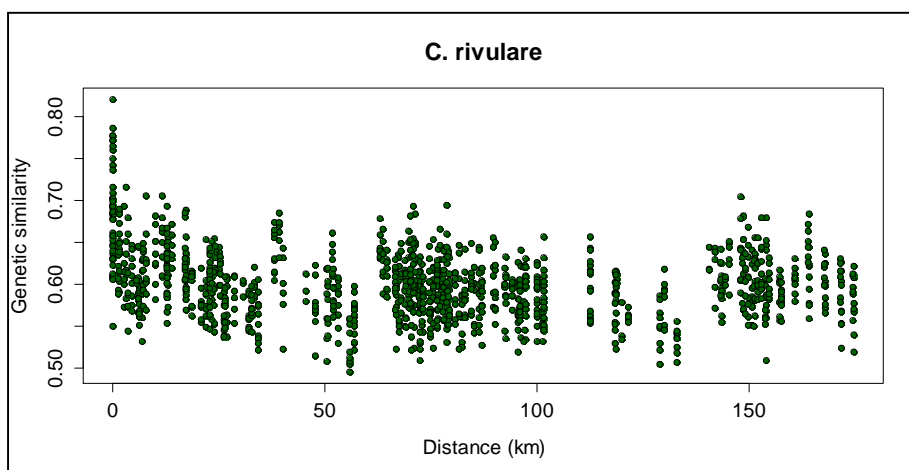
Fig. C9: Visualisation of genetic similarities and geographic distances in *C. rivulare*

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[23] The script was slightly modified to visualise individuals according to colours of different regions used in **2.4. Geography pattern identification** - **3D PCO**.

[24] Which was mostly the case since we took 3 samples per locality as described in chapter Sampling strategy.

[25] Calculated as 1 – Jaccard´s dissimilarity.

When visualised as a scatter plot we could observe (in most species) that the similarity is the largest at the same locality and then it decreases slowly[26]. There is also a gain in genetic similarity among samples over larger distances (see **Fig. C9**), but it is given by similar processes (such as hybridization events, bottle neck, clonality etc.) that randomly took place on these remote localities and result in higher genetic similarity between their individuals than we may observe at close localities. These similarities are, however, just some kind of "homoplasies" – they are identical by state, but not by descent.

To get rid of these "artefacts" we visualised the data as boxplots. Drawing of boxplots was done in software STATISTICA 12 (StatSoft CR s.r.o.). Genetic similarities between samples were divided into 5 groups according to respective intervals of geographic distances. These intervals were: less than 1 km, 1 – 4 km, 4 – 16 km, 16 – 64 km and over 64 km, i. e., the ranges of groups were increasing exponentially[27]. The boxplots were done for all species respectively (see Results **Fig. D50**).

2. 7. Genetic diversity

When we create a heat map of distances between samples (see **Fig. C10**), we can see that genetic distances differ across the studied species. There are species with obviously smaller distances between individuals (e. g., distances within *C. vulgare* are deep red) and species with much larger distances (e. g., distances within *C. oleraceum* and *C. rivulare* are much lighter in colour).

One of the possible reasons for this uneven genetic diversity could be the tendency of species create hybrids. To estimate the relationship between average genetic similarity per species and species promiscuity we produced two matrices. Data for species promiscuity were taken from BUREŠ et al. 2004[28].

We performed Spearman´s correlation coefficient testing not only the relationship between average genetic similarity per species and species promiscuity, but also other variables – species genome size (published in BUREŠ et al. 2004) and average number of females per species (BUREŠ, unpublished data) that could possibly affect the genetic similarity (see **Tab. C4**). The correlation was performed in R r package Hmisc (HARRELL et al. 2015); (see Supplements, **2.5. Spearman´s coefficient calculation**). The variables were plot into a diagram.

We also drew boxplots of genetic similarities for each species (see Results, **Fig. D51**) and compared the the variability of intraspecific genetic similarity among all species using Kruskal-Wallis test (see Results, **Tab. D2**).

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[26] We also drew these scatter plots for each region within the species respectively. The data usually showed similar trend as when we put all regions together. There were no apparent regional differences. The only exceptions (when the regional pattern looked very differently from the one when all regions were put together) were sometimes in the case that there were too few samples representing the region.

[27] The only species where we had only 4 categories was *C. heterophyllum,* because it was sampled only in Žďárské vrchy Hills and the distance between localities never exceeded 64 km.

[28] Number of hybrids was calculated from number of herbarium specimens.
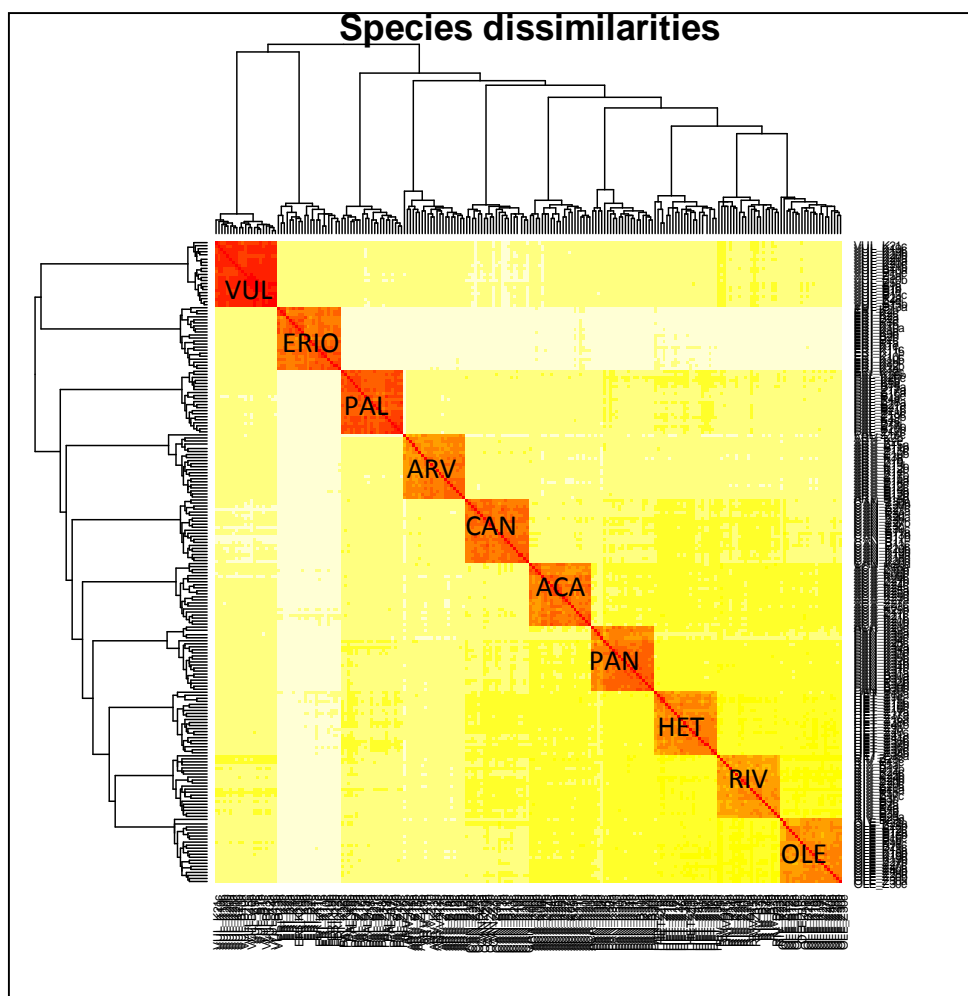
Fig. C10: Samples dissimilarities visualised as heat map for all species (represented by 21 samples per species), the darker the colour is, the smaller genetic distance between two individuals are – the central diagonal is representing identical samples with 100% similarity and has therefore the darkest colour; (R 3.2.3)

Tab. C4: Variables possibly affecting the average genetic similarity

Species	Average genetic similarity	Promiscuity[29]	Genome size[30]	Average number of females
C. acaulon	0.69	248	4.98	22.79
C. arvense	0.66	12	5.31	–[31]
C. canum	0.69	518	4.22	1.61
C. eriophorum	0.72	3	6.39	0.21
C. heterophyllum	0.63	80	4.06	54.2
C. oleraceum	0.61	884	4.27	29.15
C. palustre	0.78	418	4.69	17.21
C. pannonicum	0.68	80	4.51	33.95
C. rivulare	0.60	375	4.4	29.42
C. vulgare	0.88	14	10	0.55

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[29] Number of herbarium specimens.
[30] In arbitrary units (BUREŠ et al. 2004)
[31] Contrastingly to all other taxa, *C. arvense* is dioecious therefore it was not included in the analysis.

2. 8. Interspecific gene flow identification

As described in chapter **Study areas and potential hybridization within them**, not all sampled species were present in all regions. For instance *C. heterophyllum* can be found only in Žďárské vrchy Hills, *C. acaulon* is absent in Brno and *C. pannonicum* is missing in Žďárské vrchy Hills[32]. We wanted to compare average genetic similarity between two potentially hybridizing species considering the fact that these species can only meet in a certain region and therefore they can hybridize only there. Our idea was, that if there was interspecific gene flow taking place, the species would be more genetically similar within the region where they are both present than in a region where one of these species is missing. E. g., *C. heterophyllum* is growing only in Žďárské vrchy Hills. In this region it could hybridize with *C. oleraceum, C. palustre* and *C. rivulare.* In the other two regions it is missing, therefore there is no hybridization and no gene flow can take place (at least gene flow *in situ*). If our assumption was correct, we would have smaller genetic distances between samples of *C. heterophyllum* and the three species within Žďárské vrchy Hills than within the other regions. The pilot analysis was tested only on several species combinations where we believed the pattern could be best observed (see **Tab. C4**)

Tab. C4: Combinations of species used for interspecific gene flow identification

Analyzed species pair	Contrasting regions	Co-occurrence	Expected similarity
C. canum, C. pannonicum	White Carpathians	yes	higher
	Žďárské vrchy Hills	only can	lower
C. acaulon, C. oleraceum	Brno	only ole	lower
	White Carpathians Žďárské vrchy	yes	higher
C. heterophyllum, C. oleraceum	Brno	only ole	lower
	White Carpathians	only ole	lower
	Žďárské vrchy	yes	higher
C. heterophyllum, C. palustre	Brno	only ole	lower
	White Carpathians	only ole	lower
	Žďárské vrchy	yes	higher
C. heterophyllum, C. rivulare	Brno	only ole	lower
	White Carpathians	only ole	lower
	Žďárské vrchy	yes	higher

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[32] Another species that is found only in two regions (Brno and Žďárské vrchy Hills) is *C. eriophorum.* This species however does not hybridize, therefore it was not included in our study.

D. Results

1. Molecular approval of samples

PCO analysis showed a very good resolution of studied species (**Fig. D1**). Comparing to the analysis based on raw data (see Material and Methods, **Fig. C4**), the clusters of species are much more compact. The PCO also shows that species *C. arvense, C. eriophorum* and *C. arvense* – members of sections *Eriolepis* and *Cephalonoplos* – form three remote clusters whereas the other species (from section *Cirsium*) tend to cluster with two exceptions – *C. canum* and *C. palustre.* These species, although they belong to the same section, tend to make their own clusters and do not join the main cluster of other species from *Cirsium* section.
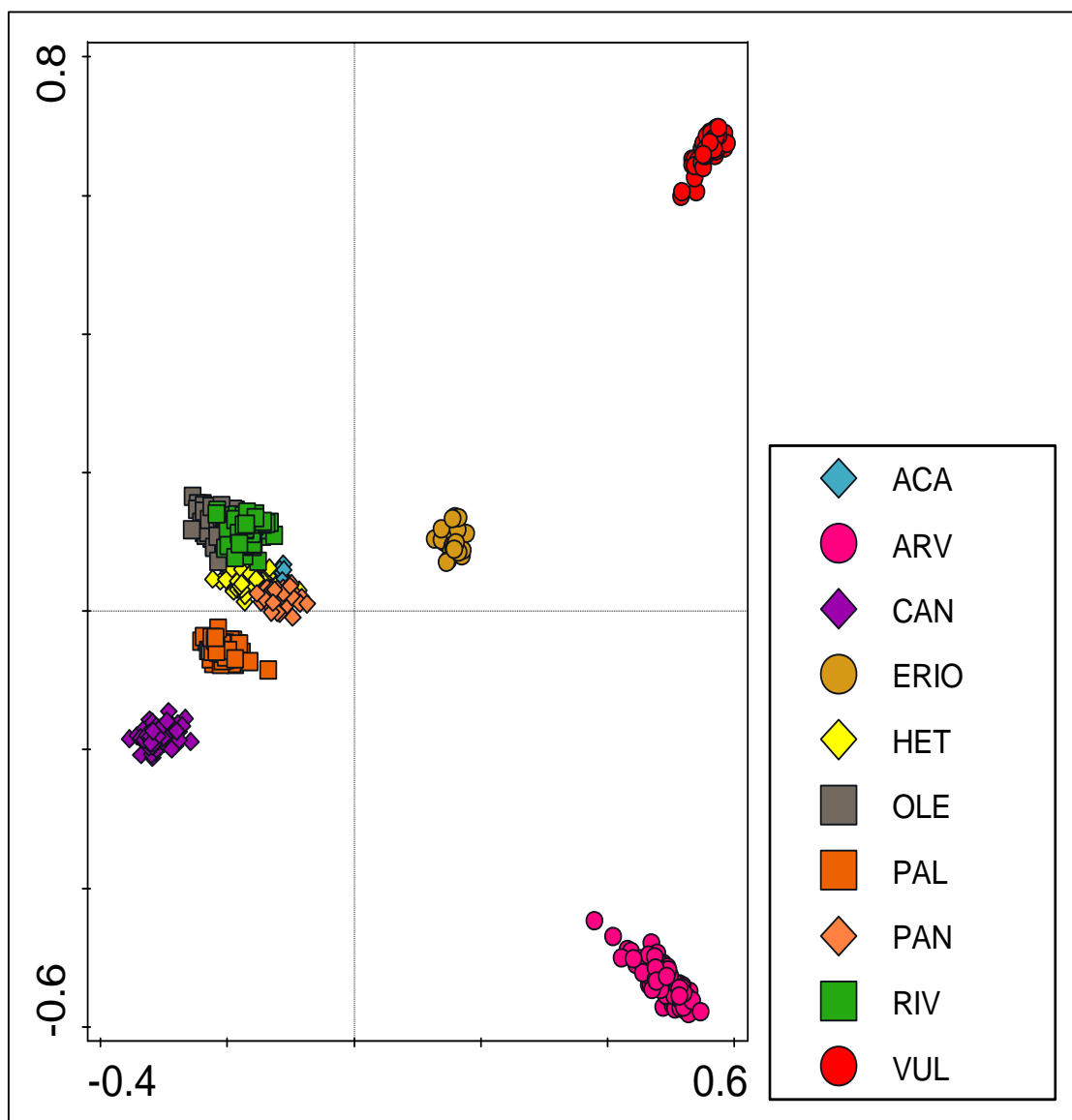
Fig. D1: PCO analysis based on manually checked data, eigenvalues: 1st axis: 0.103, 2nd axis: 0.095; (Canoco5)

Agglomerative clustering analysis – neighbour-joining (NJ) – visualised as an unrooted tree also divided all samples into species clusters according to their membership (see **Fig. D2**).

To test the tree quality, we examined cophenetic correlation of original distance in matrix and reconstructed distance used for NJ construction; linear model estimated $R^2 = 0.9704$, $p < 2.2*10^{-16}$ indicating high tree quality (See **Fig. D3**).
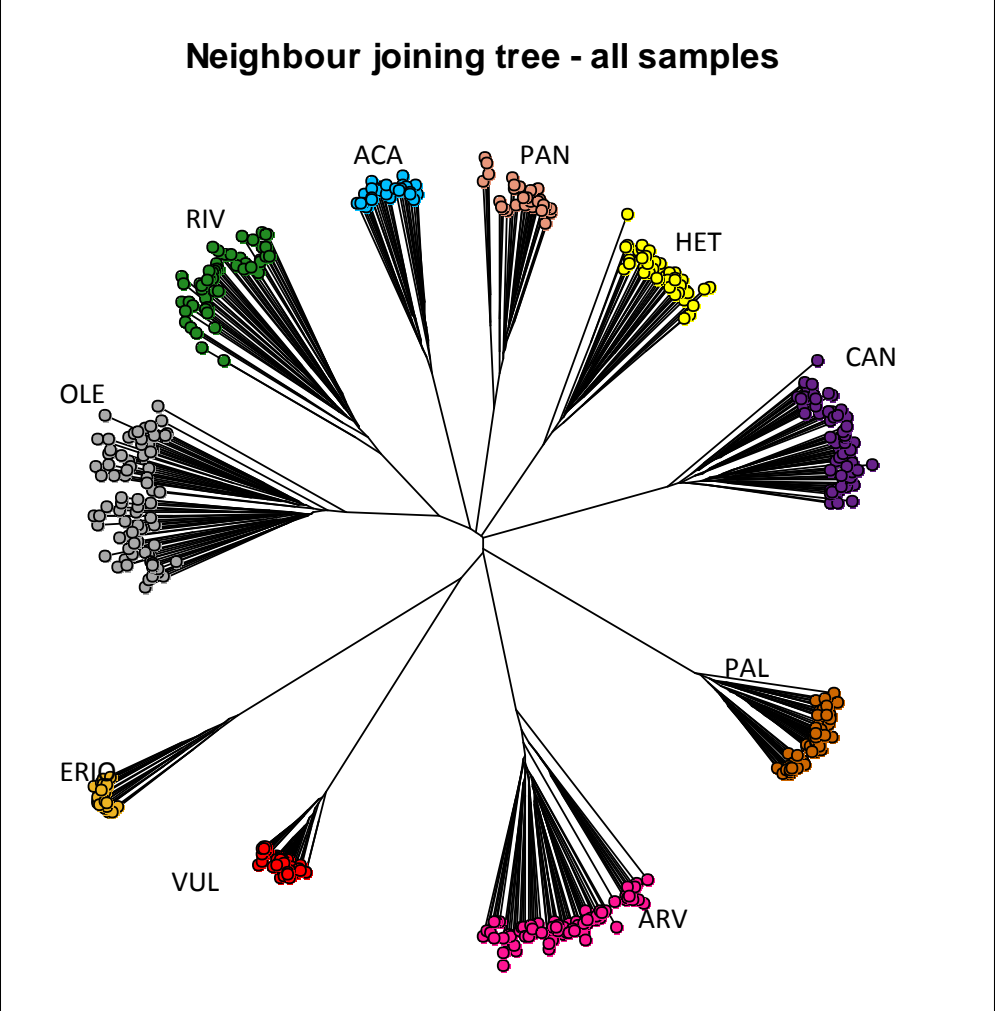
Fig. D2 : Neighbour joining tree clustering analysis of all samples (for legend see Fig. 2); (R3.2.3)
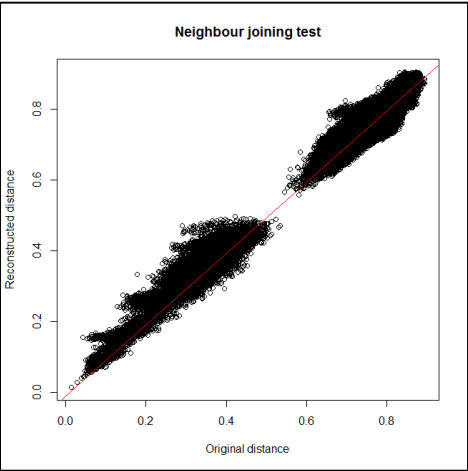
Fig. D3: NJ tree quality test

Species identity values according to STRUCTURE and our own script are visualised in **Fig. D4 – Fig. D13**. Both analyses were able to recognise membership of an individual to species. STRUCTURE analysis approved to show better results than our script. There is always one single peak indicating the species to which individuals belong. The profile of all individuals within one species is almost identical so the overlaps seem to look like one single line. The results of our script are slightly worse. The individuals do not form one perfect line as there is in case of STRUCTURE. On the other hand they are still copying the same pattern and the line shapes show the same trend. The script, however cannot completely get rid of the phylogenetic background. Individuals belonging to species, that are having very close relatives (most visible in *C. oleraceum* and *C. rivulare*), show another peak tendency on position of the close relative (see **Fig. D9** and **Fig. D12**). E. g., samples of *C. oleraceum* form a large peak at *C. oleraceum* position, but there is also a remarkable increase at *C. rivulare* position. This trend is not given by the fact that all of our sampled individuals were backcross hybrids of these two species. This trend can be also seen in *C. eriophorum* and *C. vulgare* (see **Fig. D7** and **Fig. D13**) that belong to the same section and are relatives. These two species never hybridize so the risk of having all of our samples as backcross hybrids is unconceivable. The only species that does not show almost any phylogenetic background is *C. arvense* (**Fig. D5**), the only member of section *Cephalonoplos*. Very little phylogenetic background is also observed in *C. canum* (**Fig. D6**) and *C. palustre* that are species with no close relative.
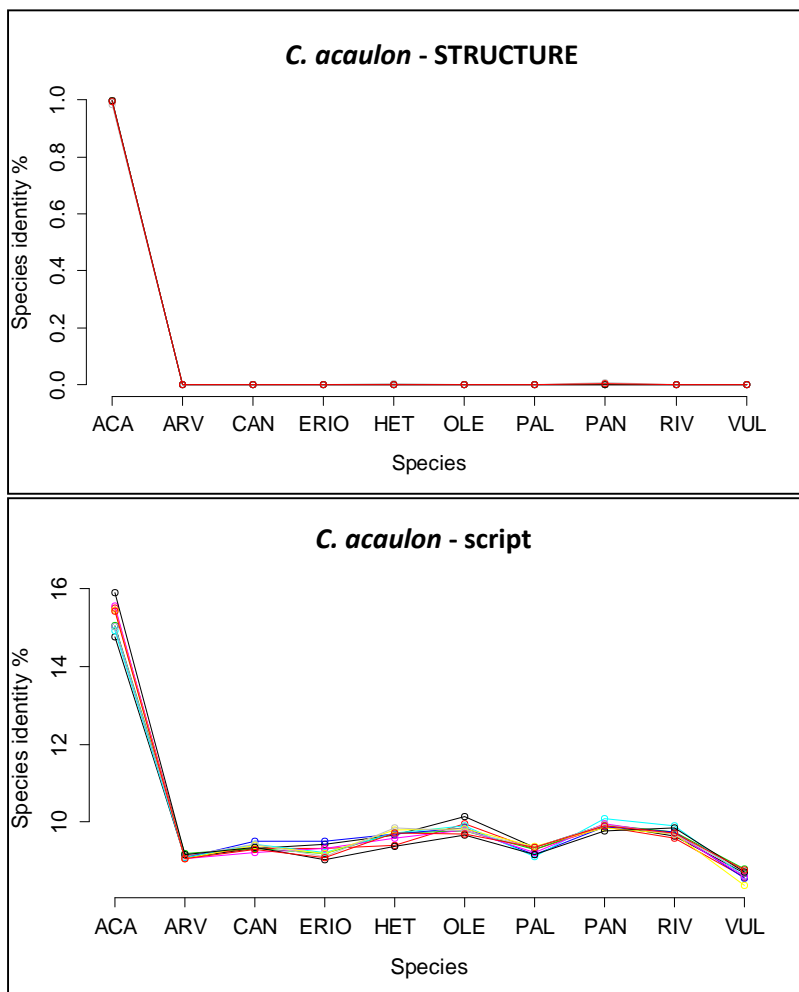
Fig. D4: Species identity calculated for samples of *C. acaulon* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
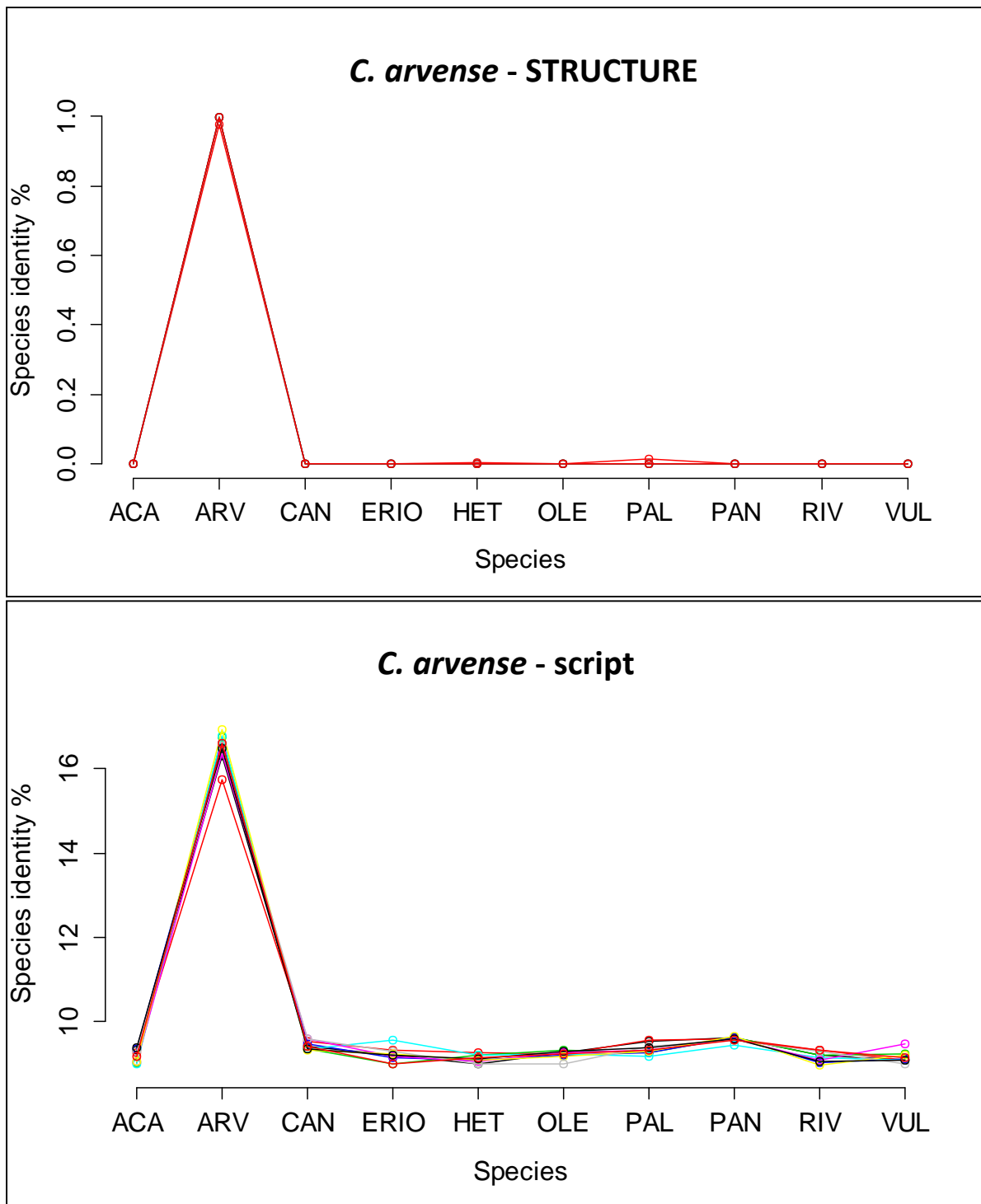
Fig. D5: Species identity calculated for samples of *C. arvense,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
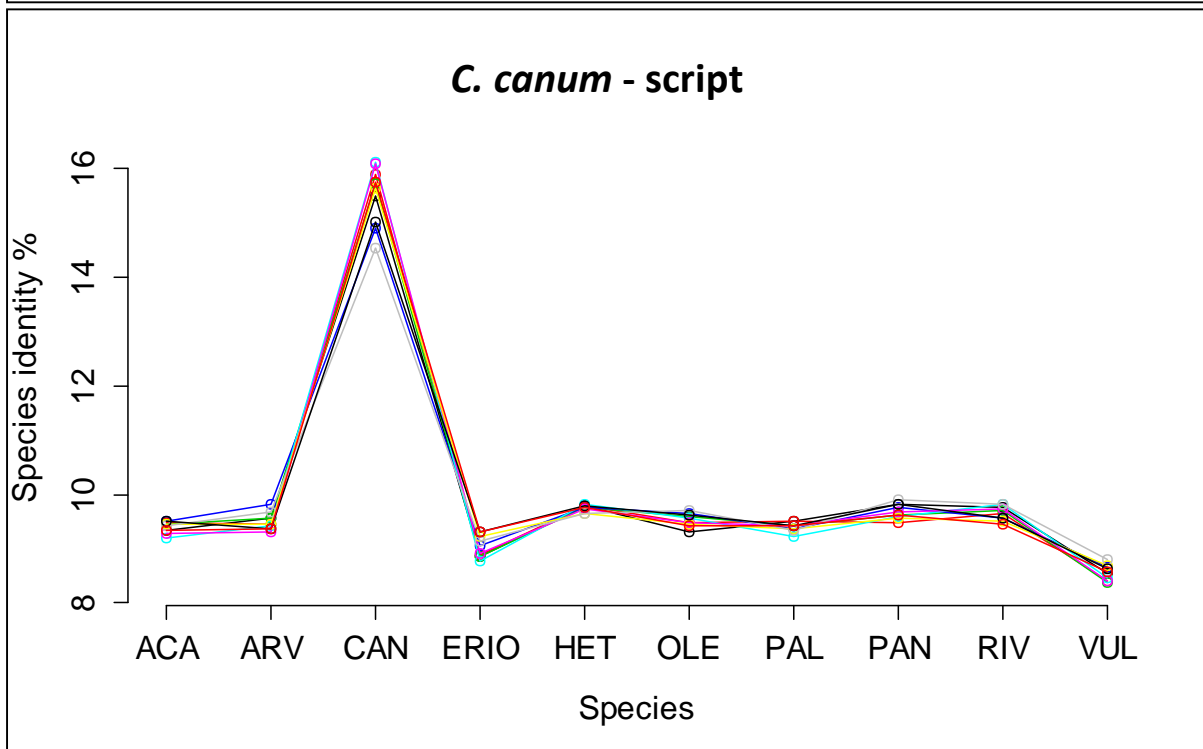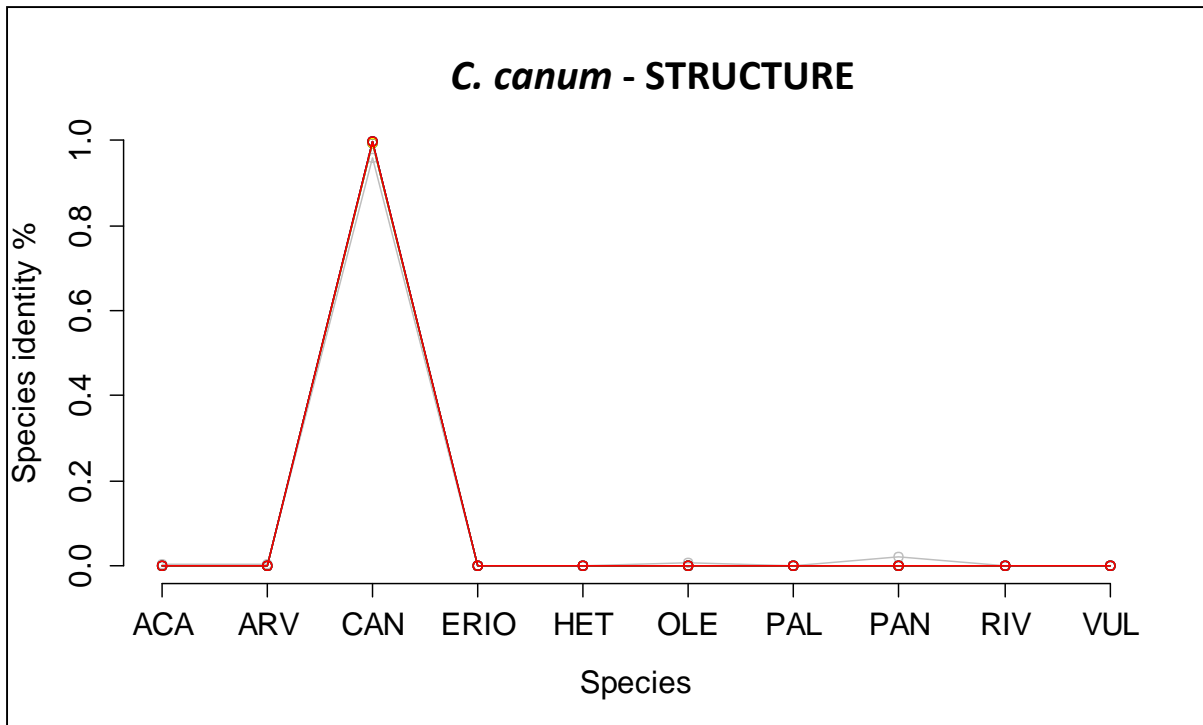
Fig. D6: Species identity calculated for samples of *C. canum,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
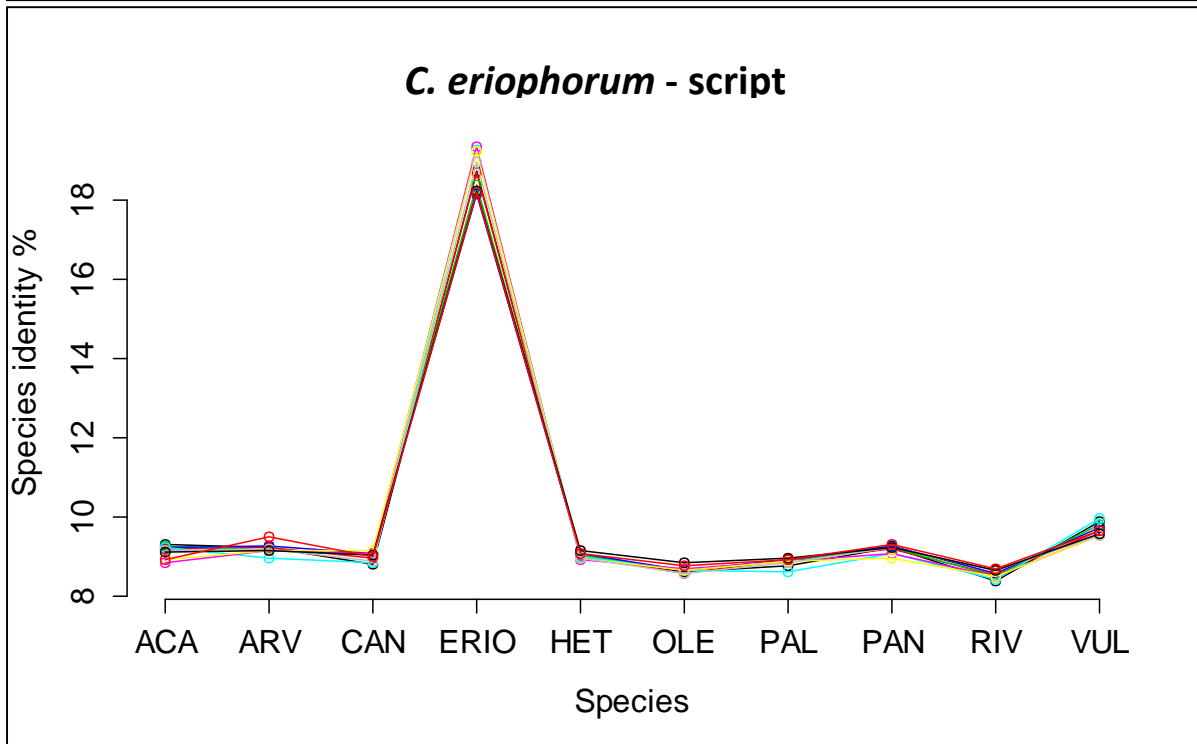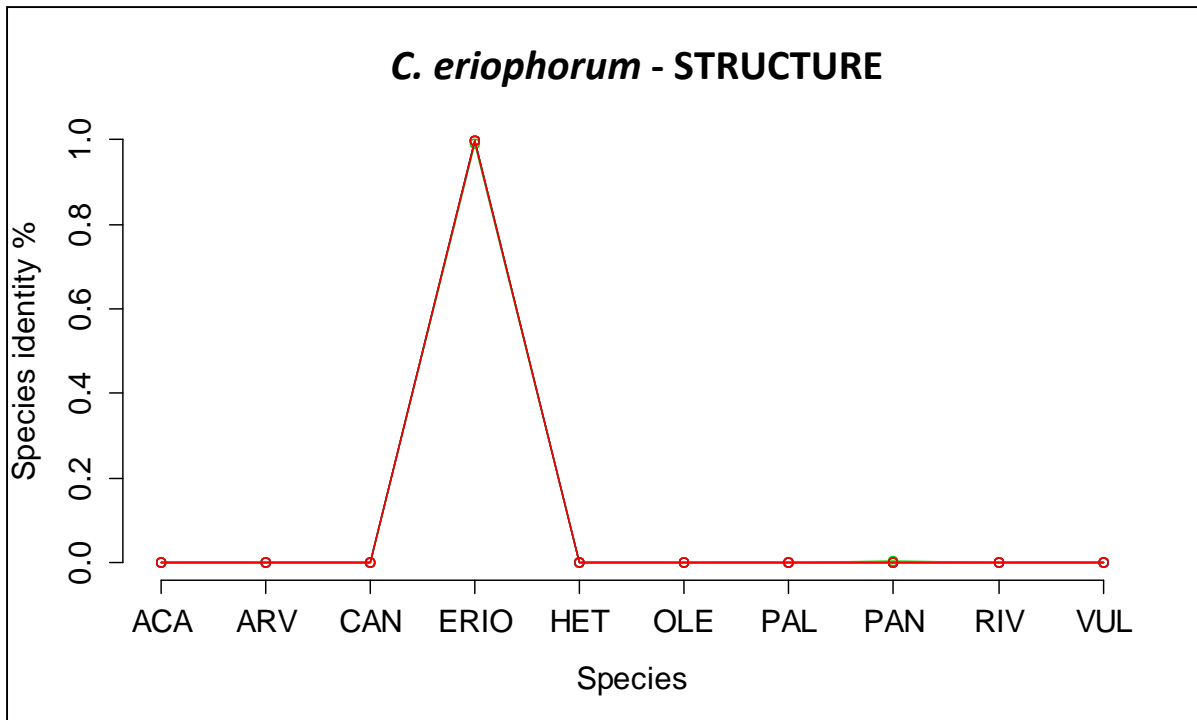
Fig. D7: Species identity calculated for samples of *C. eriophorum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
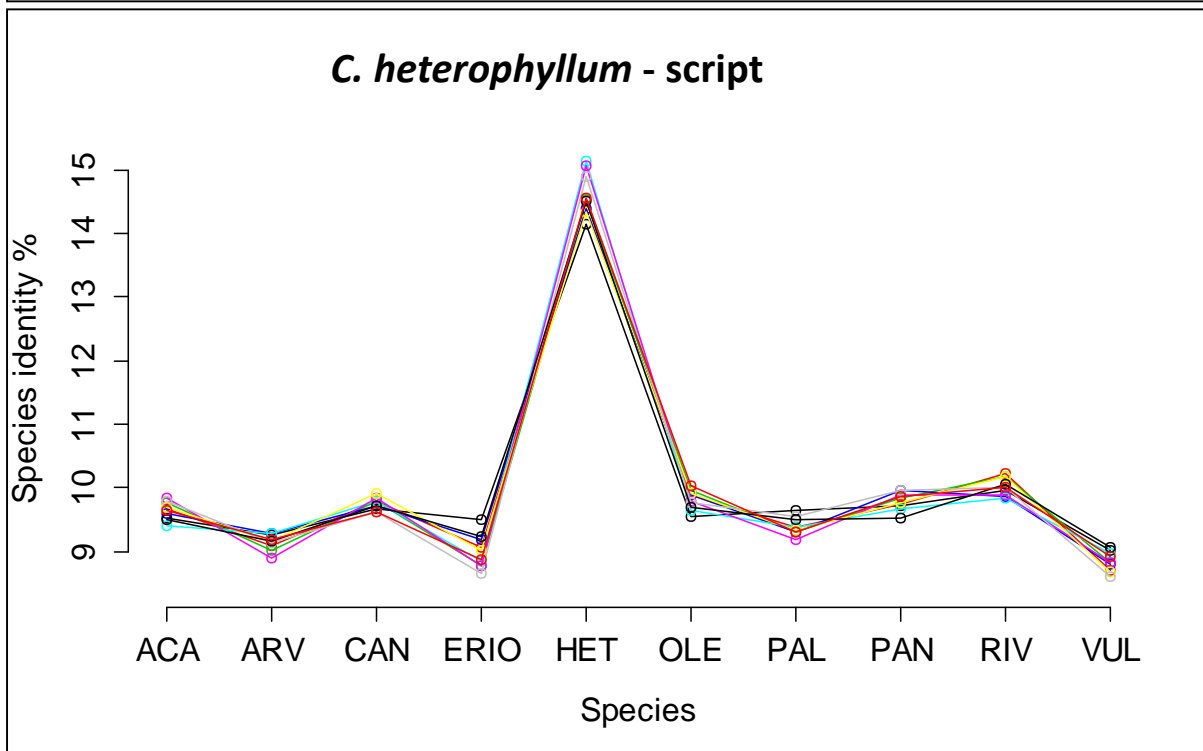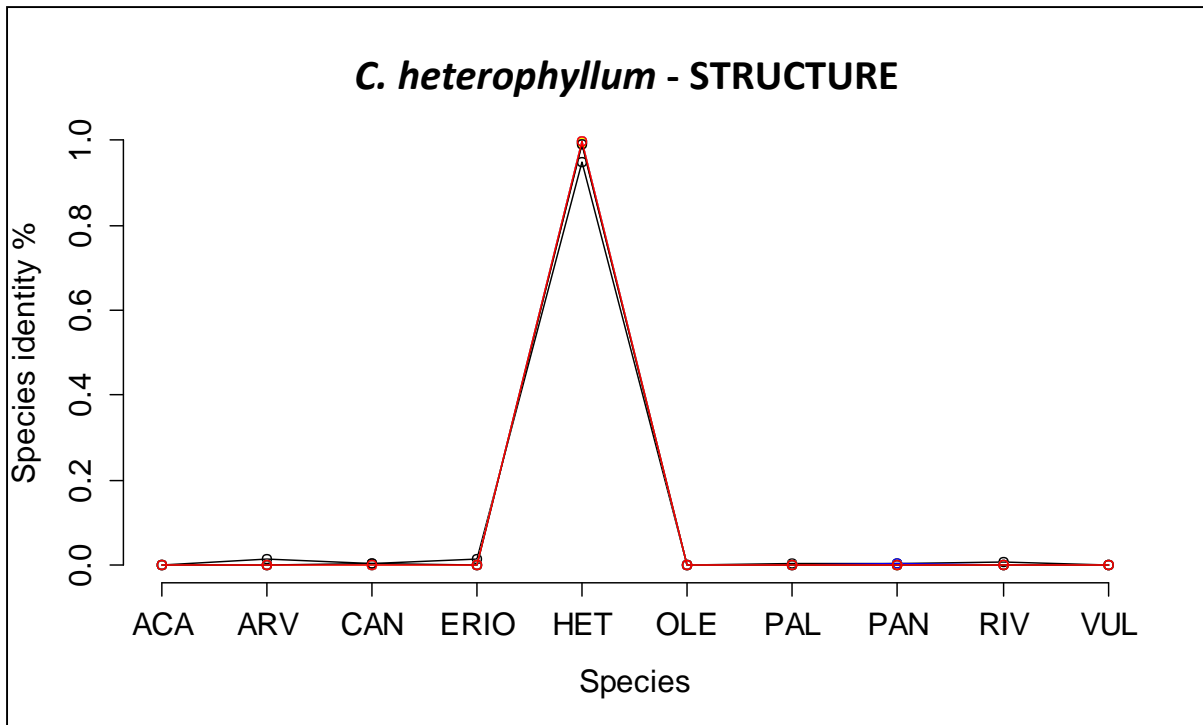
Fig. D8: Species identity calculated for samples of *C. heterophyllum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
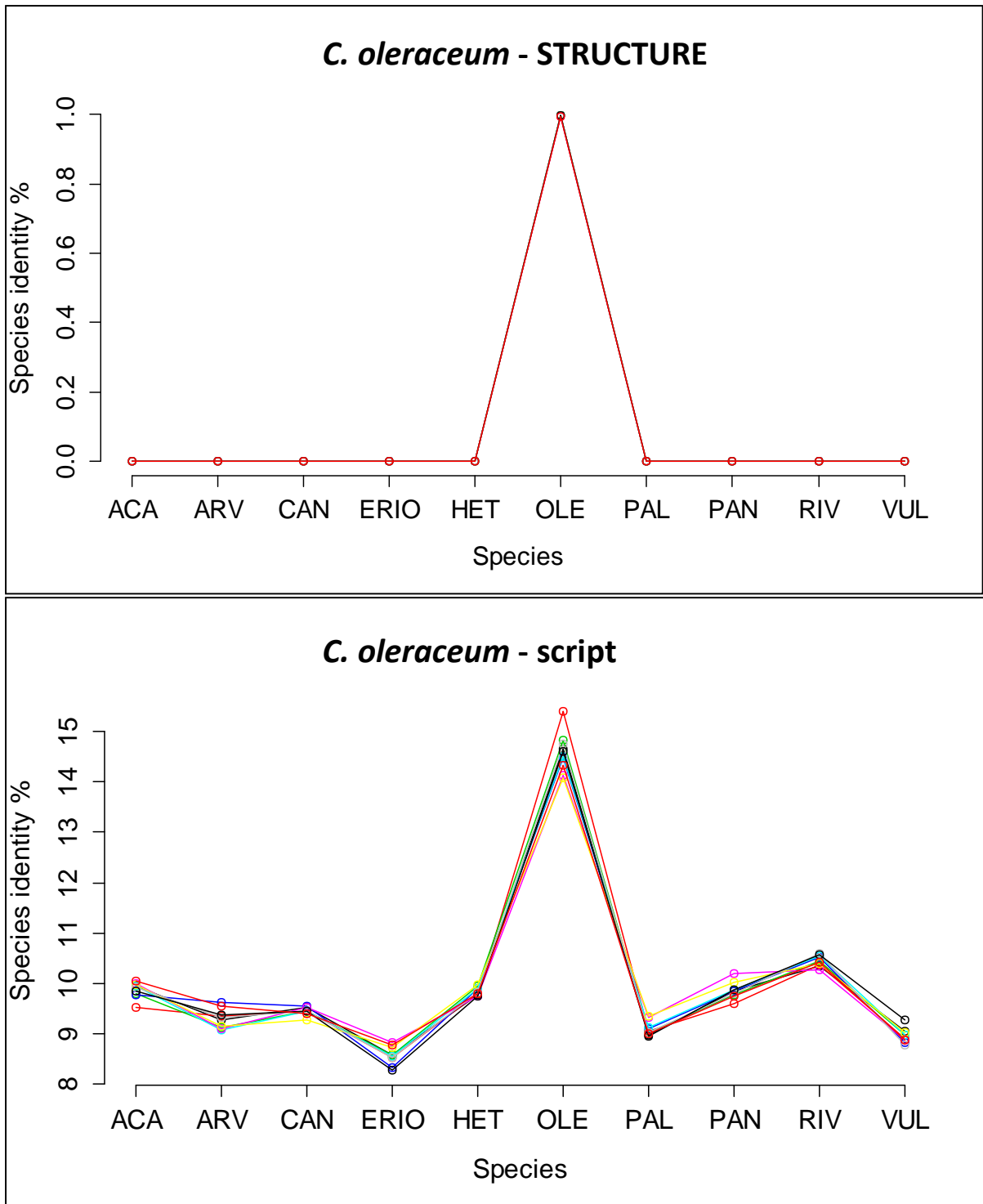
Fig. D9: Species identity calculated for samples of *C. oleraceum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
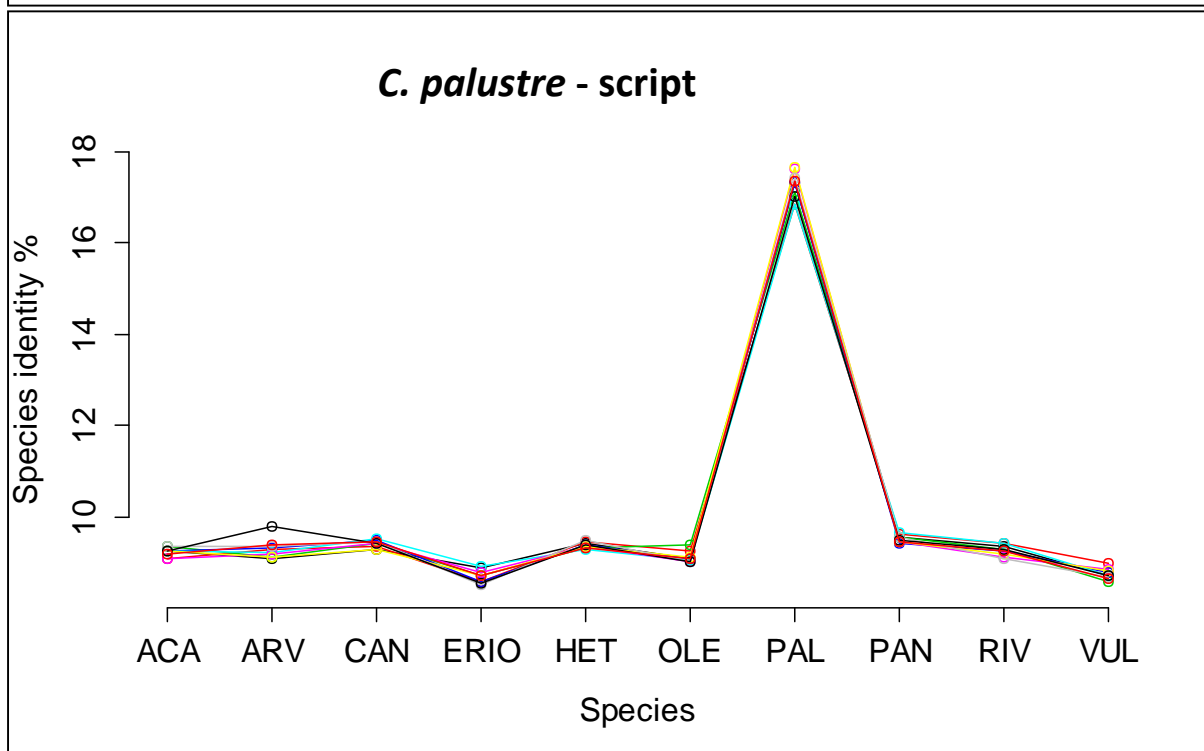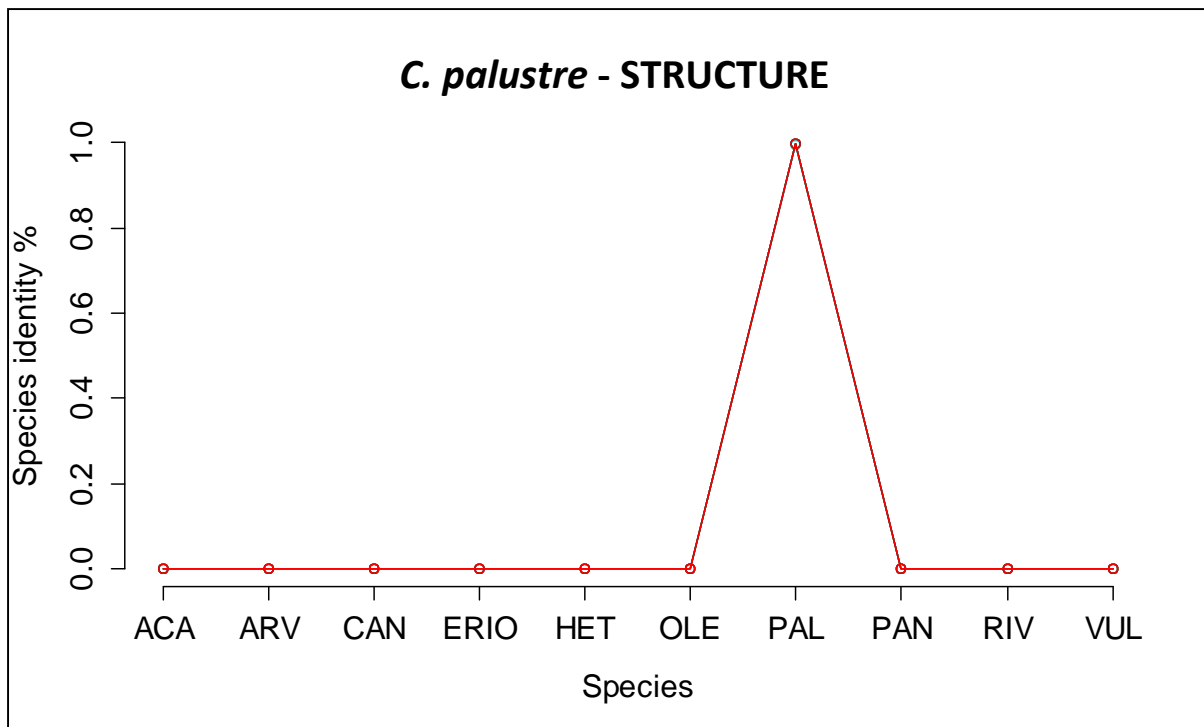
Fig. D10: Species identity calculated for samples of *C. palustre*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
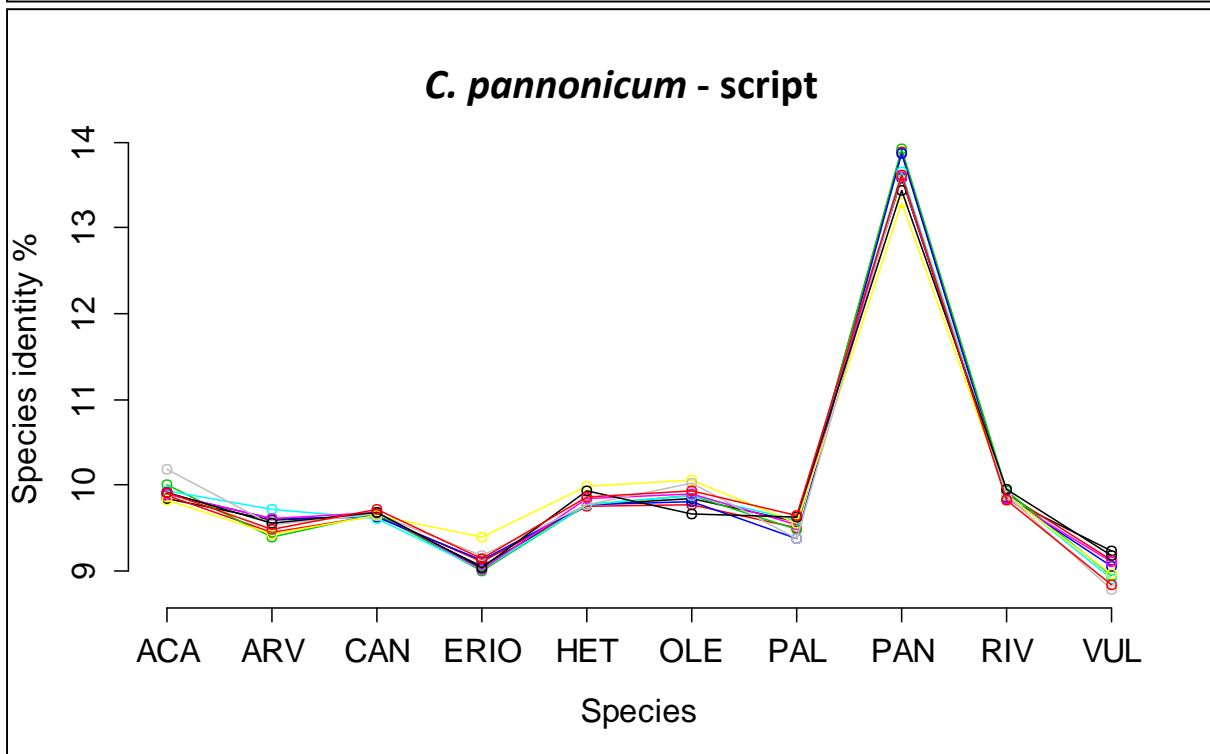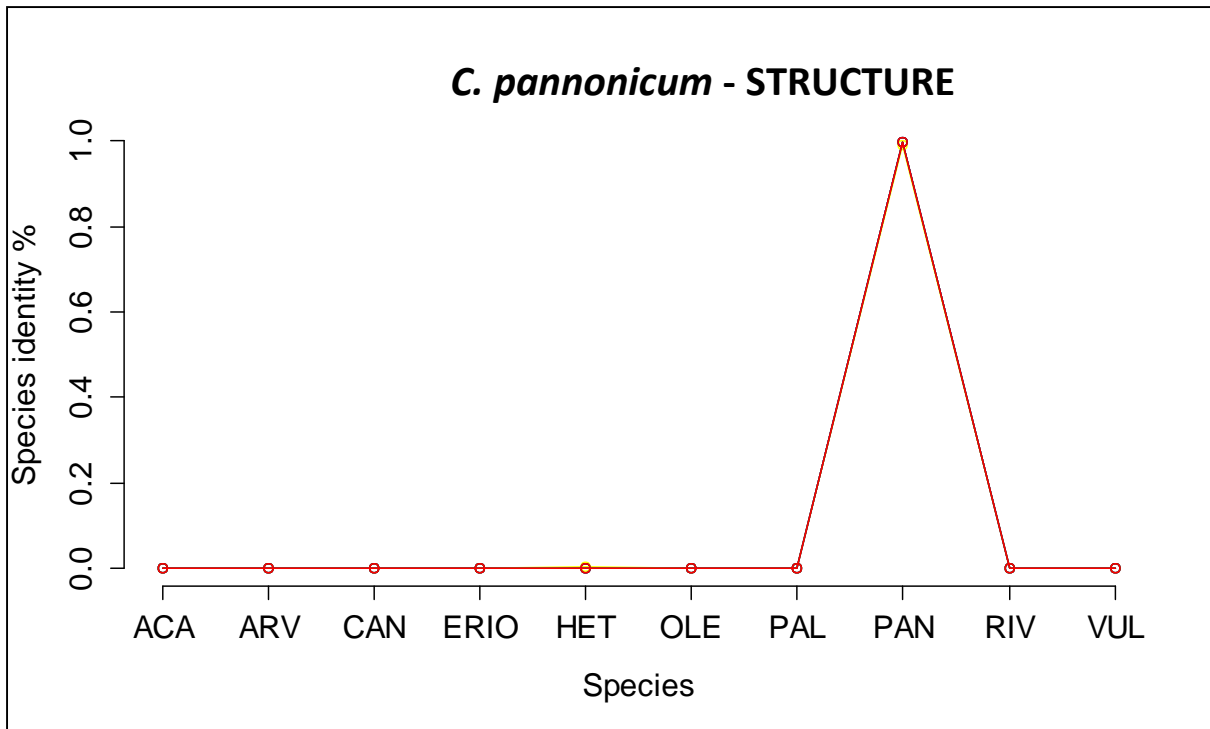
Fig. D11: Species identity calculated for samples of *C. pannonicum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
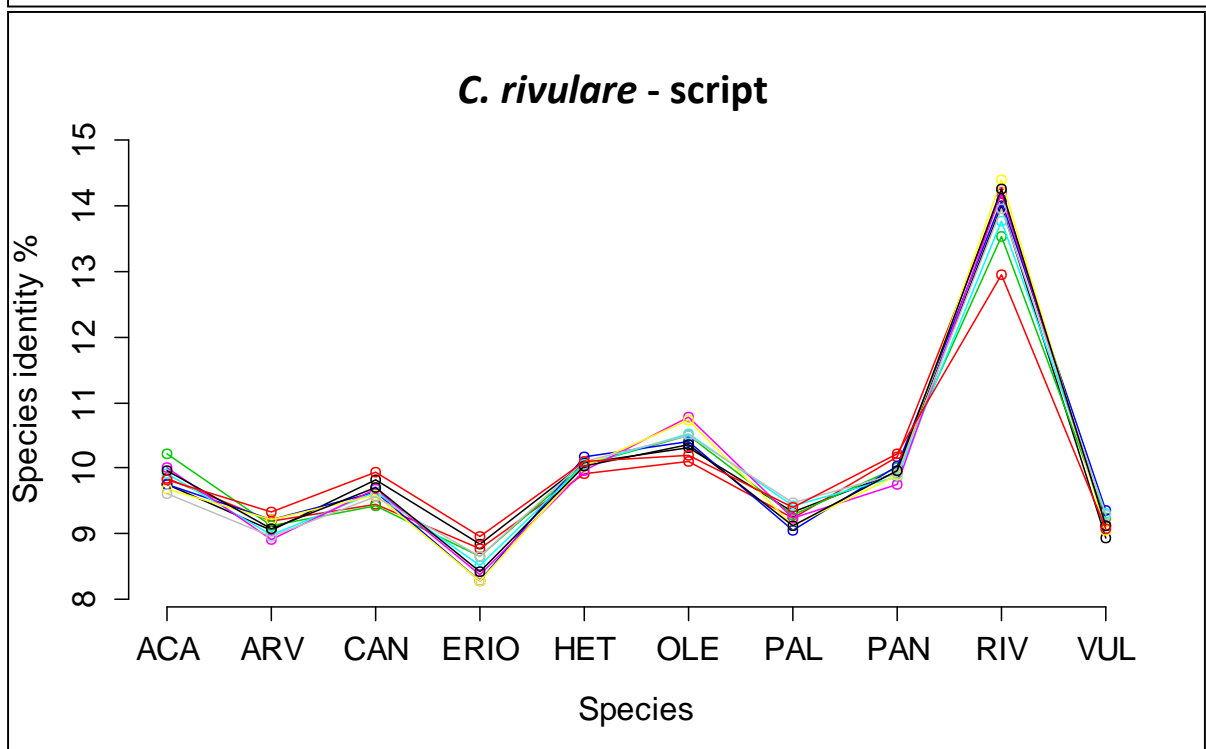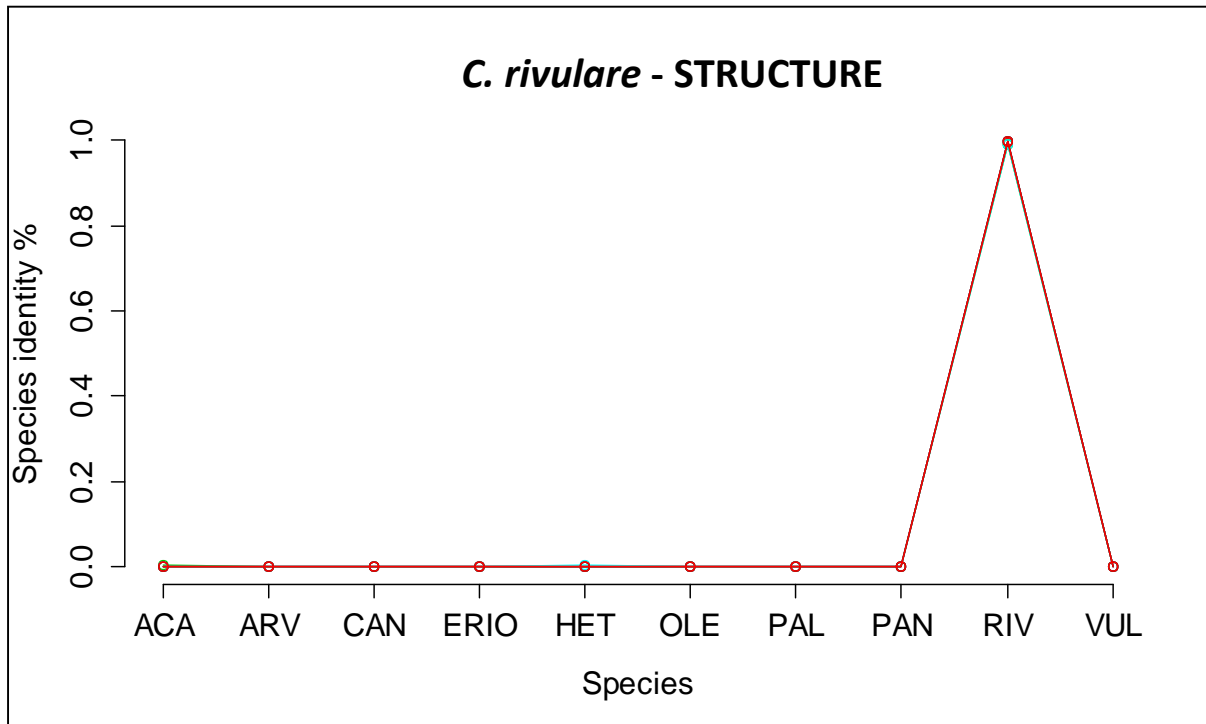
Fig. D12: Species identity calculated for samples of *C. rivulare*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
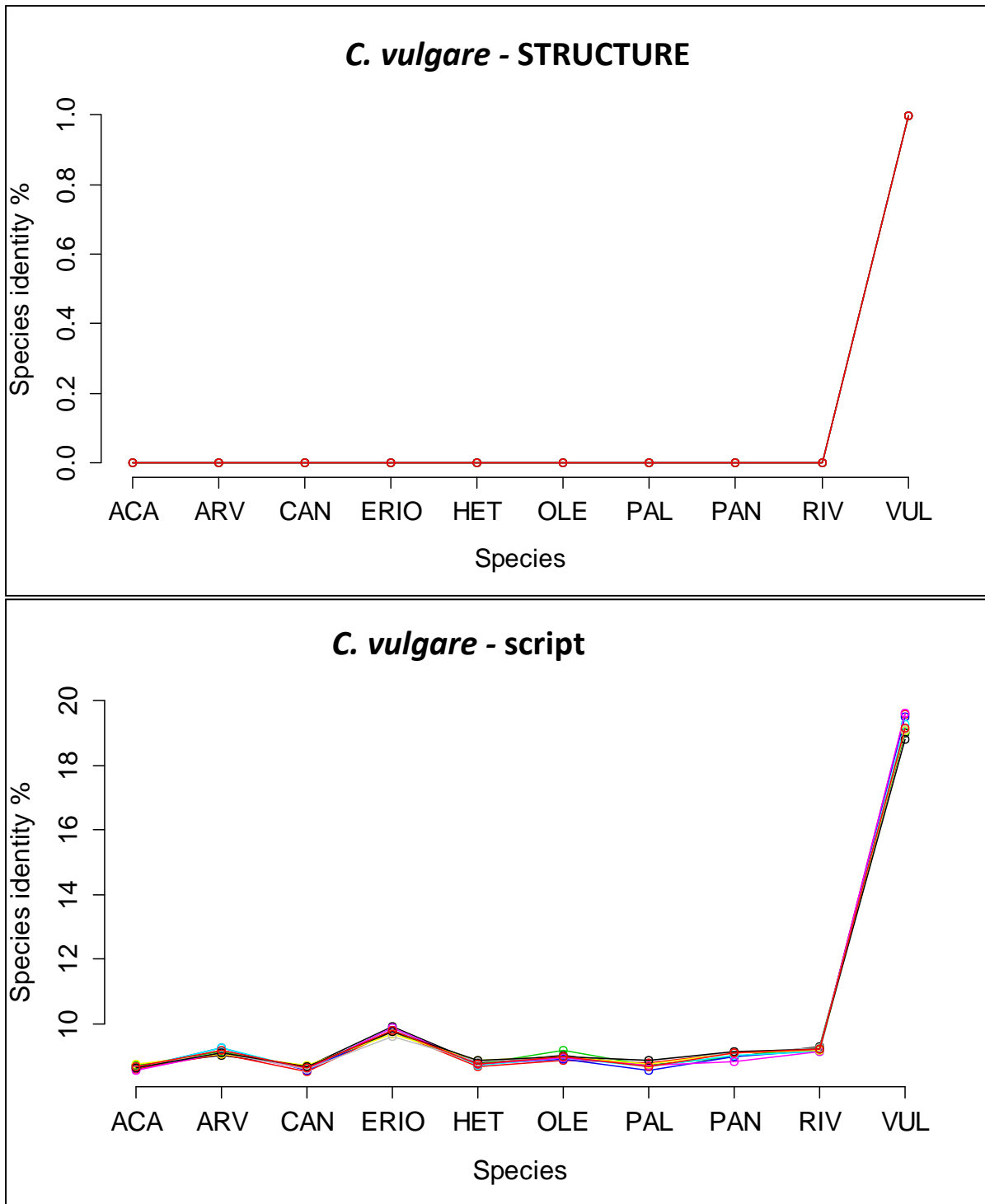
Fig. D13: Species identity calculated for samples of *C. vulgare*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)

2. Hybrids – identification of parental species

Species identity values according to STRUCTURE and our own script for hybrids are visualised in **Fig. D14 – Fig. D27**. This time STRUCTURE analysis showed much worse results comparing to our own script. Although there was again no phylogenetic background and the analysis showed clear peaks, the STRUCTURE analysis recognised correctly only part of the individuals. The species that were mistaken for parents were often even unrelated to real parents (e. g., in **Fig. D17** for one of samples of *C. canum × C. palustre* STRUCTURE found *C. oleraceum* as a putative parent and for another sample of the same hybrid it was *C. rivulare.* These species are not related to *C. canum* nor to *C. palustre.*

Our script, however, performed decent resolution of parental species. There were only 8 samples that were determined differently than we determined them during sampling (see **Discussion**). There is a phylogenetic background as there was also in the case of morphologically pure individuals (especially for hybrids of *C. oleraceum*, where there is a peak in position of *C. rivulare*, which makes them look like triple hybrids e. g., **Fig. D15 –** *C. acaulon × C. oleraceum*), but the line shapes show consistent trend – increasing steeply in the position of parental and phylogenetically related species within this hybrid. It was necessary to set a threshold specifying until which is the increased percentage just a result of phylogenetic background and from which it can be assumed as a parental species. After setting this threshold to 11 % we obtained 14 hybrid combinations where only two parental species were present. There was one case in which a third species exceeded the critical point. This happened in sample ACA_x_PAN_K20b. This sample was collected as a hybrid of *C. acaulon* and *C. pannonicum*, but after performing the analyses we could observe a significant percentage of *C. canum* present in this species (see **Fig. D28**). This sample is evidently a triple hybrid and was recognised as triple hybrid even by STRUCTURE.
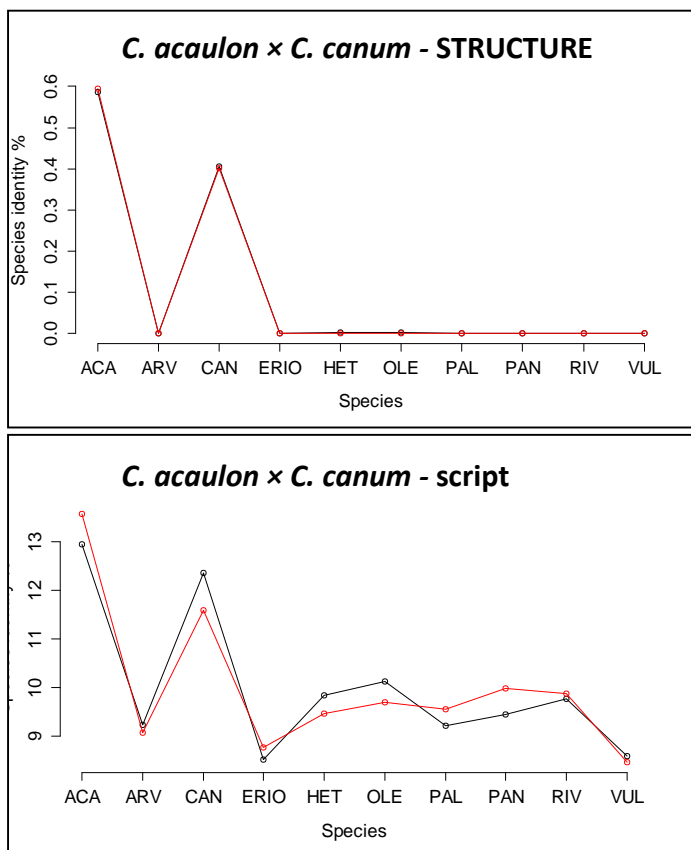
Fig. D14: Species identity calculated for hybrids of *C. acaulon* × *C. canum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
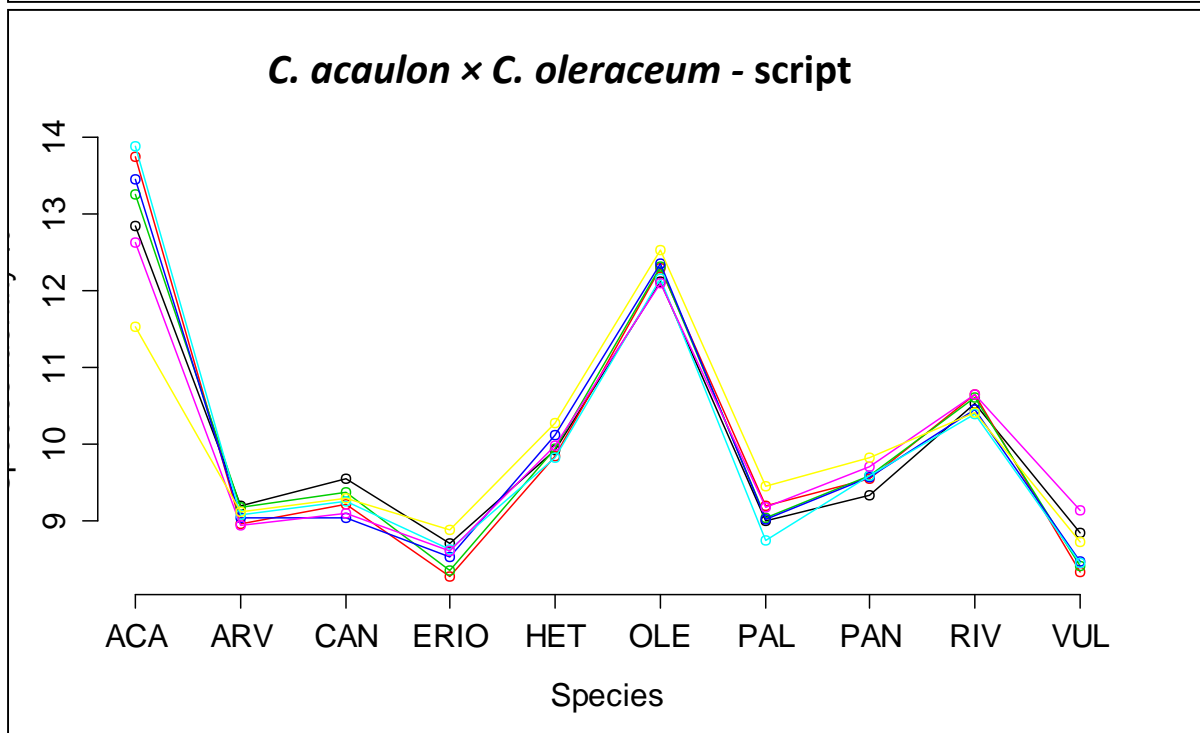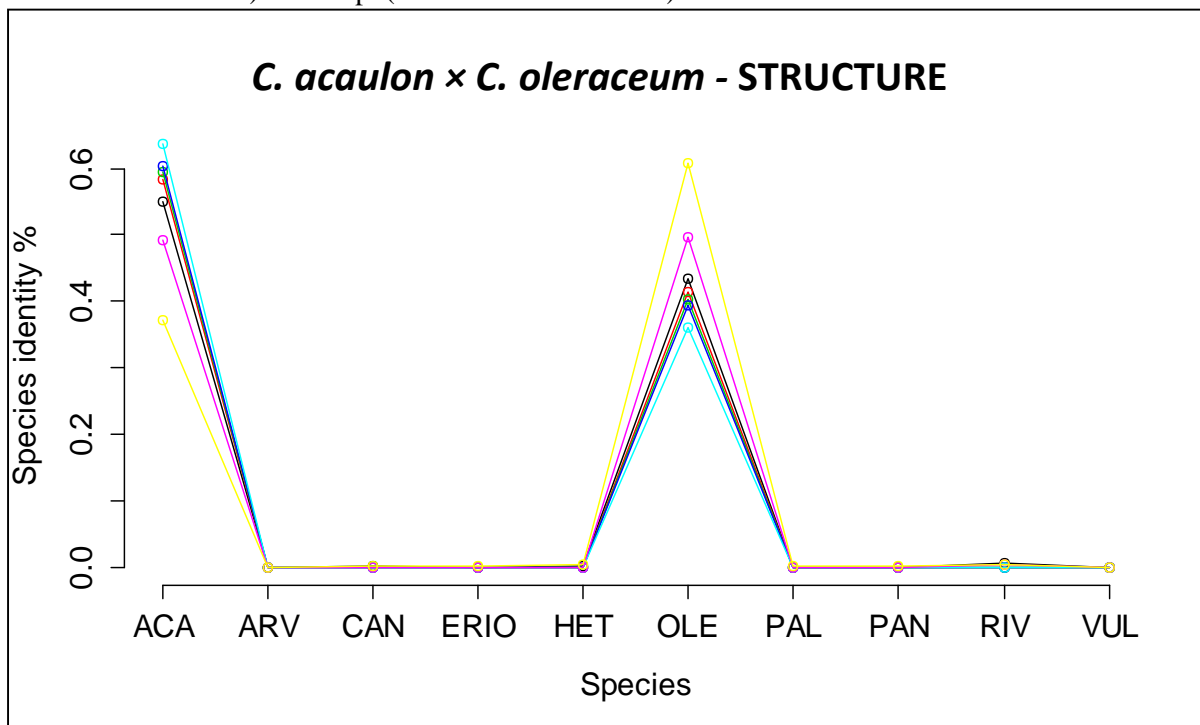
Fig. D15: Species identity calculated for hybrids of *C. acaulon* × *C. oleraceum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
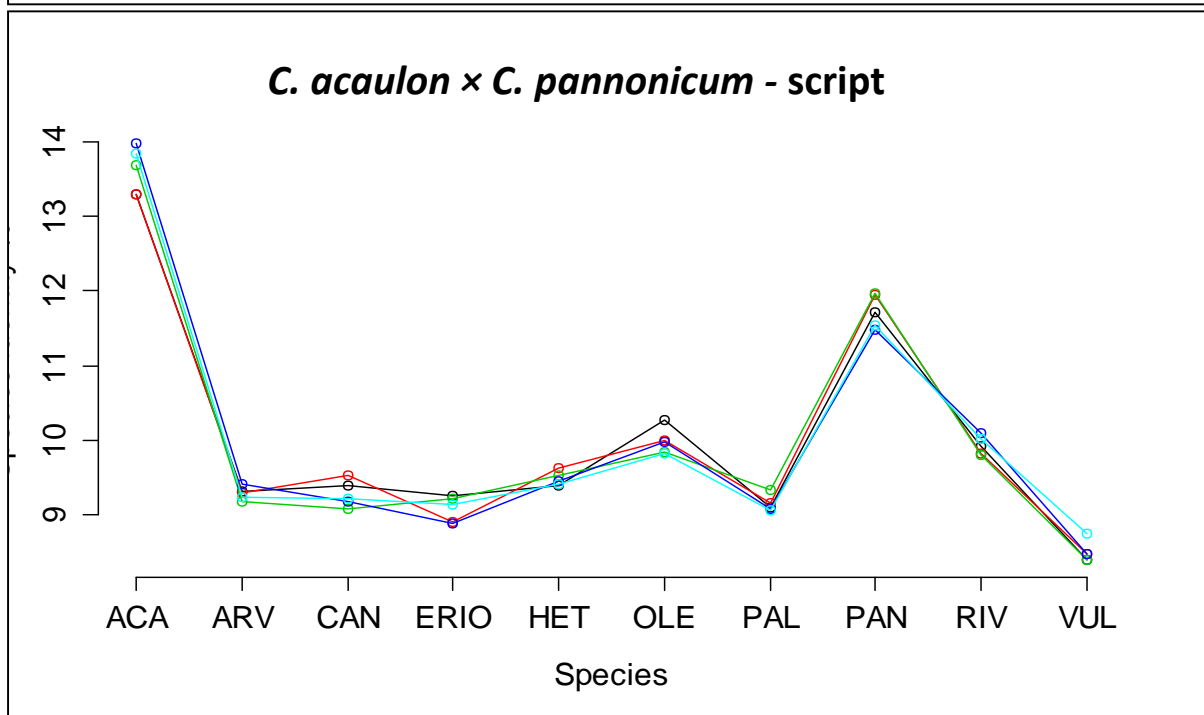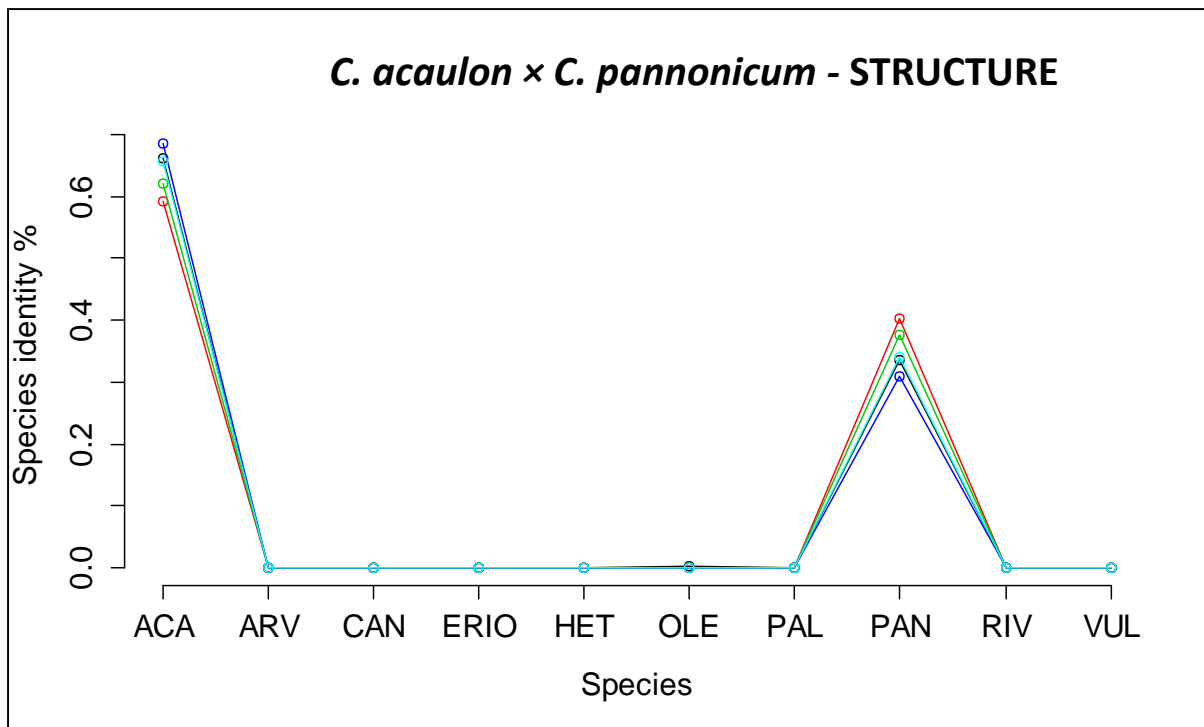
Fig. D16: Species identity calculated for hybrids of *C. acaulon* × *C. pannonicum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
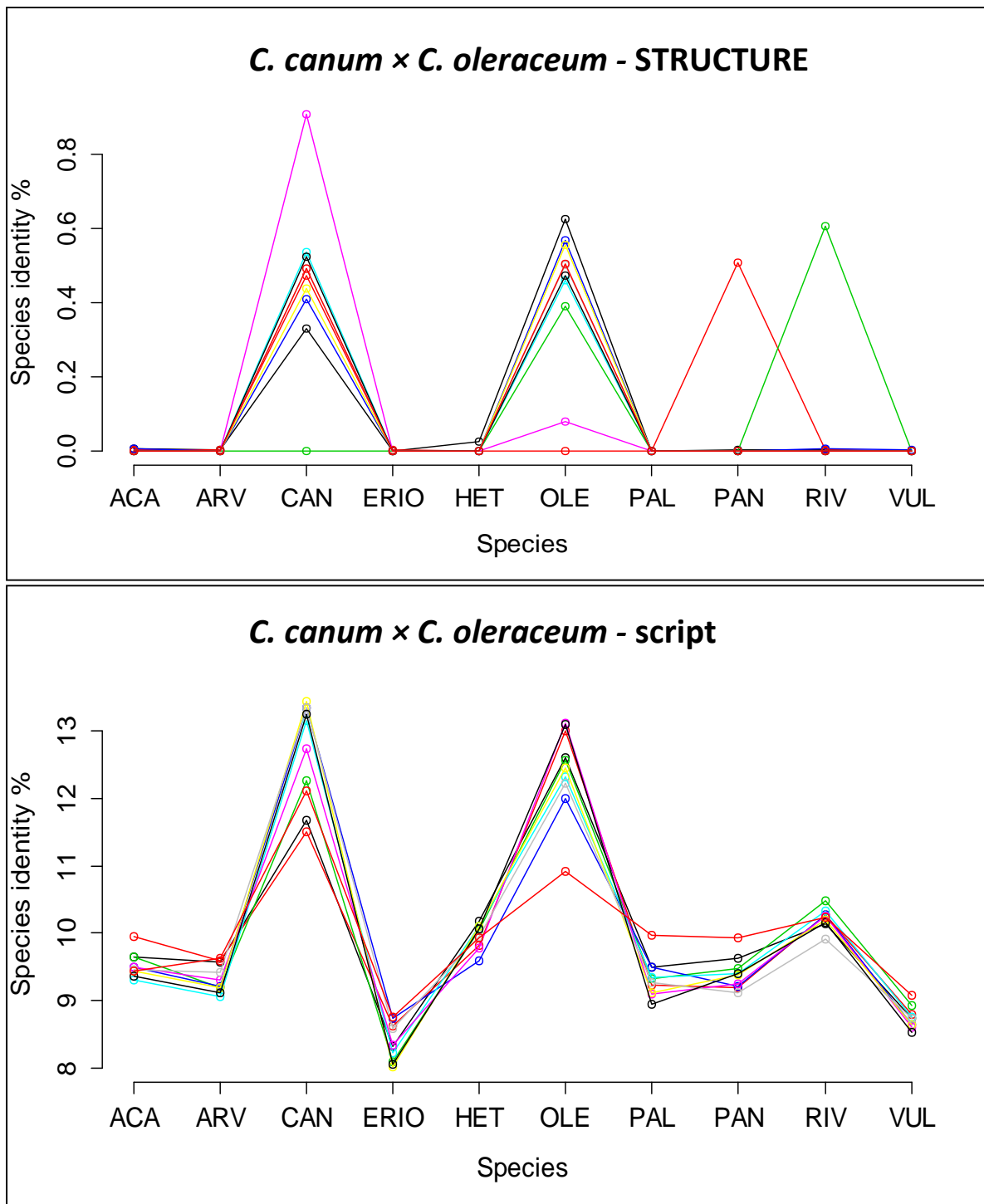
Fig. D17: Species identity calculated for hybrids of *C. canum × C. oleraceum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
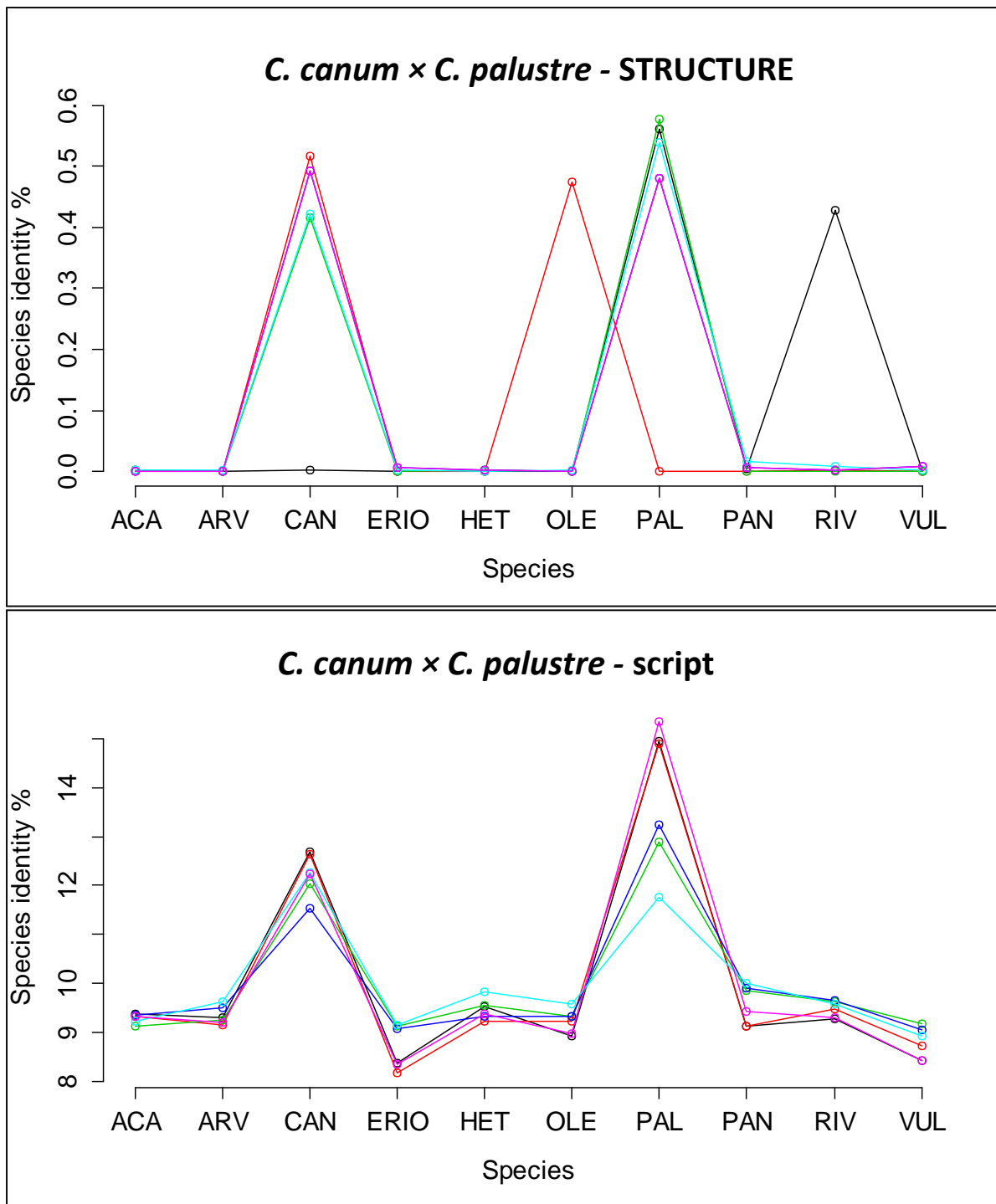
Fig. D18: Species identity calculated for hybrids of *C. canum × C. oleraceum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
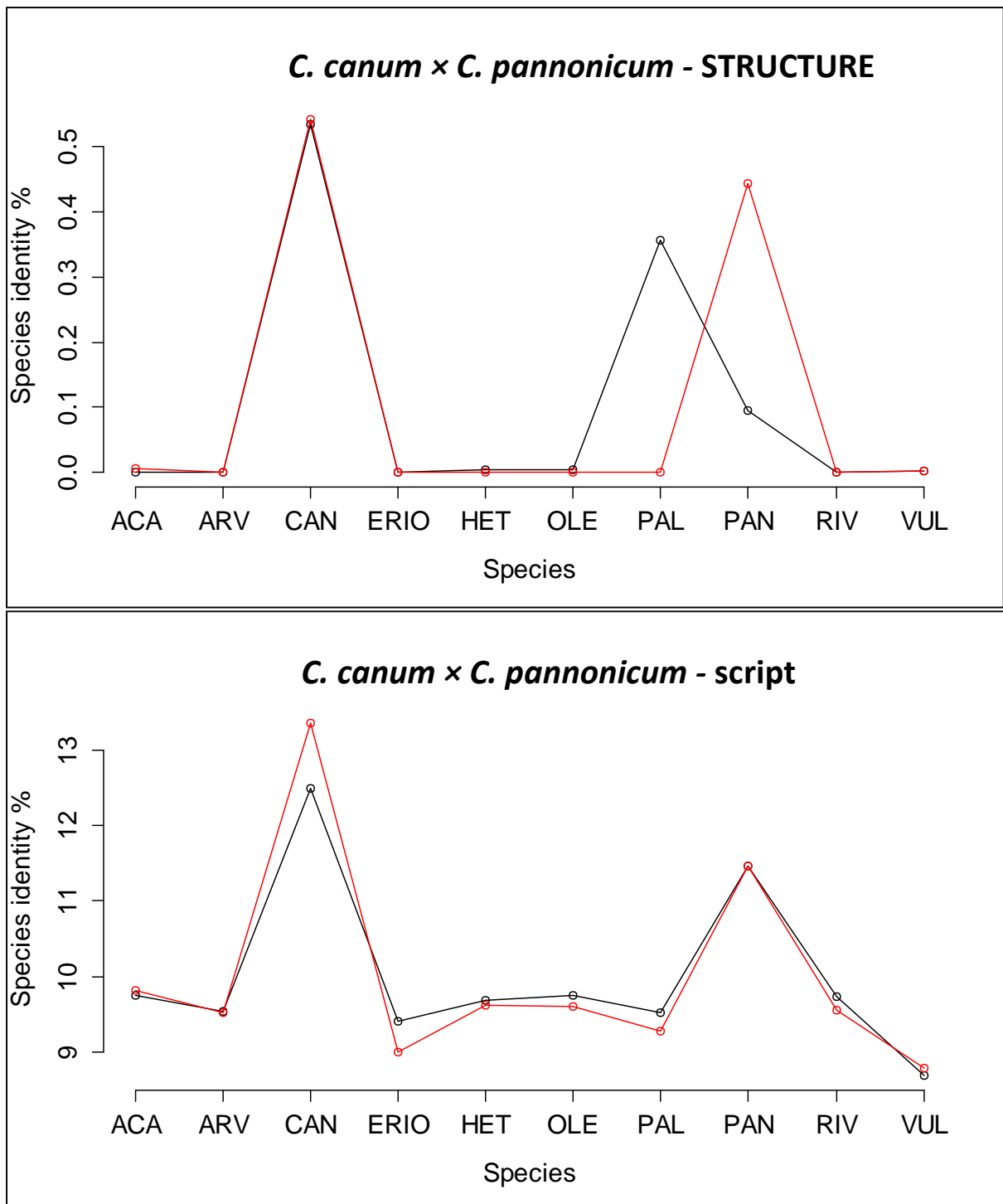
Fig. D19: Species identity calculated for hybrids of *C. canum × C. pannonicum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
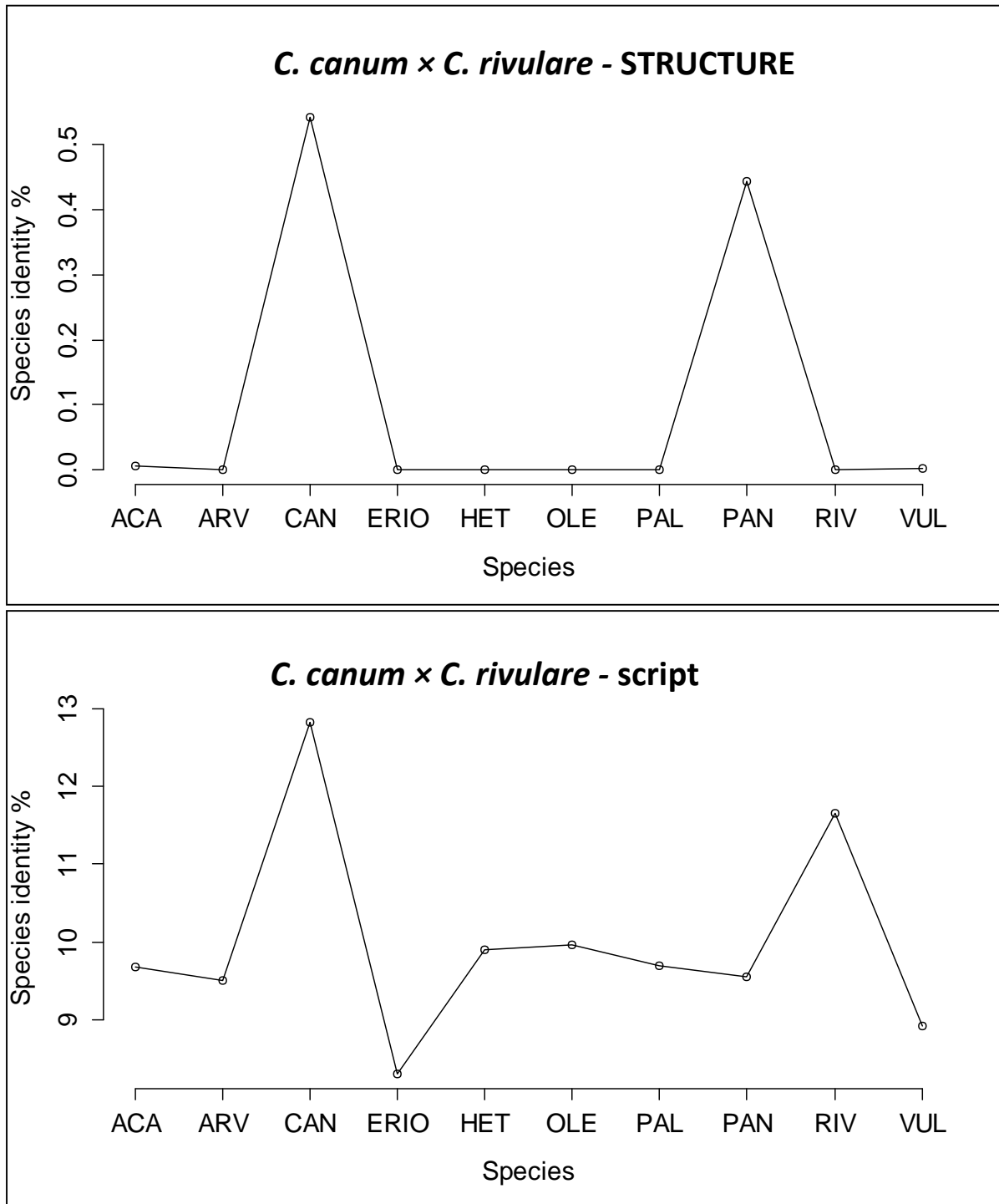
Fig. D20: Species identity calculated for hybrids of *C. canum × C. rivulare*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
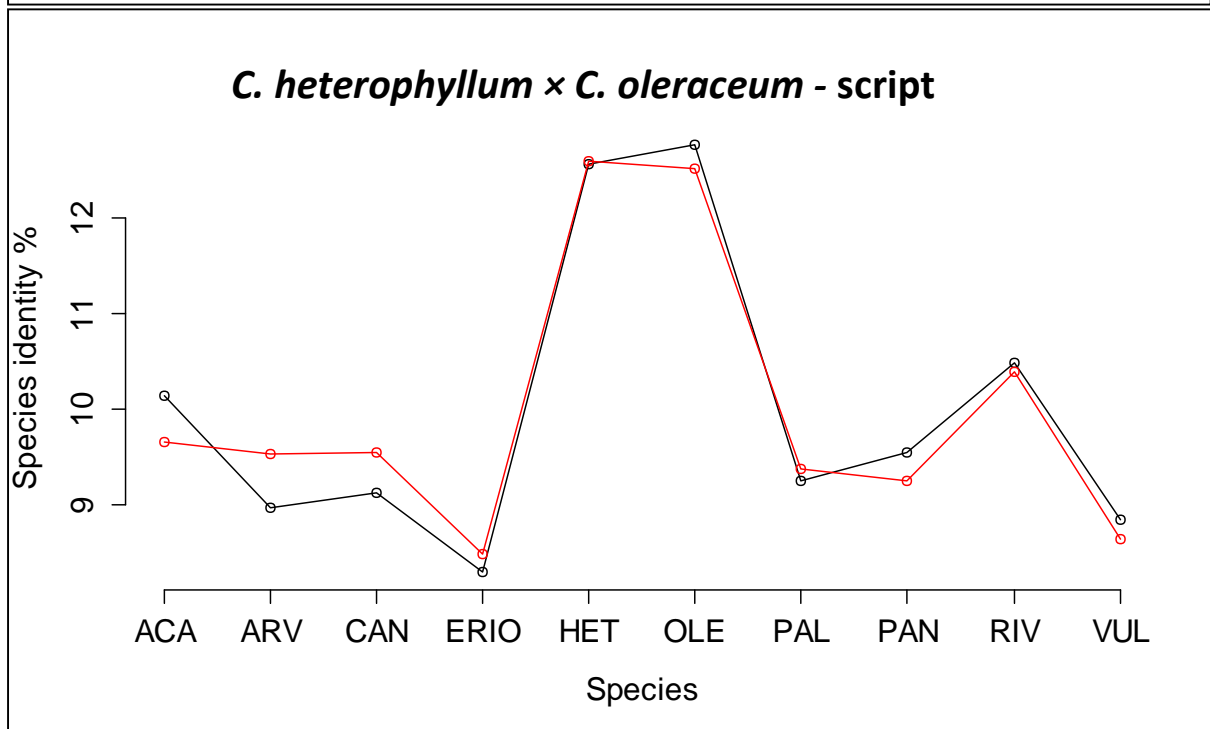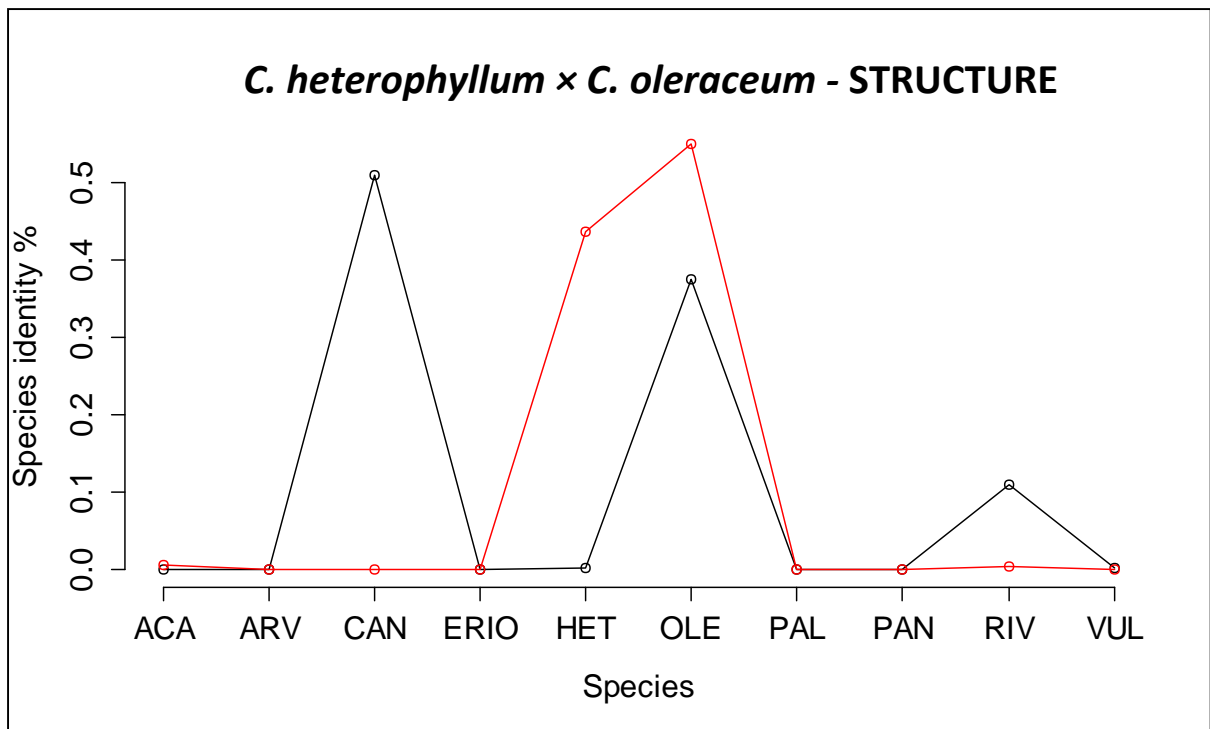
Fig. D21: Species identity calculated for hybrids of *C. heterophyllum × C. oleraceum,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
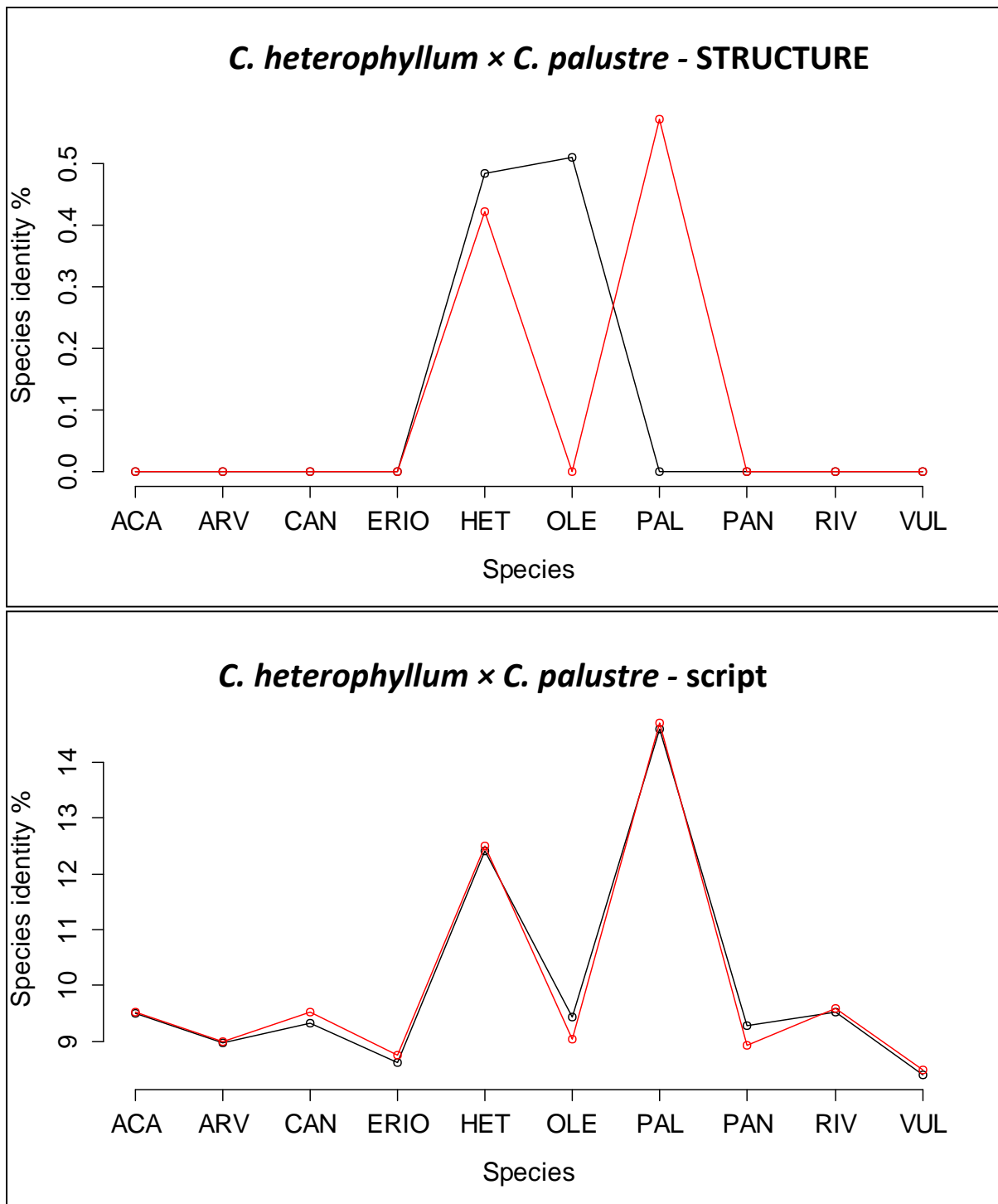
Fig. D22: Species identity calculated for hybrids of *C. heterophyllum × C. palustre,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
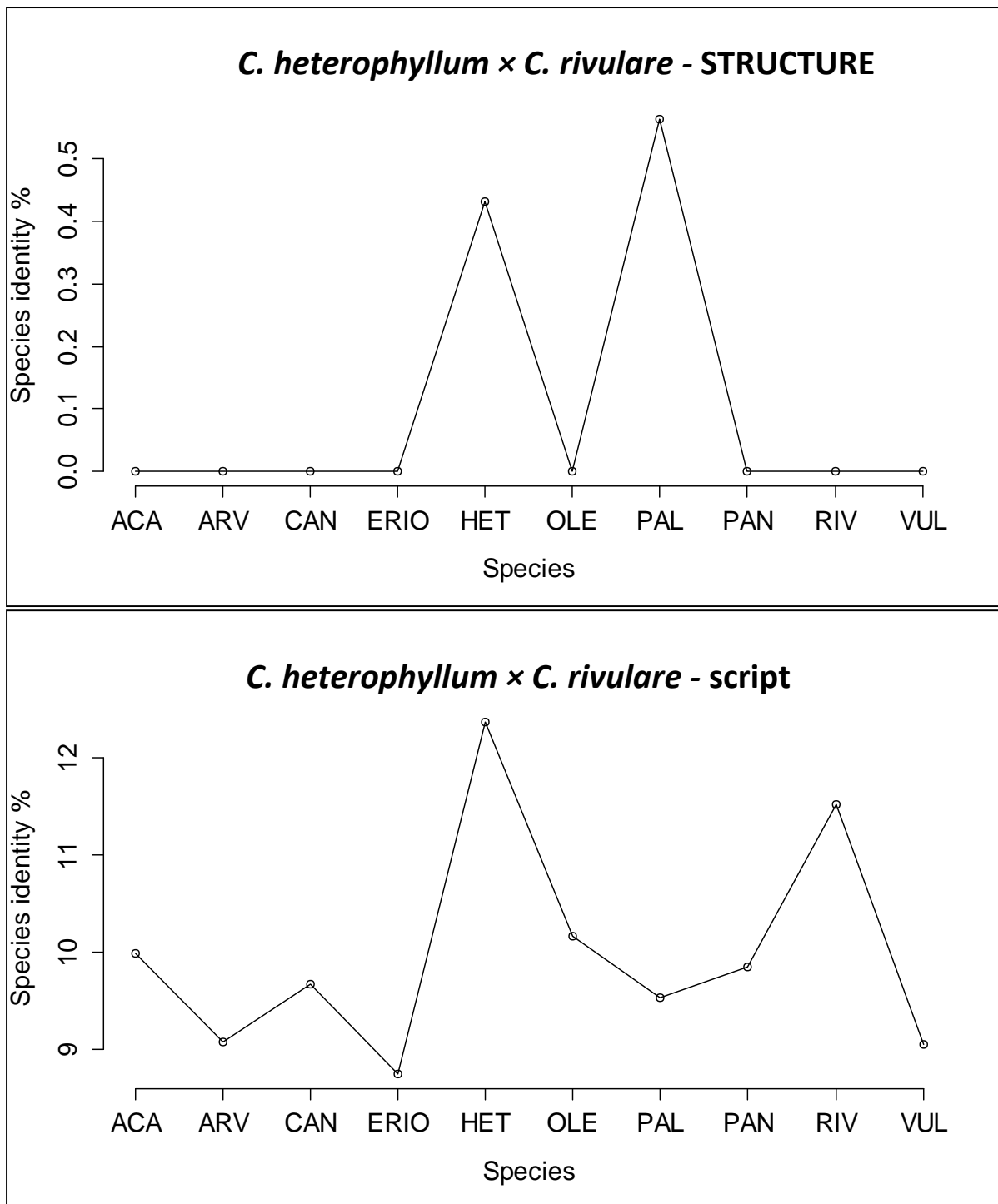
Fig. D23: Species identity calculated for hybrids of *C. heterophyllum × C. rivulare,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
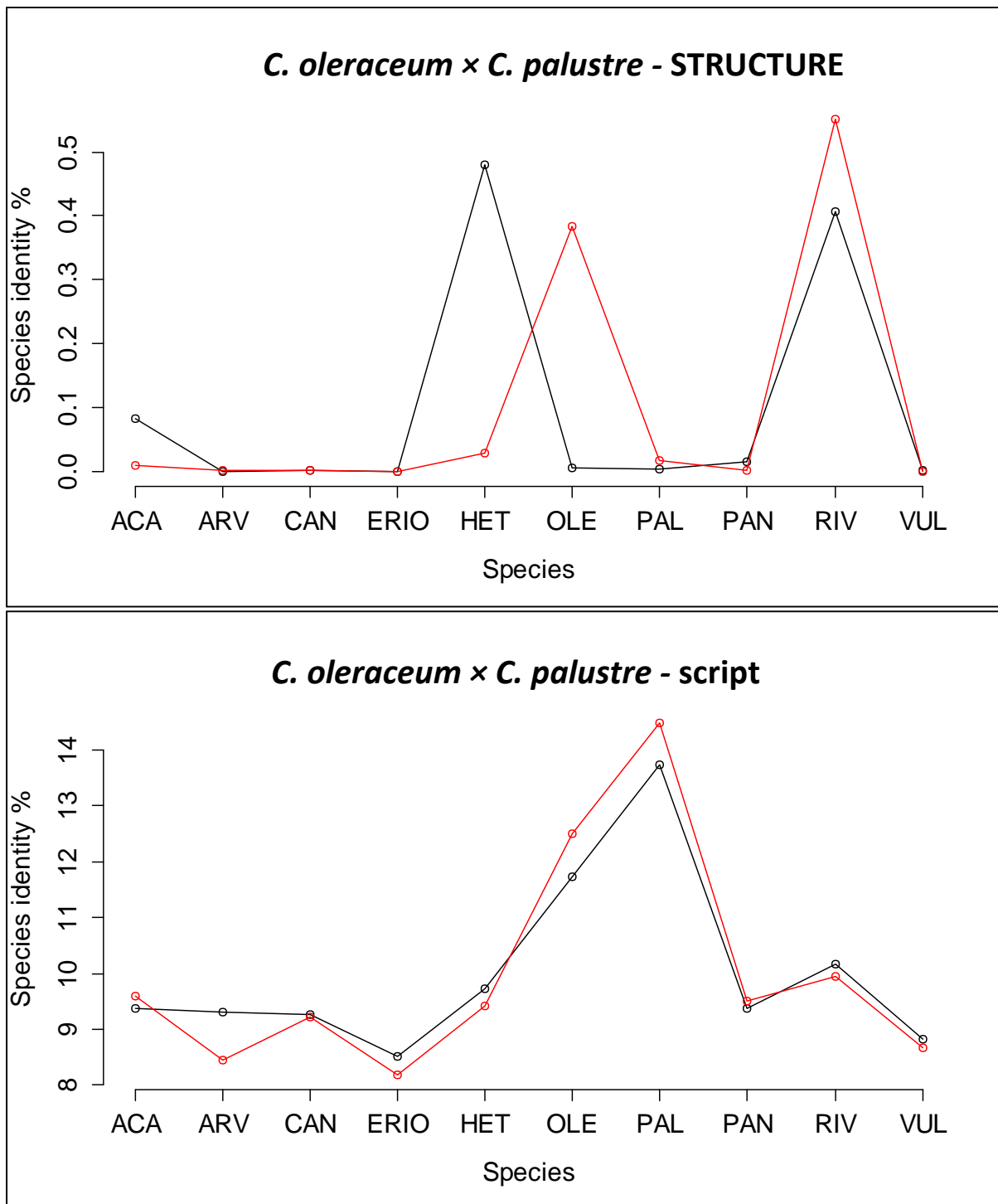
Fig. D24: Species identity calculated for hybrids of *C. oleraceum × C. palustre,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
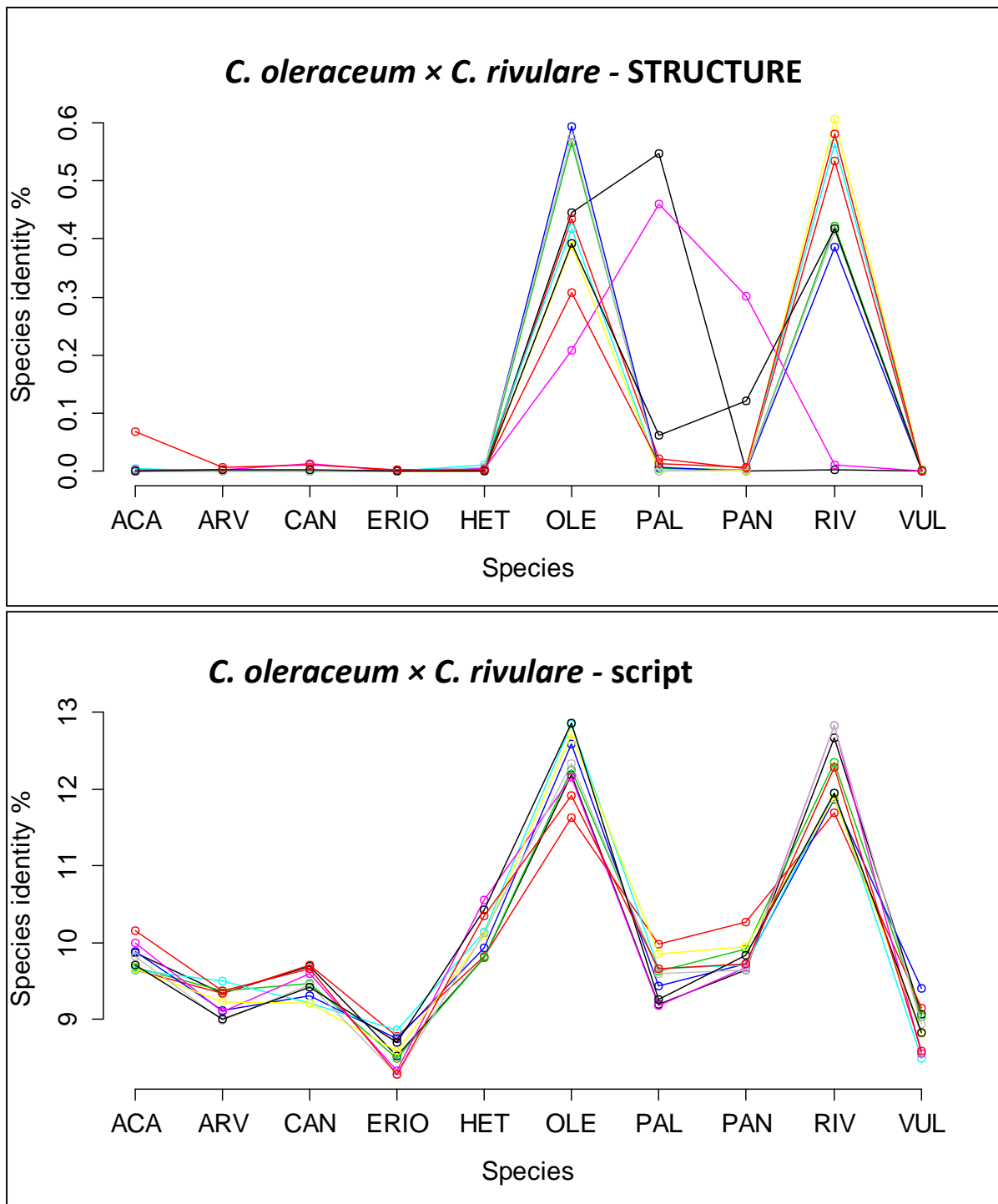
Fig. D25: Species identity calculated for hybrids of *C. oleraceum × C. rivulare,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
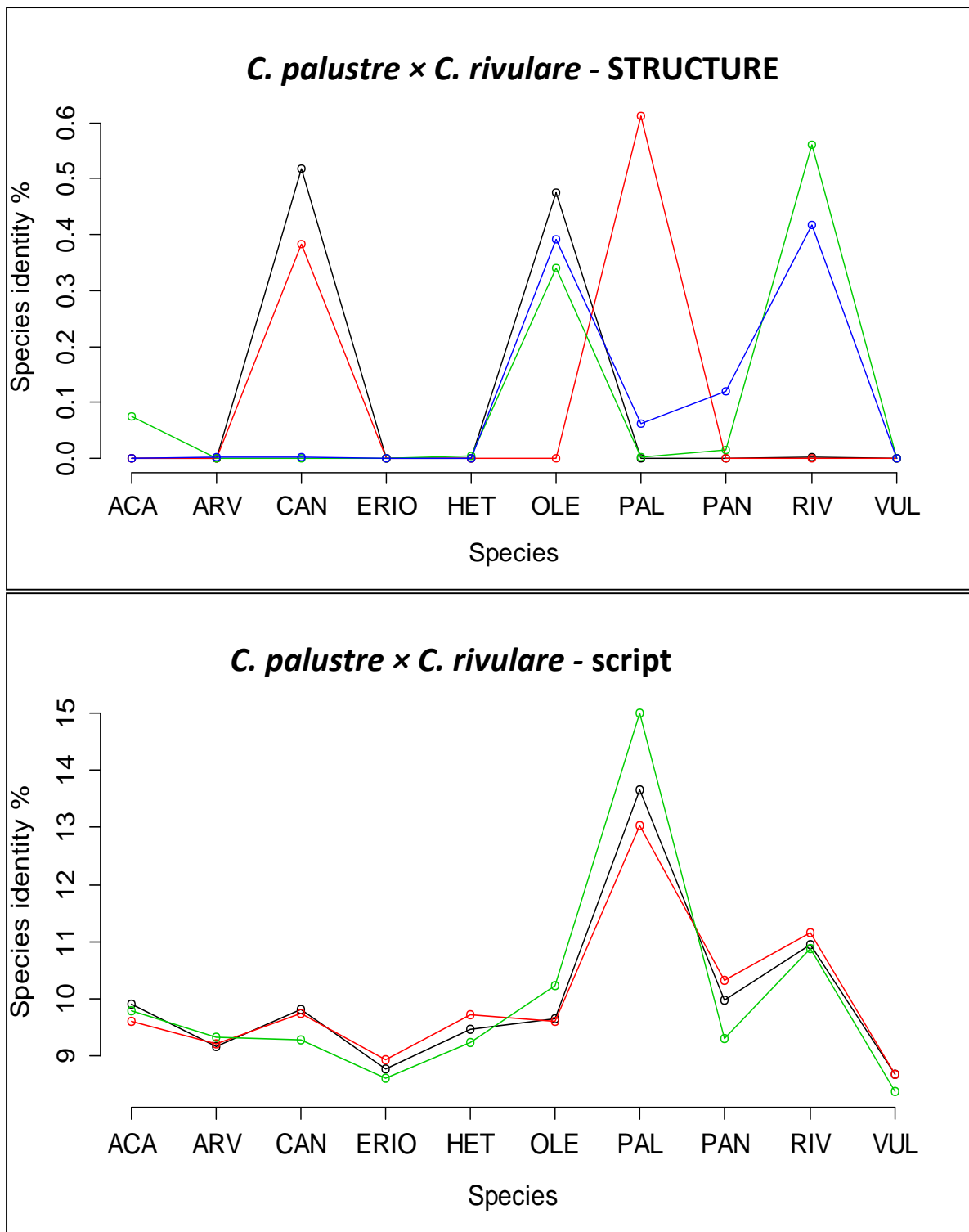
Fig. D26: Species identity calculated for hybrids of *C. palustre × C. rivulare,* according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
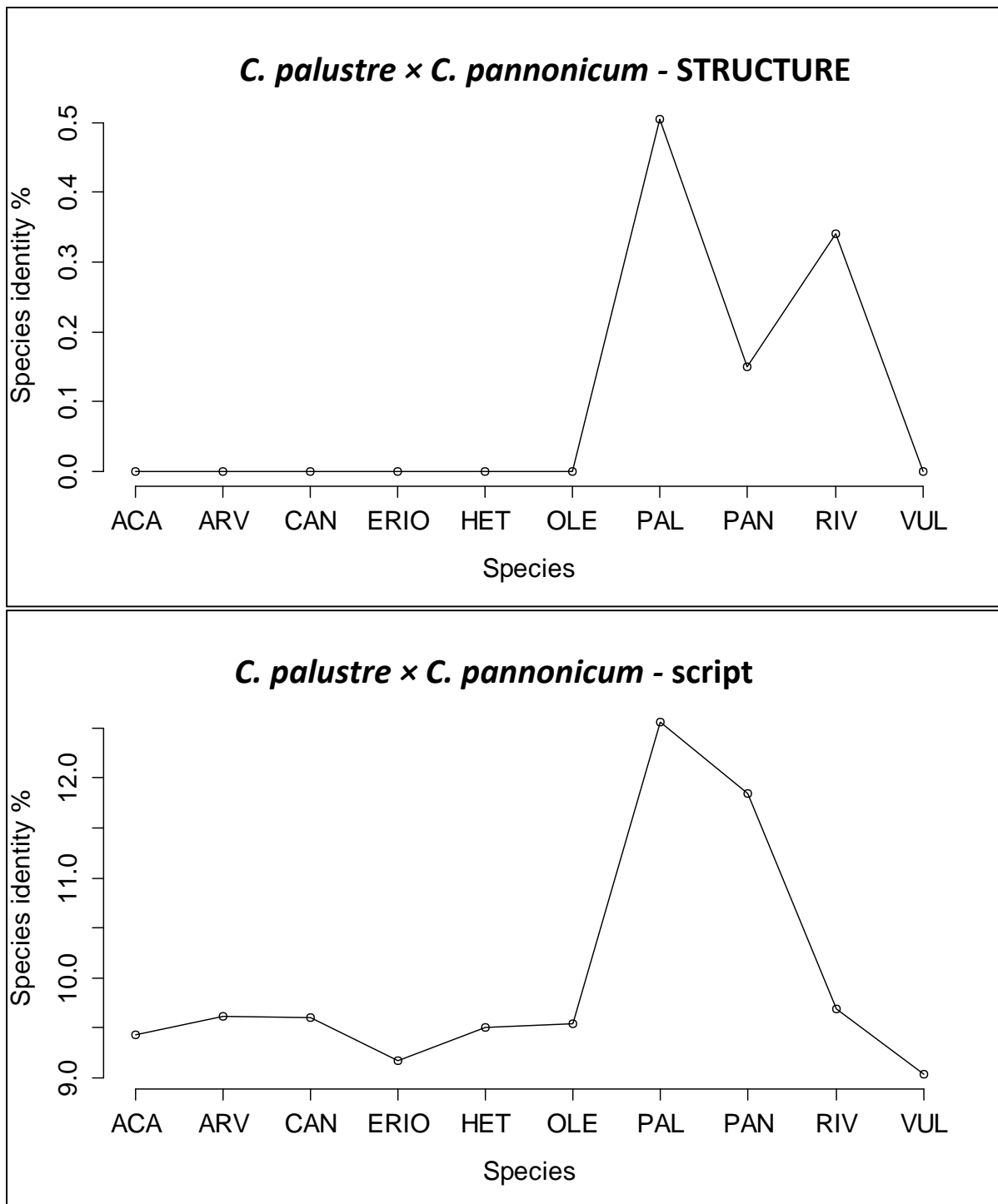
Fig. D27: Species identity calculated for hybrids of *C. palustre × C. pannonicum*, according to i) STRUCTURE and ii) our script (visualisation in R 3.2.3)
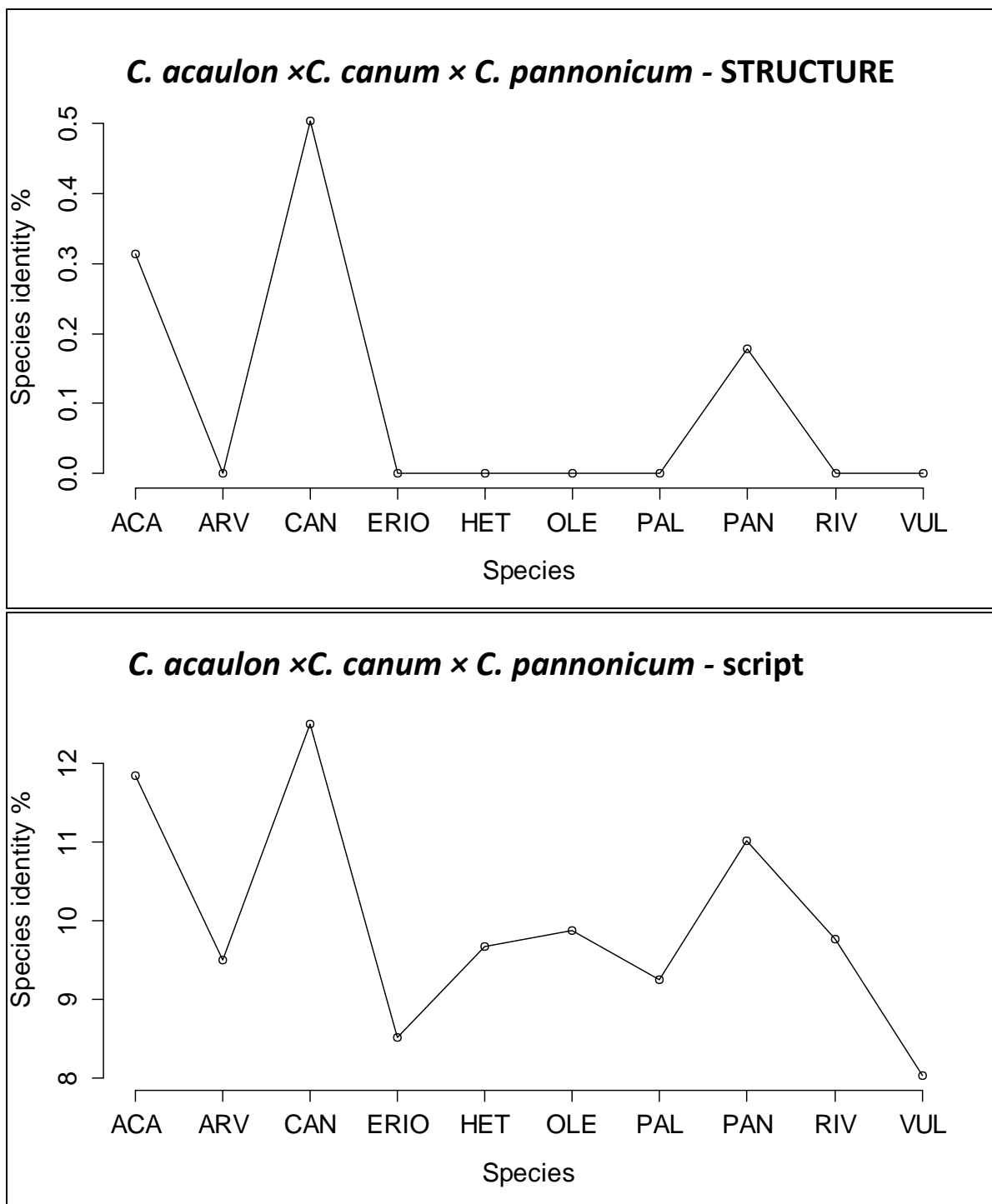
Fig. D28: Species identity calculated for triple hybrid of *C. acaulon, C. canum* and *C. pannonicum*

3. Geographical analysis of genetic variance

3D PCO plots based on intraspecific genetic distances (Jaccard´s dissimilarity) with three different colours representing each region (red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills); showed some geographic resolution (**Fig. D29–D38**). The geographic pattern is apparent in some species (e. g., *C. acaulon* – although there are three samples from White Carpathians in the cluster of Žďárské vrchy Hills (see **Fig. D29**), *C. canum* – the resolution seems to be the best among all studied species (see **Fig. D31**) and *C. rivulare* (see

Fig. D37), but to some extent we are able to observe it in all species. The worst resolution was in *C. arvense* where there was a gradual mixing up of samples from different regions.
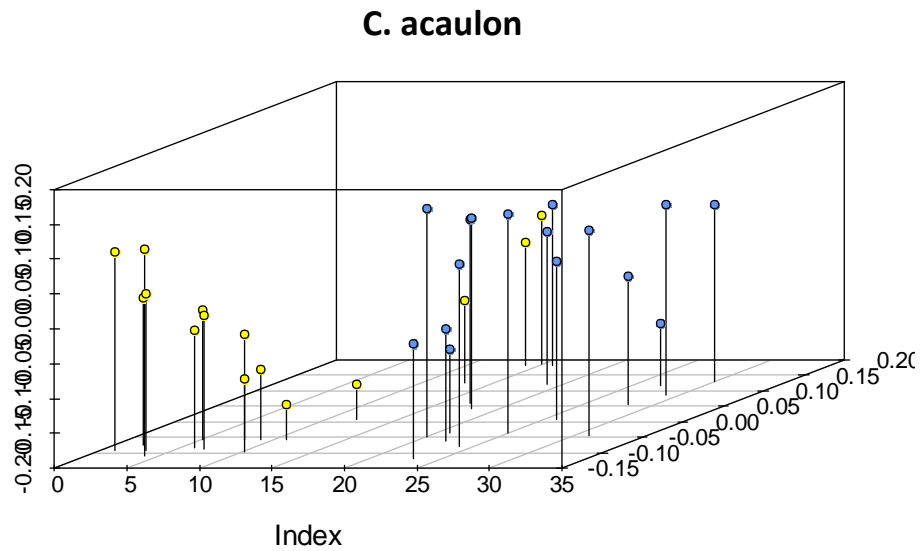
C. acaulon

Fig. D29: PCO based on genetic distances visualising geographical pattern of *C. acaulon*, eigenvalues: 1st axis: 0.279, 2nd axis: 0.1764, 3rd axis: 0.150, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)
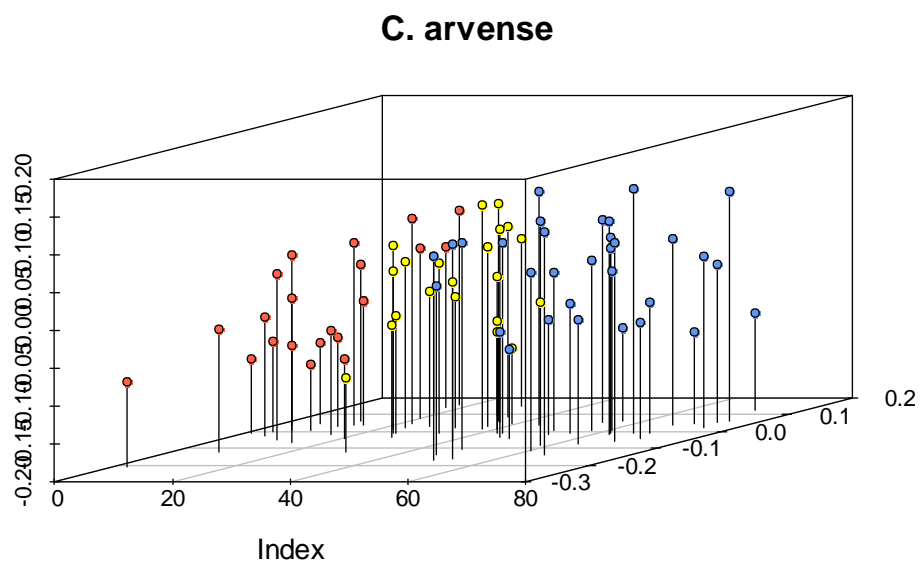
C. arvense

Fig. D30: PCO based on genetic distances visualising geographical pattern of *C. arvense*, eigenvalues: 1st axis: 0.397, 2nd axis: 0.2922, 3rd axis: 0.222, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)

C. canum
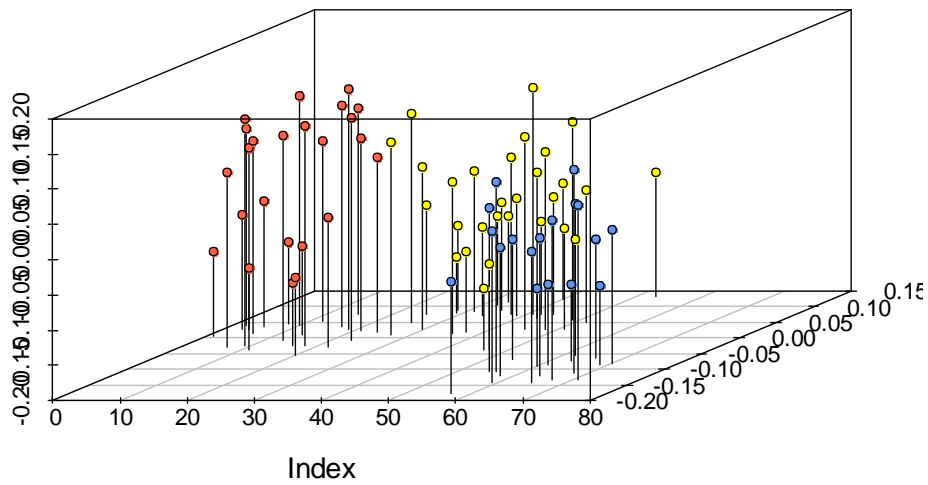
Fig. D31: PCO based on genetic distances visualising geographical pattern of *C. canum,* eigenvalues: 1st axis: 0.395, 2nd axis: 0.3708, 3rd axis: 0.269, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)

C. eriophorum
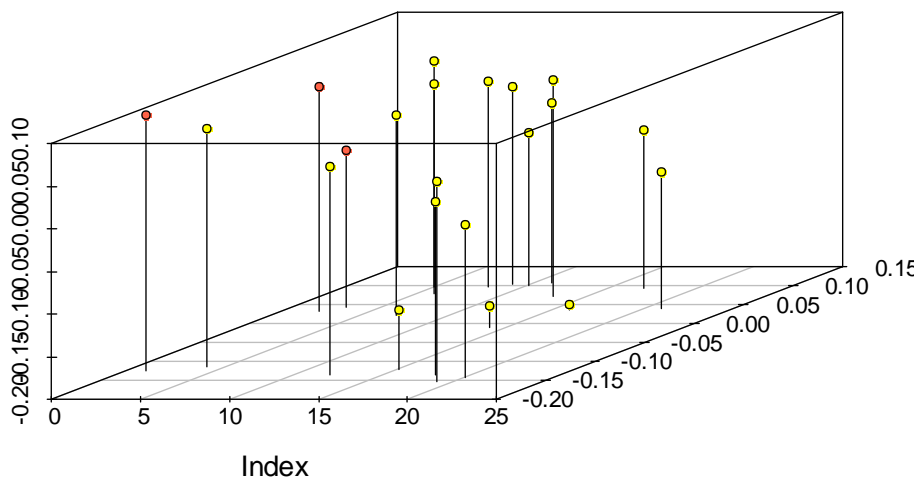
Fig. D32: PCO based on genetic distances visualising geographical pattern of *C. eriophorum,* eigenvalues: 1st axis: 0.201, 2nd axis: 0.125, 3rd axis: 0.111, red= Brno, yellow = White Carpathians; (R 3.2.3)
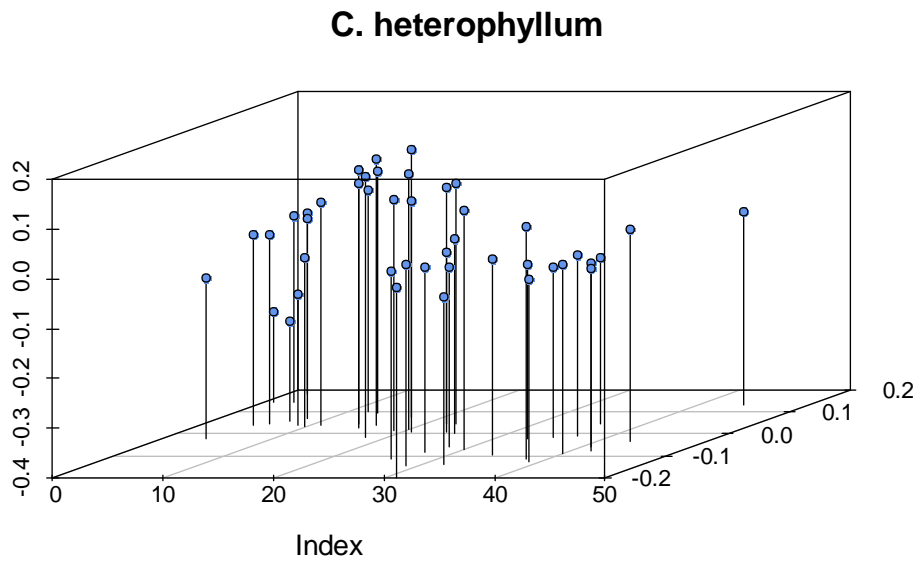
C. heterophyllum

Fig. D33: PCO based on genetic distances visualising geographical pattern of *C. heterophyllum,* eigenvalues: 1st axis: 0.308, 2nd axis: 0.272, 3rd axis: 0.269, blue = Žďárské vrchy Hills; (R 3.2.3)
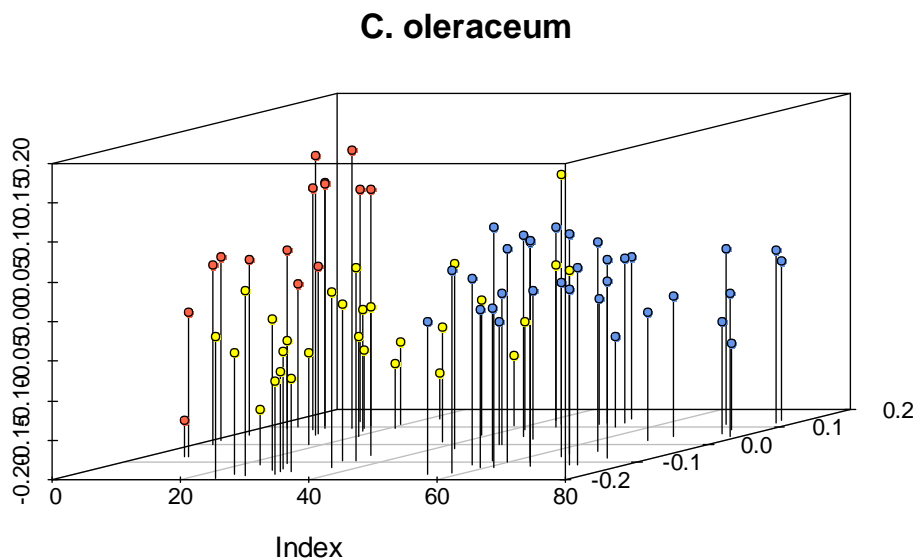
C. oleraceum

Fig. D34: PCO based on genetic distances visualising geographical pattern of *C. oleraceum,* eigenvalues: 1st axis: 0.865, 2nd axis: 0.362, 3rd axis: 0.277, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)

C. palustre
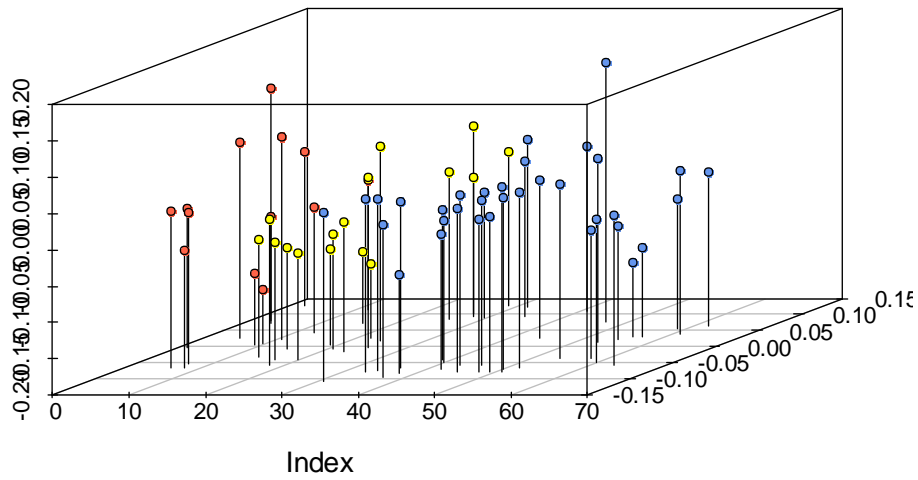
Fig. D35: PCO based on genetic distances visualising geographical pattern of *C. palustre,* eigenvalues: 1ˢᵗ axis: 0.275, 2ⁿᵈ axis: 0.173, 3ʳᵈ axis: 0.101, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)

C. pannonicum
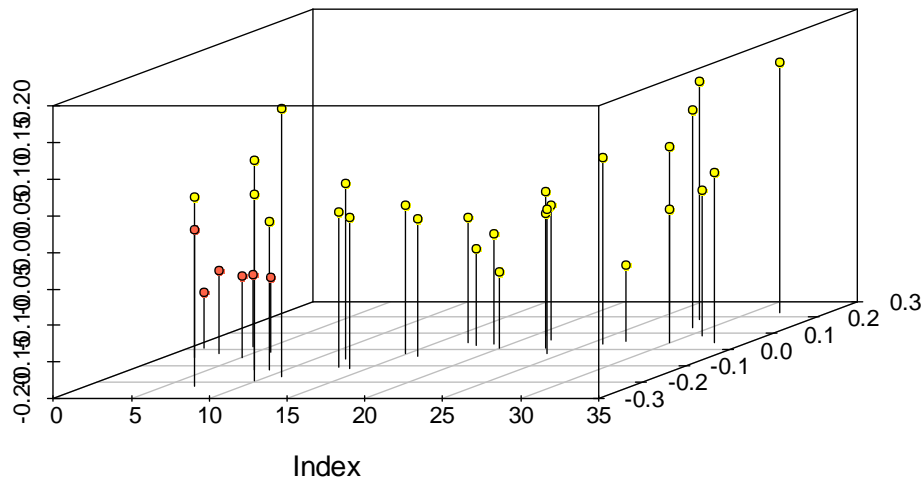
Fig. D36: PCO based on genetic distances visualising geographical pattern of *C. pannonicum,* eigenvalues: 1ˢᵗ axis: 0.370, 2ⁿᵈ axis: 0.176, 3ʳᵈ axis: 0.131, red= Brno, yellow = White Carpathians; (R 3.2.3)
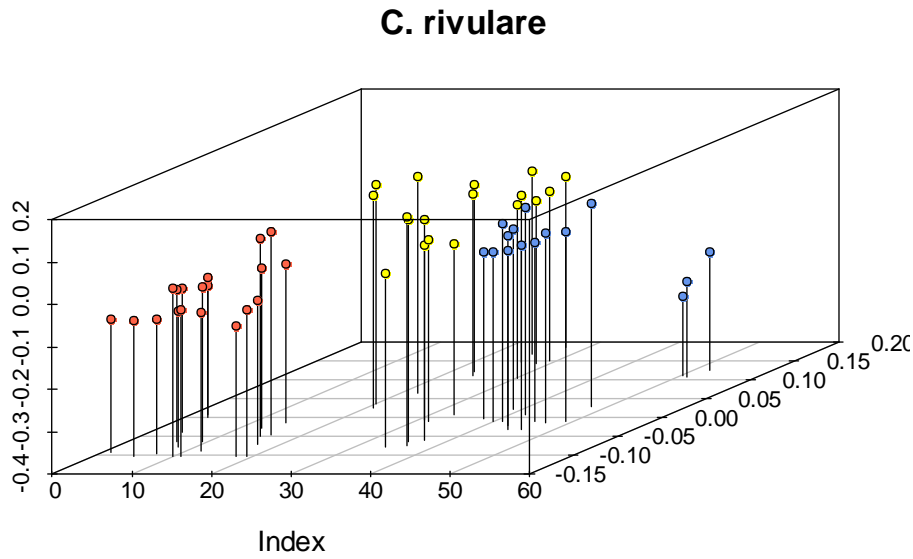
Fig. D37: PCO based on genetic distances visualising geographical pattern of *C. rivulare,* eigenvalues: 1st axis: 0.344, 2nd axis: 0.293, 3rd axis: 0.259, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)
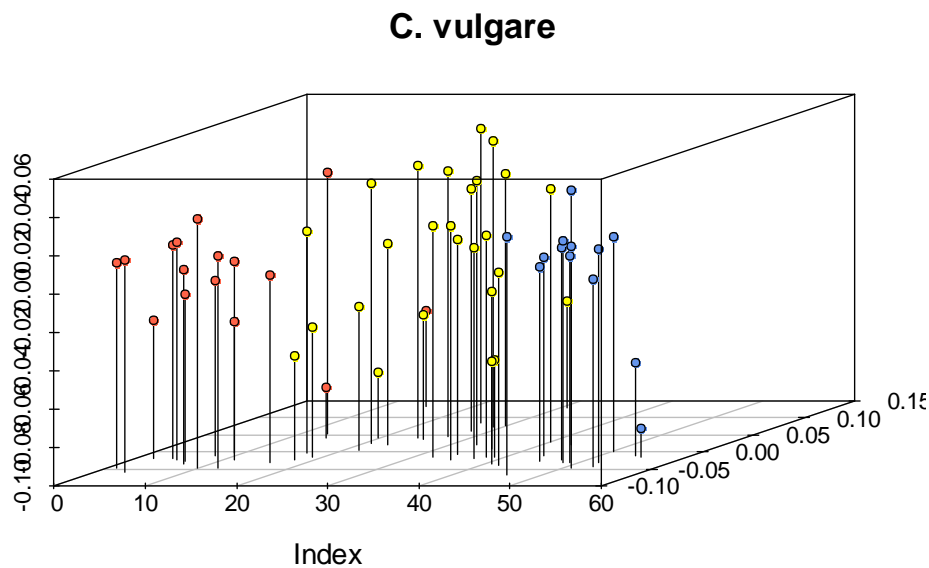
Fig. D38: PCO based on genetic distances visualising geographical pattern of *C. vulgare,* eigenvalues: 1st axis: 0.104, 2nd axis: 0.056, 3rd axis: 0.040, red= Brno, yellow = White Carpathians, blue = Žďárské vrchy Hills; (R 3.2.3)

The unrooted neighbour-join trees (constructed using genetical distances between samples – Jaccard´s coefficient) allow us to observe not only the regional differences within species (regions are again represented by three colours – red: Brno, yellow: White Carpathians, and blue: Žďárské vrchy hills), but also checking whether samples collected at same locality are clustered together (see **Fig. D39 - D48**). The regional resolution visualised by NJ trees was

worse than in the case of PCO 3D plots. Within cluster of one region there were often samples from different regions. This was also the case of *C. canum* which was seemingly well resolved when visualised as PCO diagram (see **Fig. D31** and **Fig.41**).

Although the regional resolution is poor, we can see that samples from the same localities have a tendency to cluster together (e. g., three samples of *C. acaulon* from White Carpathians that were clustered with samples from Žďárské vrchy Hills, are all from the same locality). Nevertheless, this is not a rule. Although, there are samples from the same locality that are clustered together, there are also samples that are clustered with individuals from other localities or even other regions – this is especially well seen in case of *C. arvense* (see **Fig. D40**).

We tested tree quality for each species respectively plotting original distance in matrix and reconstructed distance used for NJ construction; in all cases there was a strong relationship between cophenetic distances and therefore the trees are highly reliable (See **Fig. D49**).
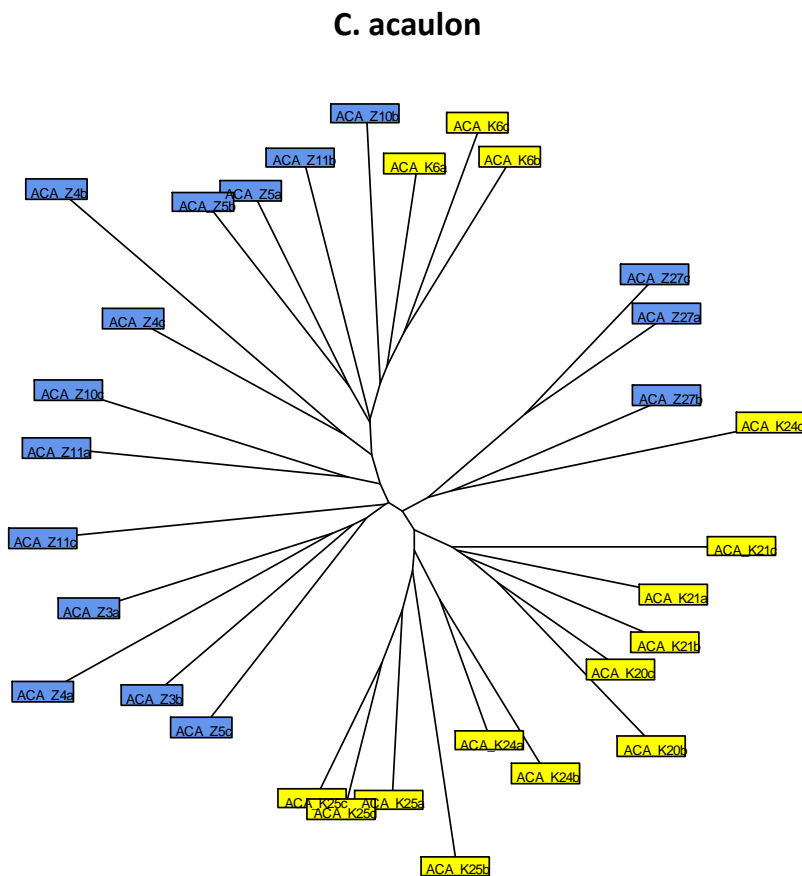
C. acaulon

Fig. D39: NJ - *C. acaulon* (R 3.2.3)

C. arvense
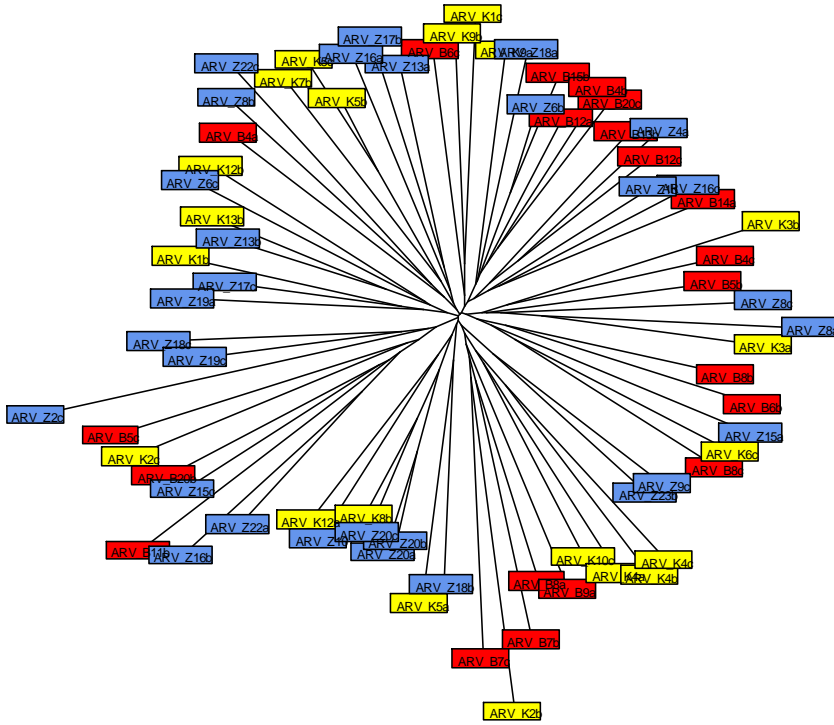
Fig. D40: NJ - *C. arvense* (R 3.2.3)
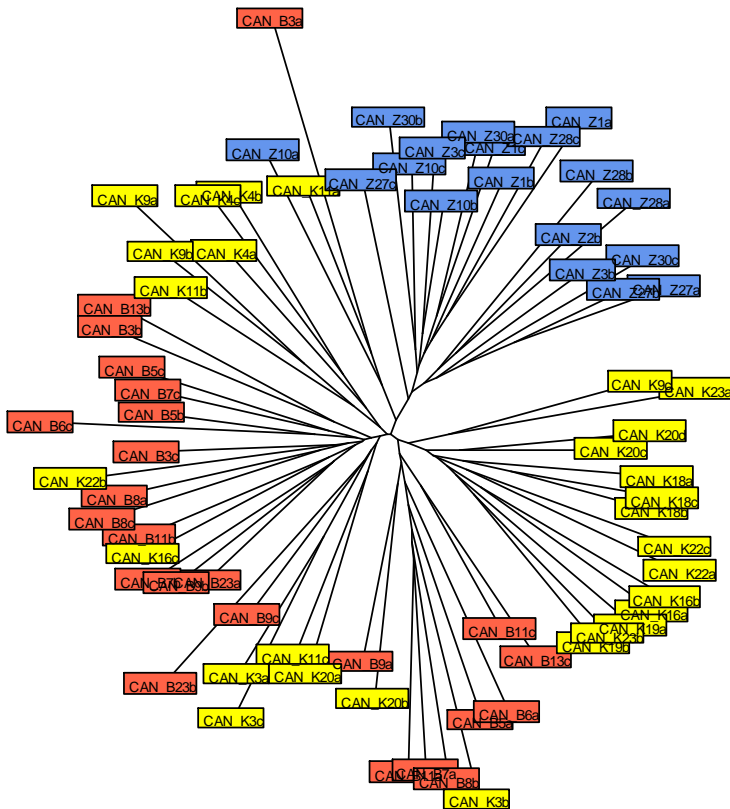
C. canum

Fig. D41: NJ - *C. canum* (R 3.2.3)

C. eriophorum
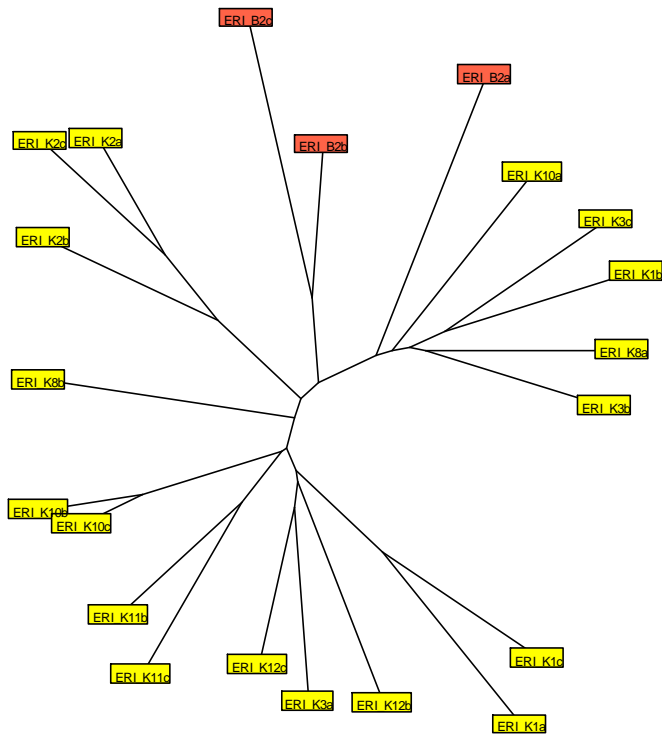
Fig. D42: NJ - *C. eriophorum* (R 3.2.3)

C. heterophyllum
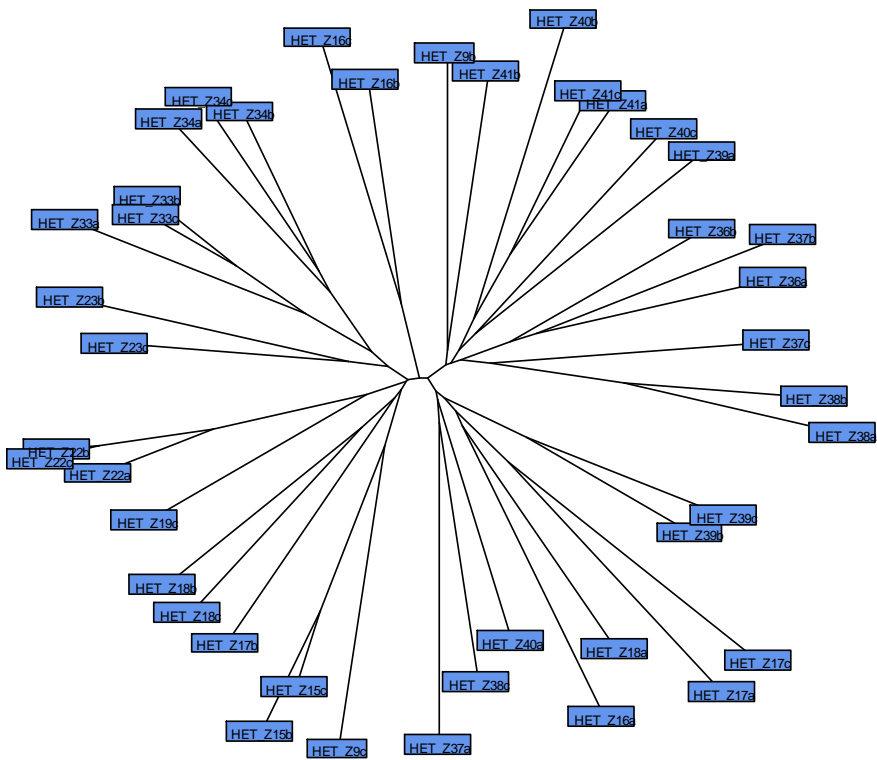
Fig. D43: NJ - *C. heterophyllum* (R 3.2.3)

C. oleraceum
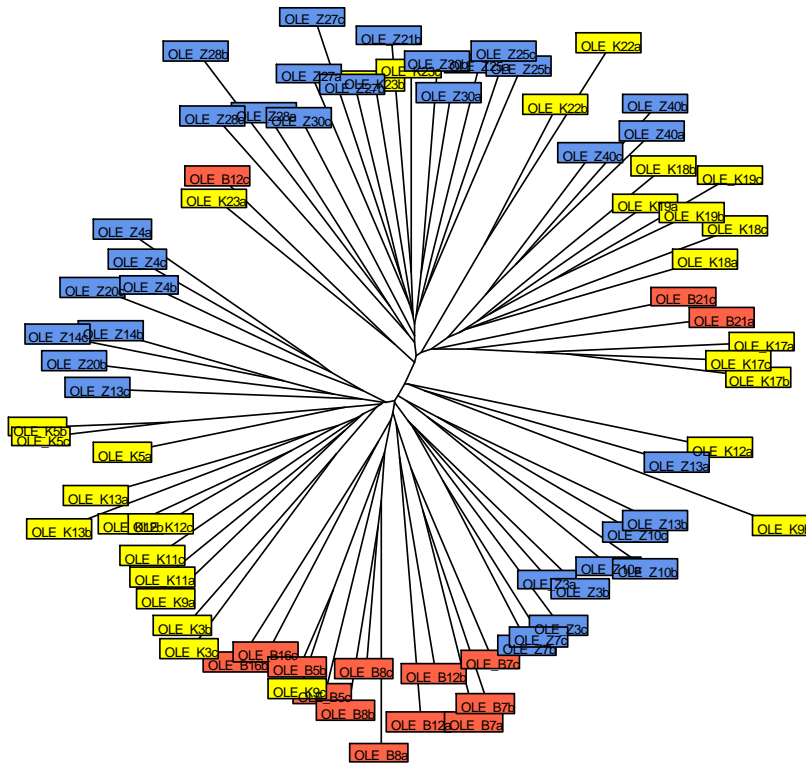
Fig. D44: NJ - *C. oleraceum* (R 3.2.3)

C. palustre
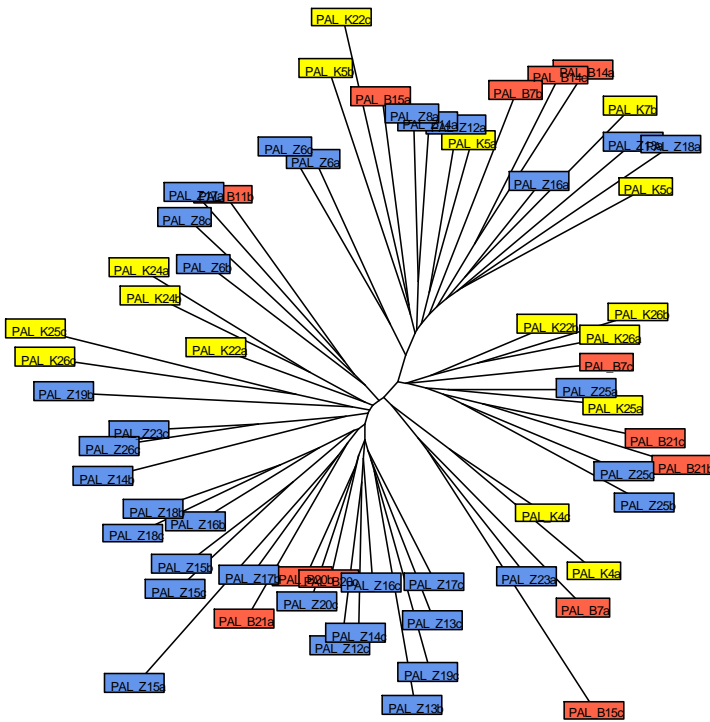
Fig. D45: NJ - *C. palustre* (R 3.2.3)

C. pannonicum
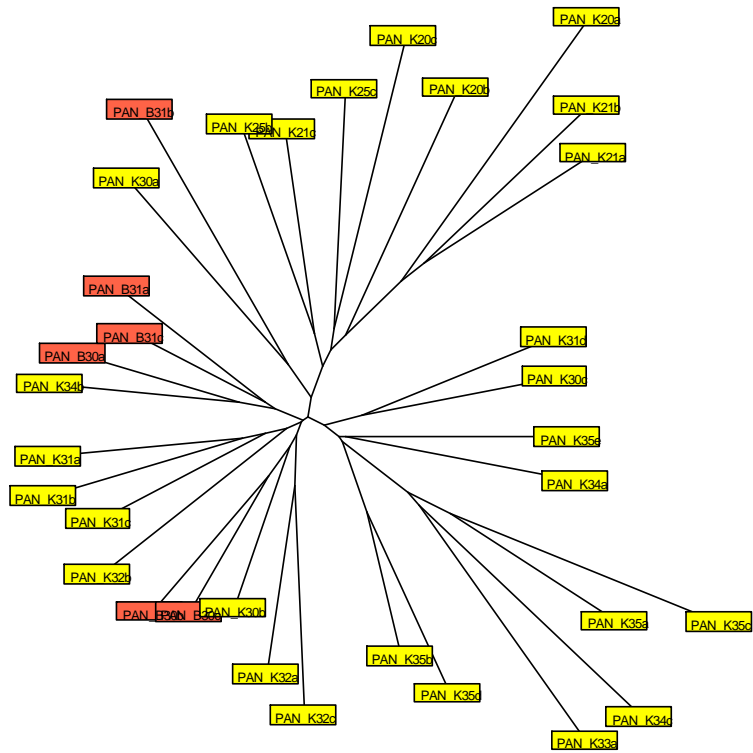
Fig. D46: NJ - *C. pannonicum* (R 3.2.3)

C. rivulare
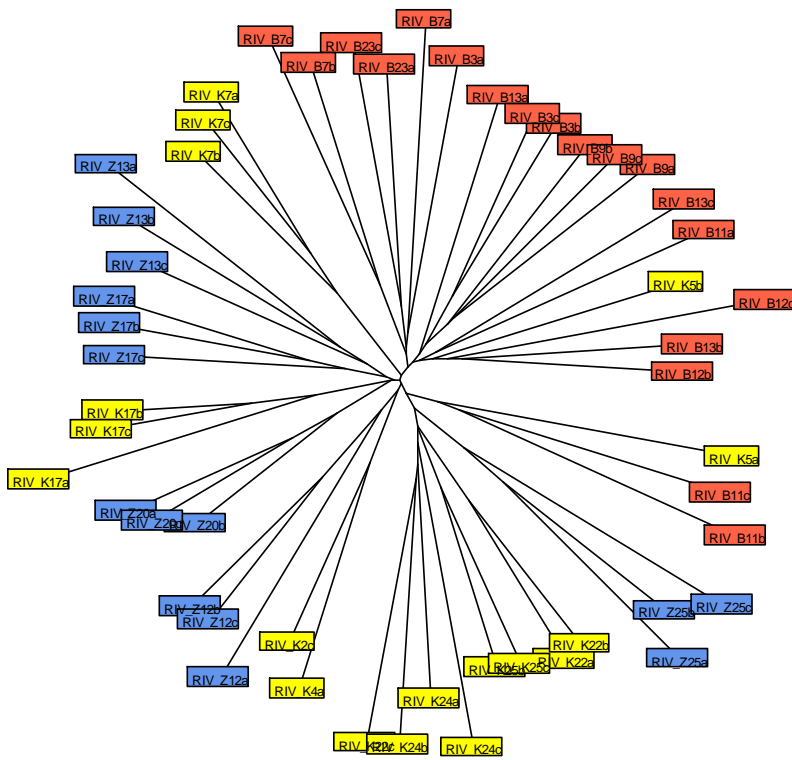
Fig. D47: NJ - *C. rivulare* (R 3.2.3)

C. vulgare
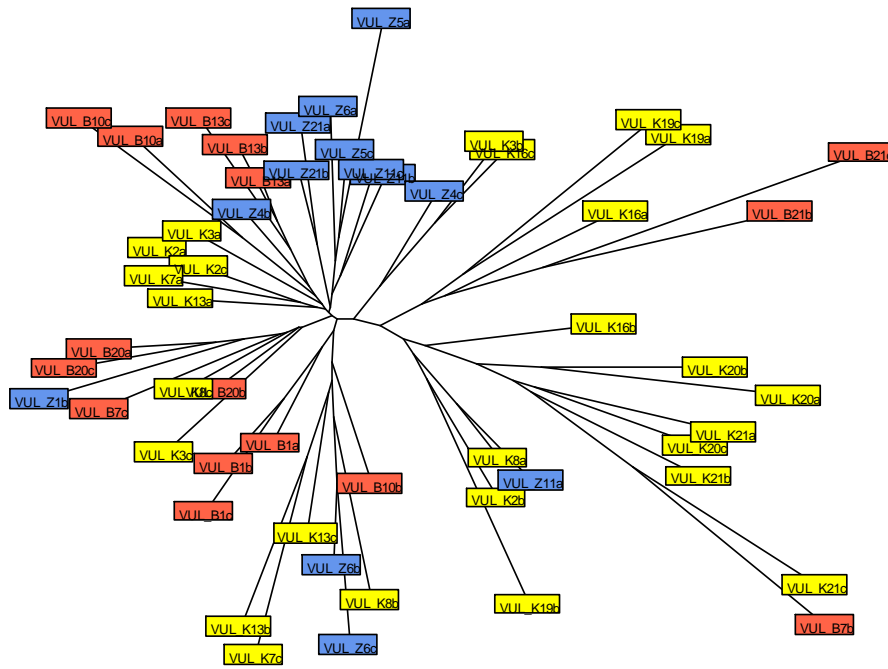
Fig. D48: NJ - *C. vulgare* (R 3.2.3)
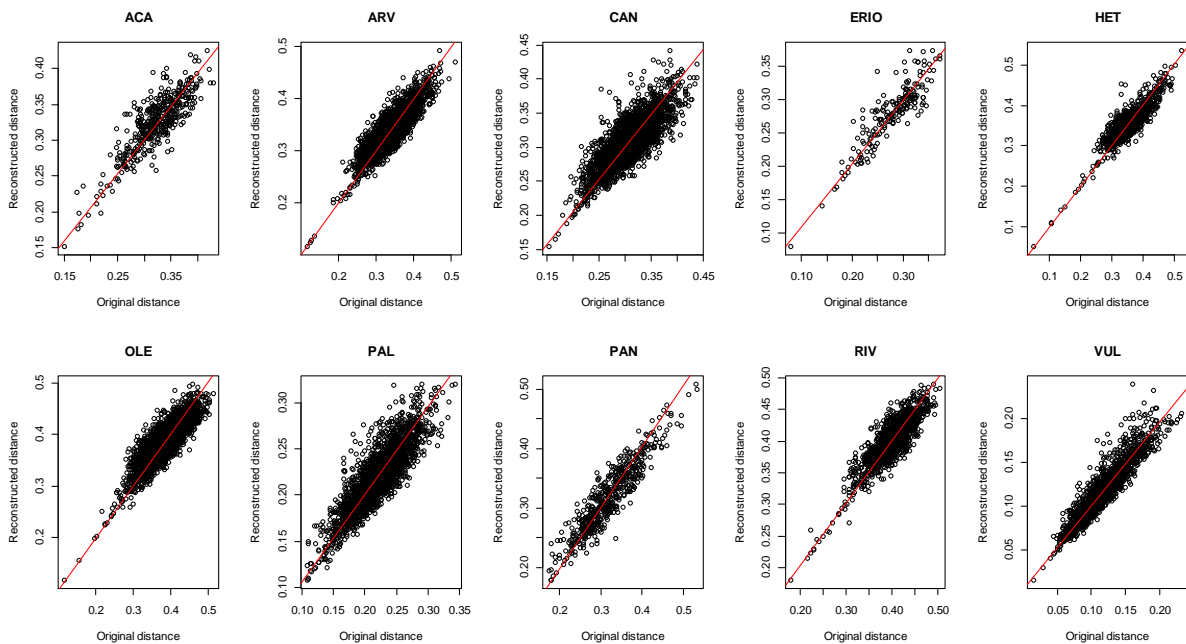
Fig. D49: NJ tree quality test for every species respectively

Although PCO projection and NJ trees provided some kind of resolution of different geographical regions, the AMOVA (analysis of molecular variance) did not approve that the regions differ from one another. The analysis partitioned the total genetic variation within regions and among regions as seen in **Tab. D1**.

The poorest regional resolution was found in *C. arvense* where the genetic variation within regions was 96.93% whereas the genetic variation among regions was only 3.07%. In other species (*C. acaulon, C. oleraceum, C. palustre, C. pannonicum, C. rivulare* and *C. vulgare*) was the variation among regions slightly higher ranging from 6.19% in *C. palustre* to 9.76% in *C. rivulare*. In *C. canum* was the variation among regions significantly higher than in other species (18.03%).

There was no case that variation among populations was greater (not even comparable) with variation within populations. Low values in ΦPT estimated that there is complete panmixis rather than separation among the studied regions.

Tab. D1: AMOVA (analysis of molecular variance) for sampling regions (Brno, White Carpathians, Žďárské vrchy Hills)

	Variation (%)		ΦPT
	Within regions	Among regions	
ACA	90.42	9.58	0.10
ARV	96.93	3.07	0.03
CAN	81.97	18.03	0.18
OLE	93.49	6.51	0.07
PAL	93.81	6.19	0.06
PAN	91.36	8.64	0.09
RIV	90.24	9.76	0.10
VUL	91.15	8.85	0.09

The visualisation of genetic similarities between individuals using boxplots showed that the genetic similarity tends to decrease with increasing distance (see **Fig. D50**). There was, however, no significant difference between the categories indicating some break point from which intraspecific gene flow (or communication between individuals) would no more take place.

This trend (gradual decreasing in genetic similarity) was the same in all species except for *C. pannonicum,* where the genetic similarity was the highest at the beginning and was decreasing till 4 - 16 km and then it started increasing again.
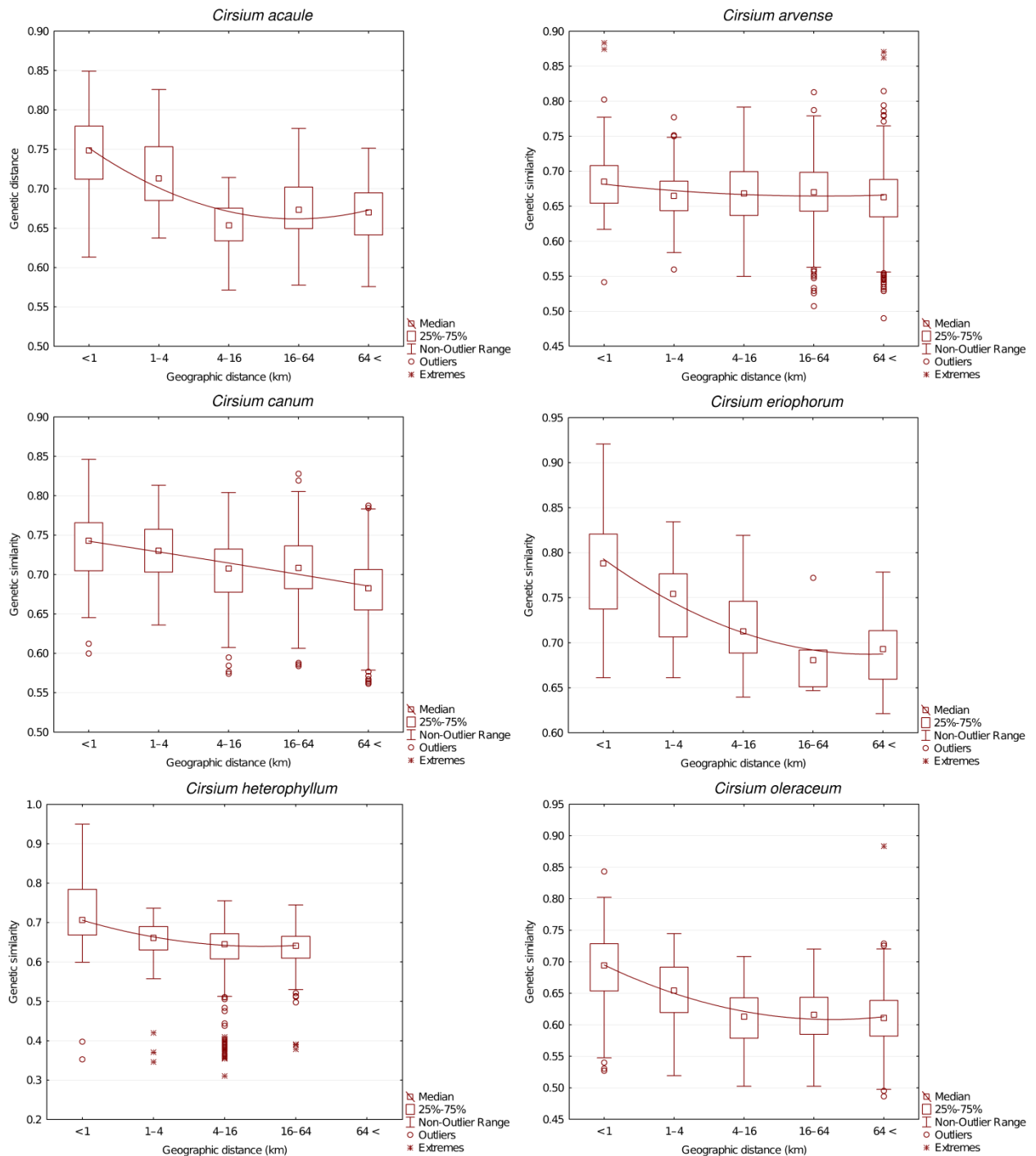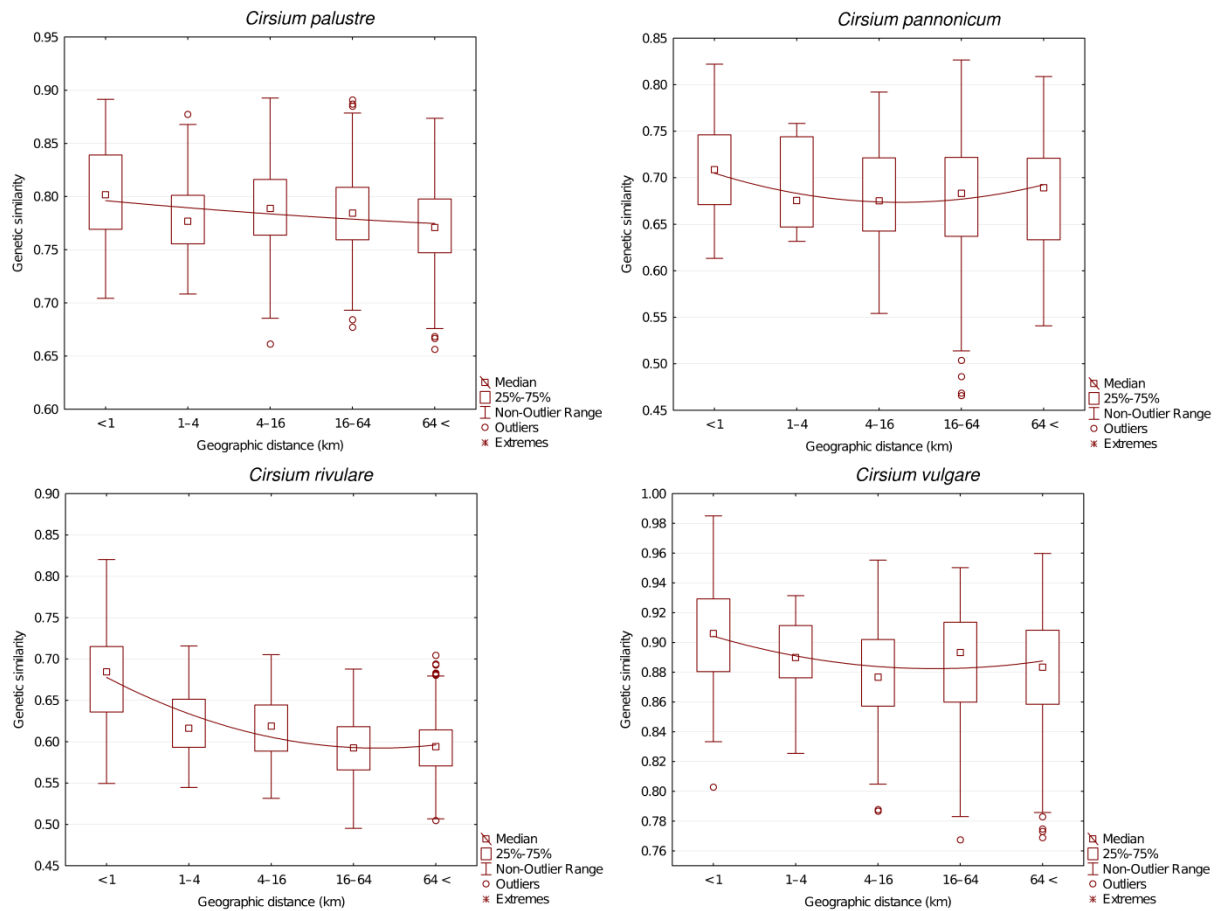
Fig. D50: Boxplots representing the influence of increasing geographic distance on genetic similarity between samples; (STATISTICA12)

The steepness of decreasing of genetic similarity (represented by the fitting line) was differing. There was quite a steep gradient in *C. acaulon, C. canum, C. eriophorum, C. oleraceum* and *C. rivulare*. Contrastingly, the narrowest gradient was in *C. arvense*.

In *C. heterophyllum* there was a large number of outliers comparing to other species. This could not be given by some wrongly determined or failed individuals within the dataset, because there were no obvious outliers in previous analyses (see PCO in **Fig. D33** and NJ in **Fig. D42**).

4. Genetic diversity

Kruskal-Wallis test approved that there is significant variability in interaspecific genetic similarities among the species (see **Fig. D51** and **Tab. D2**). The only two species with insignificant intraspecific genetic variability were *C. acaulon* and *C. pannonicum.*
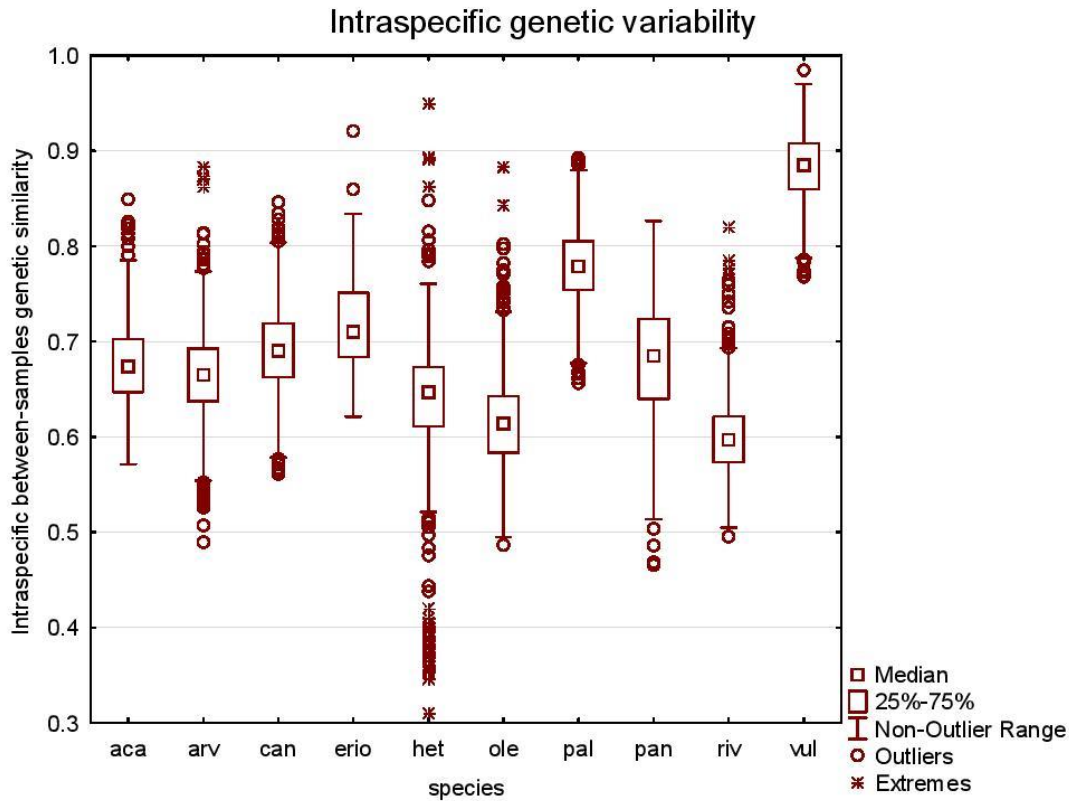
Fig. D51: Boxplots showing intraspecific genetic variability; (STATISTICA12)

Tab. D2: Kruskal-Wallis test; p = 0.00

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
ACA		0.02974	0.00033	0.00000	0.00000	0.00000	0.00000	1.00000	0.00000	0.00000
ARV	0.02974		0.00000	0.00000	0.00000	0.00000	0.00000	0.00004	0.00000	0.00000
CAN	0.00033	0.00000		0.00060	0.00000	0.00000	0.00000	0.03203	0.00000	0.00000
ERIO	0.00000	0.00000	0.00060		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
HET	0.00000	0.00000	0.00000	0.00000		0.00000	0.00000	0.00000	0.00000	0.00000
OLE	0.00000	0.00000	0.00000	0.00000	0.00000		0.00000	0.00000	0.00000	0.00000
PAL	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		0.00000	0.00000	0.00000
PAN	1.00000	0.00004	0.03203	0.00000	0.00000	0.00000	0.00000		0.00000	0.00000
RIV	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		0.00000
VUL	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

For comparison of average genetic similarities of all species with variables that would possibly explain their pattern – species promiscuity (based on the number of herbarium specimens), genome size (BUREŠ et a. 2004) and average number of females per species (BUREŠ, unpublished data) see Discussion.

5. Interspecific gene flow identification

Comparing the regional differences in average genetic diversity among chosen species (co-occurring and potentially hybridizing only in certain regions) was corresponding to our assumption only in some cases (see **Tab. D3**). The differences in similarity were also extremely small (of the order of thousandths). Our results therefore did not approve, that there is interspecific gene flow among these analysed species.

Tab. D3: Combinations of samples used for interspecific gene flow identification, if marked * the result corresponds to our expectations

Analyzed species pair	Contrasting regions	Co-occurrence	Similarity
C. canum, C. pannonicum	White Carpathians	yes	0.2470
	Žďárské vrchy Hills	only can	0.2614
C. acaulon, C. oleraceum	Brno	only ole	0.2956*
	White Carpathians Žďárské vrchy	yes	0.2985*
C. heterophyllum, C. oleraceum	Brno	only ole	0.3037*
	White Carpathians	only ole	0.3064*
	Žďárské vrchy	yes	0.3085*
C. heterophyllum, C. palustre	Brno	only ole	0.7447
	White Carpathians	only ole	0.7430
	Žďárské vrchy	yes	0.7467
C. heterophyllum, C. rivulare	Brno	only ole	0.3190*
	White Carpathians	only ole	0.3153*
	Žďárské vrchy	yes	0.3196*

E. Discussion

1. Molecular approval of samples

The molecular methods allowed us to succesfully detect genetically pure samples that we would not be able to detect using other techniques. Using ordination analysis PCO and agglomerative clustering analysis Neighbour-joining, we have approved remote clusters of species consisting of genetically pure samples with no undergone hybridization events and no incorrect amplification. More accurate analyses based on Bayesian approach performed in STRUCTURE software and our own script[33] proved the results. When testing sample membership to different species we obtained similar results from both methods. STRUCTURE output was more accurate and when plotting all members of one species into a line chart the consistence of this method was so high that the samples overlapped and created more or less a single line (see Results **Fig. D4 - D13**). Our script, even though the plotted results were not so consistent, clearly showed from which species is the sample coming. Comparing to STRUCTURE there was an increase in percentage of species identity (%) indicating falsely that other also species contribute to the genetic make-up of the sample. This was, however, only given by phylogenetic background we were not able to completely get rid of.

Our results show that our script can be used for molecular approval of samples same well as the widely used STRUCTURE software. The prior knowledge of samples membership is, however, necessary, which makes our script unusable for any sorts of analyses with no (or only faint) prior knowledge of samples membership e. g., for phylogenetic studies. It is however very suitable for controlling the dataset in order to avoid involving of genetically impure (F2 or B2 hybrids) samples that would distort further analyses.

2. Hybrids – identification of parental species

2. 1. Two models comparison

Of the two approaches (Bayesian approach using STRUCTURE and our own script) we used for finding putative parents of sampled hybrids, our script proved to be much more successful. Its resolution of various hybrids was consistent and was corresponding to our field determination (morphologically based) from 90%. The samples that were determined else wise can be seen in **Fig. E1.**

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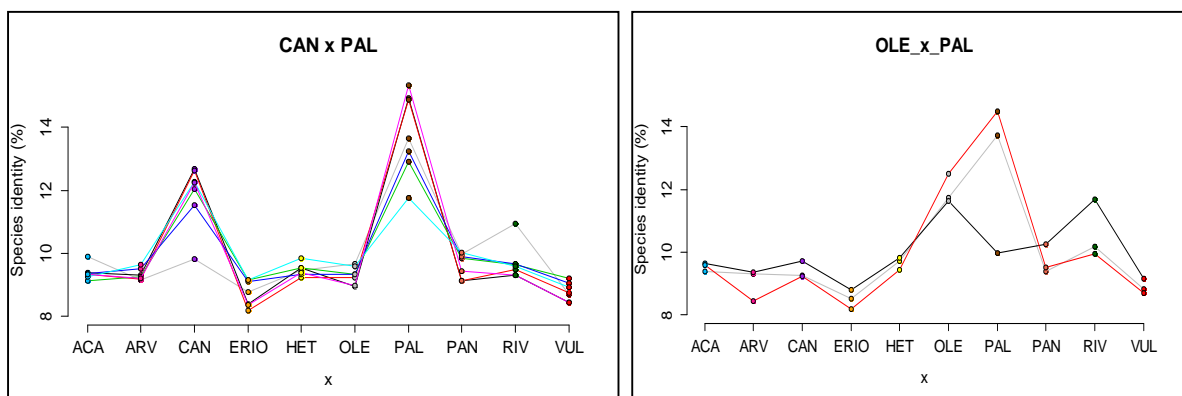
[33] Written by Jakub Šmerda
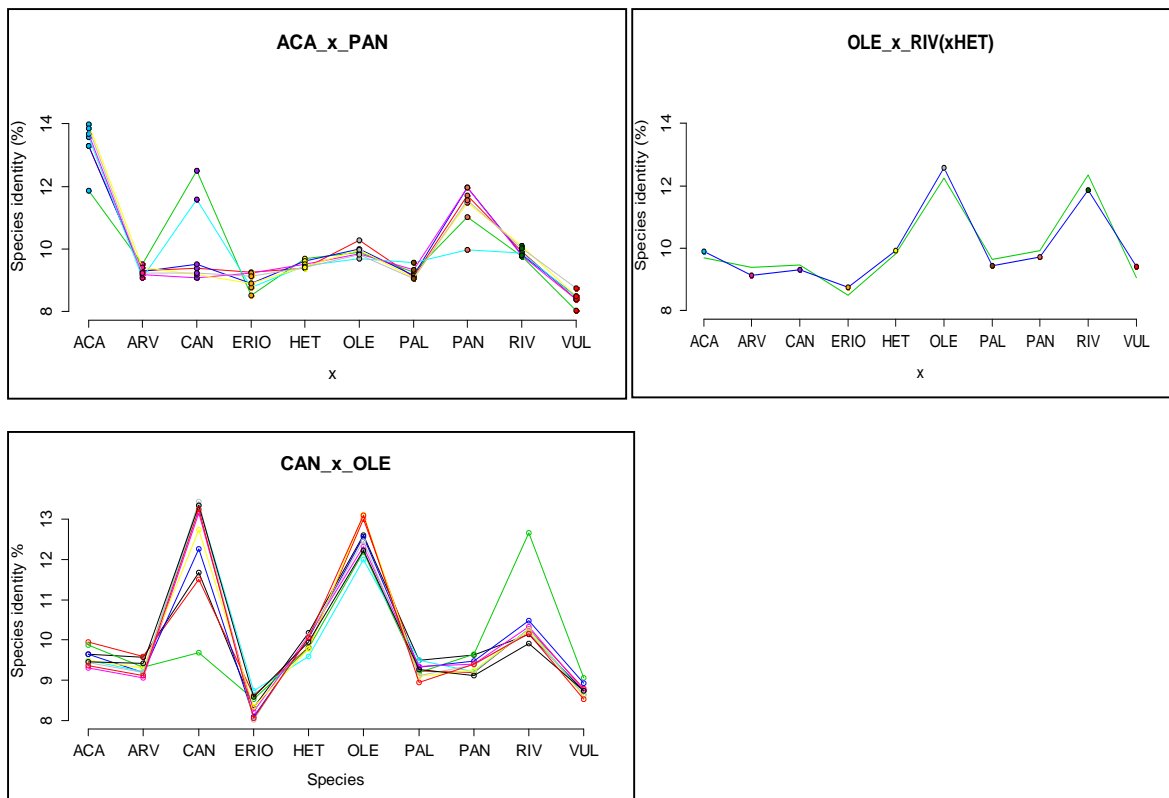
Fig. E1: Diagrams showing wrongly determined samples of hybrids according to of our own script.

This misdetermination may be given by several factors. First factor would be that we did a determination mistake while sampling the samples. This is very likely to be the case of two samples that were determined as hybrids of *C. acaulon × C. pannonicum.* Our script recognised one of the hybrids to be *C. acaulon × C. canum* and the other one a triple hybrid *C. acaulon × C. canum × C. pannonicum.* The fact that we did not recognise *C. canum* as one of the parents was probably because *C. canum* and *C. pannonicum* look morphologically very similar and their characteristics are hardly recognisable when present in a hybrid. This could be also the case of sample of *C. oleraceum × C. rivulare* which was determined as *C. canum × C. oleraceum.* The individuals of potential triple hybrid *C. oleraceum × C. rivulare × C. heterophyllum* were already sampled with prior knowledge that these species may be only simple hybrids and need to be checked molecularly. The only two problematic samples are those that should include *C. palustre,* but they do not. This situation is a little bit suspicious, because *C. palustre* is morphologically very unique and does not look like the species that were revealed to be parents by the molecular analysis. One possibility is that we sampled individuals that were not yet mature enough to determine the parental species correctly. Another possibility is, that we did some mistake during sample manipulation (e. g., during the lab work).

We can therefore assume that our script is highly reliable in recognition of putative hybrid parents and for our data is much more suitable than STRUCTURE, although it is commonly used for hybrid recognition based on AFLP data (DE HERT et al. 2012, THOMPSON et al. 2013), because it recognised correctly only 72% of samples.

2. 2. Determined hybrids

We have molecularly proved 14 out of 31 hybrid combinations within the Czech flora (BUREŠ et al. 2004) see **Fig. E2**). So far there has been no molecular evidence supporting the existence of these hybrids[34].
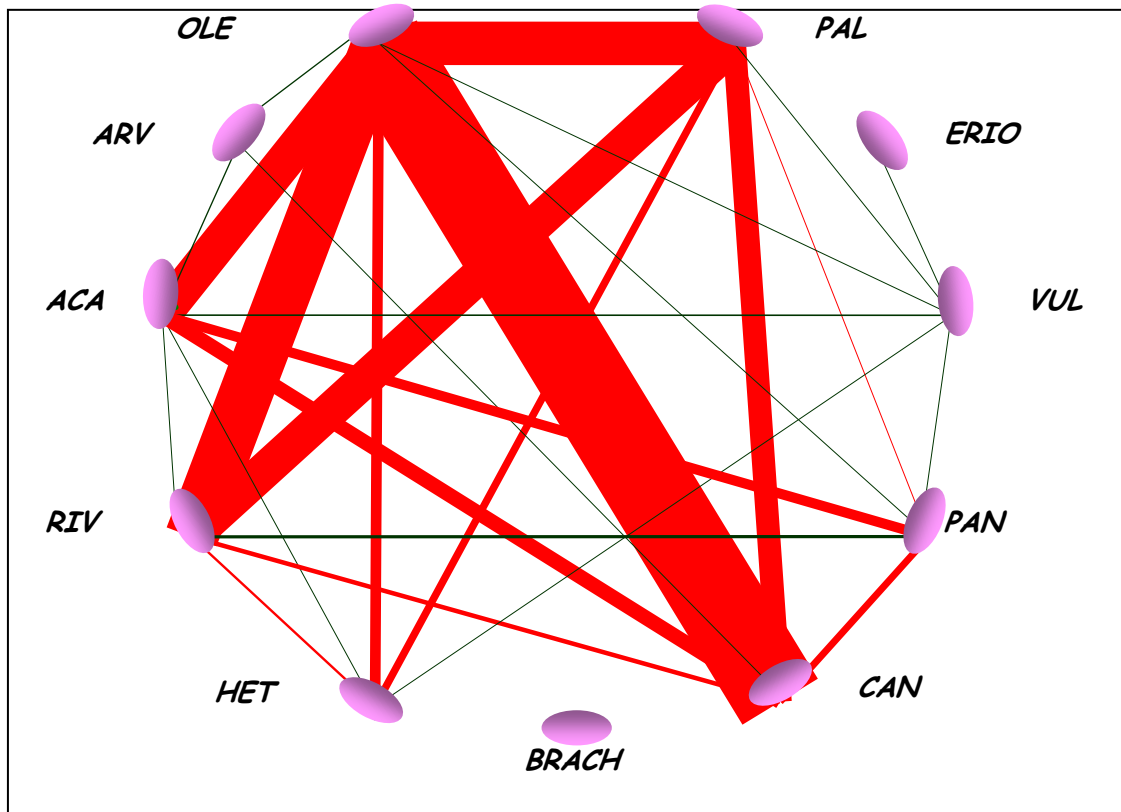
Fig. E2: Intensity of interspecific hybridization in genus *Cirsium* according to number of specimens of hybrids (BLIZNÁKOVÁ 2001, modified). The red lines are combinations that were proved molecularly, the green were not.

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[34] There has been a molecular analysis done for a Pyrenean hybrid of *C. carniolicum* subsp. *rufescens* and *C. palustre* (SEGARRA-MORAGUES et al. 2007) but none on hybrids we studied.

The hybrids we proved were rather common (see **Fig. 2**), but there were some exceptions. The list of hybrids together with their pictures and maps of their localities (black circles represent the localities taken from BLIZŇÁKOVÁ 2010, red cross represent our samples) is as follows:

Cirsium acaulon × C. canum = *Cirsium × winklerianum* ČELAK. Prodr. Fl. Böhm. 2:262, 1871.

Fig. E3: *Cirsium × winklerianum*

Cirsium acaulon × C. oleraceum = *Cirsium × rigens* (DRYANDER) WALLR. Sched. Crit. 446, 1822.
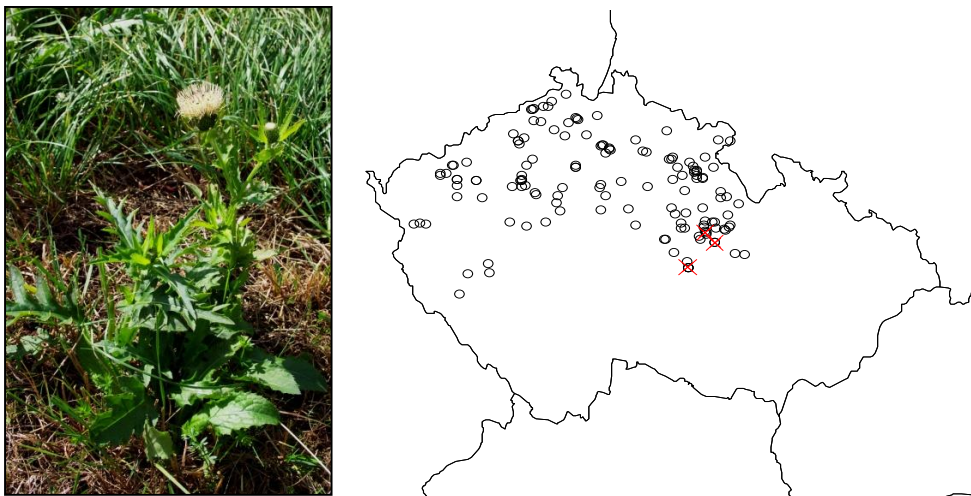
Fig. E4: *Cirsium × rigens*

Cirsium acaulon × C. pannonicum = *Cirsium × freyerianum* KOCH Taschenb. Deutsch. Schweiz. Fl. 288, 1843.
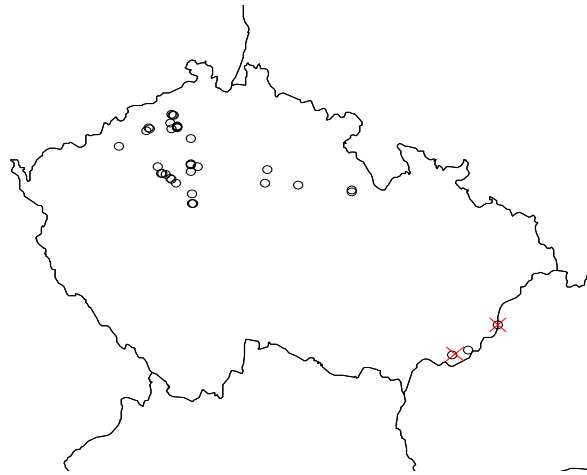
Fig. E5: *Cirsium × freyerianum*

Cirsium canum × C. oleraceum = *Cirsium × tataricum* (L.) ALL. Fl. Pedem. 1:151, 1785.
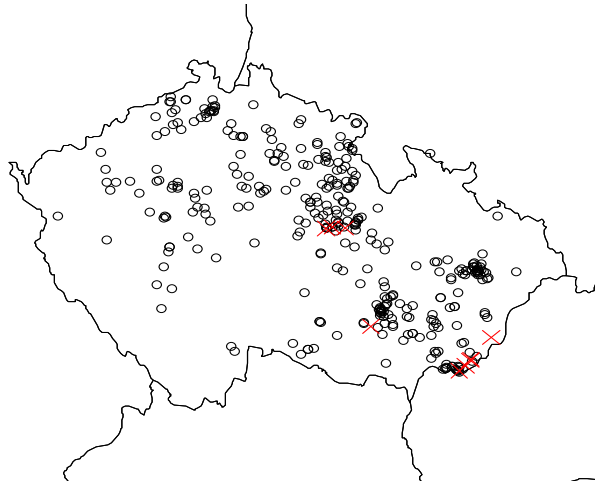
Fig. E6: *Cirsium × freyerianum*

Cirsium canum × C. palustre = *Cirsium × wimmeri* ČELAK. Prodr. Fl. Böhm. 2:263, 1871.
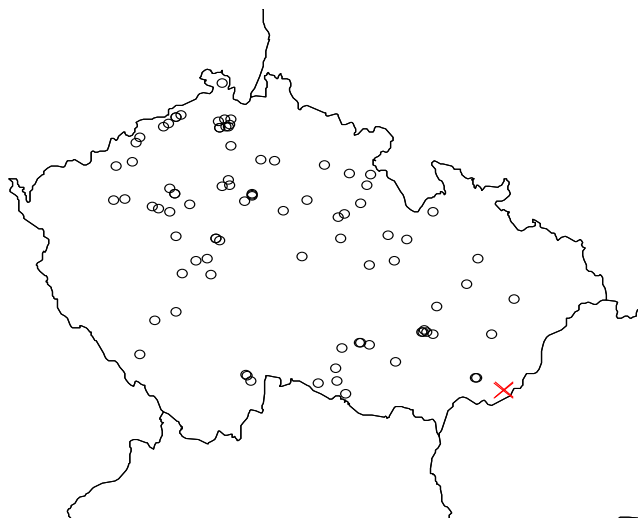
Fig. E7: *Cirsium × wimmeri*

Cirsium canum × C. pannonicum = *Cirsium × austropannonicum* SIMK. in WAISB. Köszeg Vid. Edény. Növény., ed. 2, 30, 1891.

Fig. E8: *Cirsium × austropannonicum*

Cirsium canum × C. rivulare = *Cirsium × siegertii* SCHULTZ BIP. ex REICHARDT Verh. Zool.-Bot. Ges. Wien 11:372, 1861.

Fig. E9: *Cirsium × siegertii*

Cirsium heterophyllum × C. oleraceum = *Cirsium × affine* TAUSCH Flora, Regensburg, 16:228, 1833.

Fig. E10: *Cirsium × affine*

Cirsium heterophyllum × C. palustre = *Cirsium × wankelii* REICHARDT Verh. Zool.-Bot. Ges. Wien 11:382, 1861.

Fig. E11: *Cirsium × wankelii*
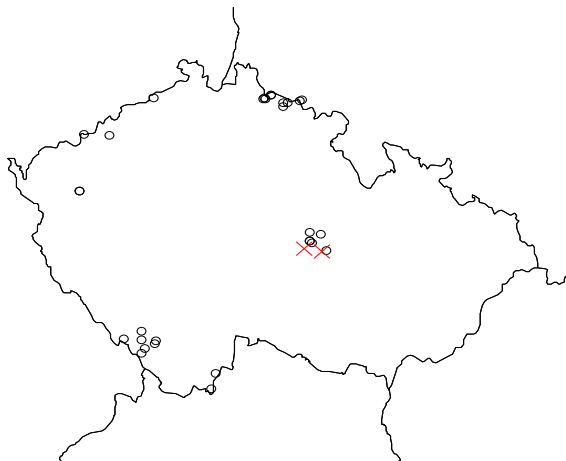
Cirsium heterophyllum × C. rivulare = *Cirsium × ambiguum* ALL. Auct. Fl. Pedem. 10, 1789.

Fig. E12: *Cirsium ×ambiguum*
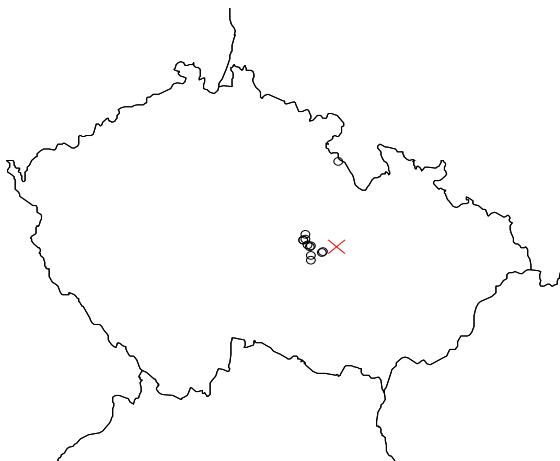
Cirsium oleraceum × C. palustre = *Cirsium × hybridum* KOCH ex DC. Fl. Franç. 6:463, 1815.

Fig. E13: *Cirsium ×hybridum*
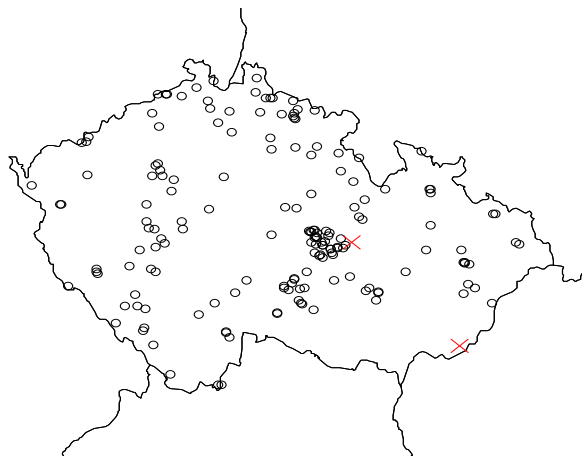
Cirsium oleraceum × C. rivulare = *Cirsium × erucagineum* DC. in LAM. et DC. Fl. Franç. 4:115, 1805.
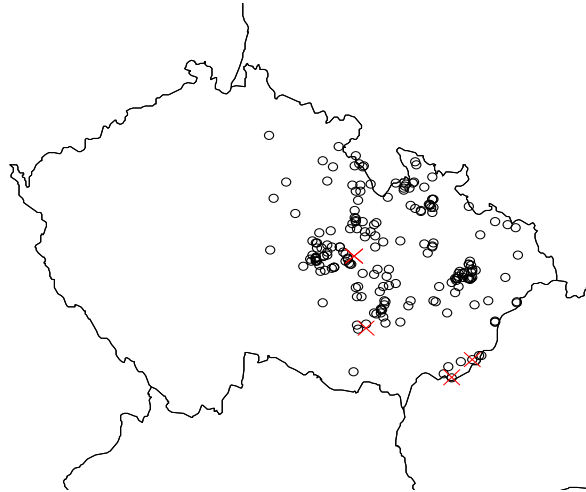
Fig. E14: *Cirsium ×erucagineum*

Cirsium palustre × C. pannonicum = *Cirsium × suspiciosum* BECK Fl. Nieder-Österreich 2/2:1243, 1893.
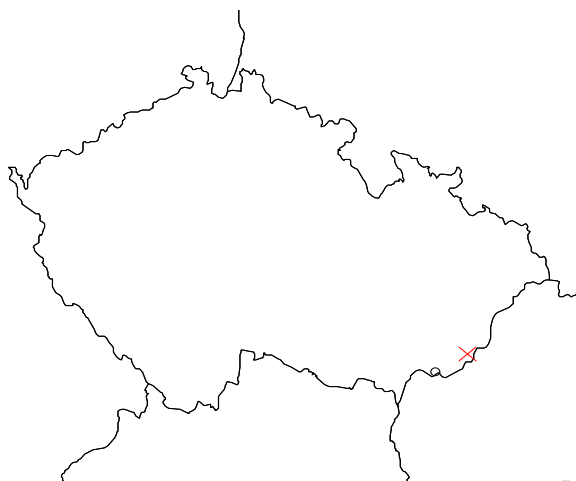
Fig. E15: *Cirsium ×subspiciosum*

Cirsium palustre × C. rivulare = *Cirsium × subalpinum* GAUDIN Fl. Helv. 5:182, 1829.
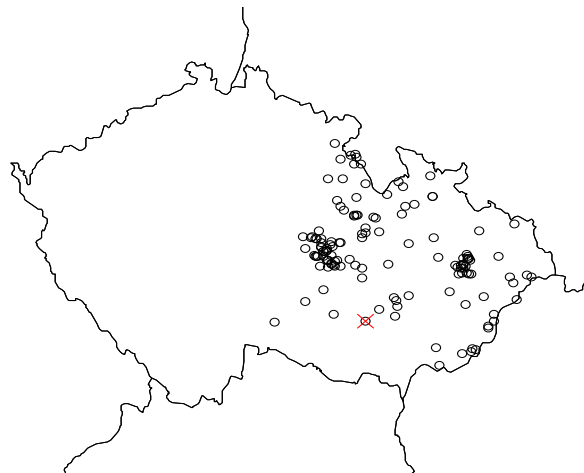
Fig. E16: *Cirsium ×subalpinum*

During our sampling we found new localities for some rarer hybrids that were not known when the distribution of thistles within Czech Republic was studied by BLIZŇÁKOVÁ 2010. E. g., there were no localities of *C. acaulon × C. canum* known from Moravia (see **Fig. E3**)[35]. It is possible that occurrence of this hybrid is underestimated in Moravia, because the hybrids look very similar to hybrids of *C. acaulon × C. pannonicum,* because *C. canum* and *C. pannonicum* look morphologically very similar. When we sampled this individual we also believed it was a hybrid of *C. acaulon × C. pannonicum,* but the molecular analysis clearly showed the sample was a hybrid of. *C. acaulon × C. canum.*

Probably the best record was a hybrid of *C. palustre × C. pannonicum* which was so far discovered only in proximity of Radějov (BUREŠ 2004). Our discovery was in the quarry of Rasová. These two localities, however, are close from one another and they belong to the same phytogeographic district – 78 (see **Fig. E15**). Although these two species frequently hybridize with other species, their hybrids are exceptionally rare. Since *C. palustre* is preferring wet, acidic soils and is found mostly in mountainous regions and *C. pannonicum* is found on dry, calcareous soils in pannonian lowland, they are both having very different ecological preferences and thus do not have much chance to meet and hybridize in the Czech Republic. The effect of post-zygotic barriers may also be the reason why do these species hybridize so rarely.

2. 3. New triple hybrid

Triple hybrids have been found across several plant genera, e. g., *Viola* (KIRSCHNER & SKALICKÝ 1990), *Aesculus* (DE PAMPHILIS & WYATT 1990), *Iris* (ARNOLD 1993), *Ephilobium* (KITCHENER, 1997), *Knautia* (ŠTĚPÁNEK 1997), *Senecio* HODÁLOVÁ 2002, *Quercus* (DODD and AFZAL-RAFII 2004), *Potamogeton* KAPLAN 2007, *Dactylorhiza* (DE HERT et al. 2011 and 2012), *Salix* (MEIKLE & ROBINSON 2000), *Fallopia* (BAILEY 2013) [36]. Although there are such studies, triple hybrids are actually very rare. Also only some of the above mentioned studies used molecular analysis to prove the existence of the putative triple hybrids. Most of them were only based on morphological examination. KAPLAN 2007 claimed that molecular evidence for three different species contributing to recent natural hybrid individuals is relatively scarce mentioning two articles. Nowadays more molecular evidence studies are published (DE HERT et al. 2011 and 2012, BAILEY 2013).

There have been also several reports of triple hybrids within genus *Cirsium.* Three triple hybrids have been reported within genus *Cirsium* in Czech flora (BUREŠ 2004:

Cirsium acaulon × C. canum × C. oleraceum = Cirsium × trigeneum BORNM. Mitt. Thüring. Bot. Ver., nov. ser., 36:41, 1925 nom. illeg., non PETRAK 1908.

Cirsium canum × C. oleraceum × C. rivulare = Cirsium × podperae FLEISCHER Österr. Bot. Z. 50:48, 1900.

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[35] Even though this hybrid is not mentioned in BLIZŇÁKOVÁ 2010, it has been found in Suchá Loz (White Carpathians) by Petr Filipov in 2005 (data from http://florabase.cz/).

[36] There are also experimentally produced hybrids, e. g., MRÁZ & PAULE 2006 produced triple hybrids of *Hieracium.*

Cirsium canum × C. oleraceum × C. palustre =Cirsium ×wettsteinii PETRAK Repert. Spec. Nov. Regni Veg. 5:329, 1908.

More hybrid combinations for central Europe are mentioned by WAGENITZ (1986). There are 31 triple hybrid taxa that were reported in central Europe. Five of these taxa are composed of species that are also growing in the Czech Republic:

Cirsium acaulon × C. oleraceum × C. palustre

Cirsium. acaulon × C. oleraceum × C. rivulare

C. heterophyllum × C. oleraceum × C. palustre = C. × fritschianum

C. oleraceum × C. palustre × C. rivulare

C. palustre × C. pannonicum × C. rivulare

The triple hybrid of *Cirsium acaulon × C. canum× C. pannonicum* (**Fig. E17**) we have discovered and proved using AFLP has never been reported before. Therefore we believe we found a new hybrid not only for flora of the Czech Republic, but also at least for flora of Central Europe.

Fig. E17: Triple hybrid of *C. acaulon, C. canum* and *C. pannonicum* (herbarium specimen)

We assume that the reason why this triple hybrid has not been reported is not its extreme rarity. WAGENITZ (1986) reported triple hybrids even among species that hardly ever hybridize and it is very scarce to find even their simple hybrids. This is the case of *C. palustre × C. pannonicum × C. rivulare.* Hybrids of the two species with *C. pannonicum* are extremely rare[37] In the case of our triple hybrid, all parental species are relatively commonly creating hybrids (see the maps above).

The reason, why this hybrid has not been discovered might be, that it is very inconspicuous and hard to determine. The parenthood of *C. acaulon* is easily determinable, because it is the only thistle within the Czech flora with almost no stem and it is very spiny. The other two species are, however, difficult to tell apart. *C. canum* is more robust –especially its roots are thick and the flower heads are rather big. The edges of its leaves are compact, and the veins are inconspicuous. *C. pannonicum* on the other hand is more slender, its roots are not thick and the leaf edges are mildly serrated. The leaf veins are more noticeable and there is a dot-like structure on the adaxial side of a leaf (BUREŠ 2004). If these characters, however, get combined with characters of short and spiny *C. acaulon*, they could be rather hidden or masked. Generally hybrids of *C. acaulon* and *C. canum* are more robust, whereas hybrids of *C. acaulon* and *C. pannonicum* are more slender. To determine this triple hybrid using only morphological characteristics is therefore very challenging and a careful morphological study will have to be done to describe it as a new taxon. Based on the molecular evidence, however, we may conclude, that this hybrid combination has been declared in the nature.

3. Geographical analysis of genetic variance

3.1. Trends in genetic variance within the whole genus

All approaches we used – PCO, NJ trees and AMOVA showed, that geographic pattern within our data is very small. Although PCO visualised some differences among studied regions (Brno, White Carpathians and Žďárské vrchy Hills) in our data[38], AMOVA (analysis of molecular variance) gave too little ΦPT values denying that the regions would be genetically diversified (see Results, **Tab. D1**). The reason why the analyses failed to distinguish different regions could be given either by the fact that the regions are not distinctive in reality or by too high marker variability. It is generally known that a common problem of phylogeographic analyses (such as AMOVA) is insufficient resolution ability of the used molecular marker (ZEISEK 2009).

Constructing NJ trees (see Results, **Fig. D39-D48**) we have remarked that samples from same localities tend to cluster together. This has leaded us to an assumption that although there is no significant genetic difference among the regions, the geographic pattern in genetic variation may be given by respective localities[39]. Intraspecific gene flow among

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[37] BLIZŇÁKOVÁ 2010 gives only 1 locality for *C. palustre × C. pannonicum* and 9 localities for *C. pannonicum × C. rivulare.*

[38] In some cases the pattern was more obvious, e. g., in *C. canum* (see Results, **Fig. D31**) in some cases it was less e. g. in *C. arvense* (see Results, **Fig. D30**).

[39] It would be interesting to test molecular variance (AMOVA) within and among respective localities in order to see whether the populations communicate with one another and intraspecific gene flow between the localities

thistle populations can be given by interpopulation pollen or seed dispersal (by insect or by wind; FREELAND et al. 2010) or other diaspores dispersal such as anthropogenic displacement of vegetative material by tillage (HETTWER & GEROWITT 2003).

According to KLEINJANS et al. (2012) the distance up to which bees may range up to is 2, 5, 10-14 km respectively. In practice, however, most pollen is transported over small distances and dropped again. Often only a small fraction (less than 1%) is carried long enough to mediate pollen transfer between plants more than 1 km apart. The probability of pollen transport and potential cross-pollination is expected to decrease exponentially with distance, not only because only few bees tend to fly long distances, but also because pollen is lost and replaced by new local pollen and because of loss of pollen viability. The thistles, however, can be also pollinated by bumblebees (DURKA et al. 2004) that can fly over larger distances (WALTHER-HELLWIG & FRANKL 2000). Thistles are also pollinated predominantly by a wide scale of other generalist pollinators[40] (PROCTOR et al., 1996) by Lepidoptera (e.g., Grass Skippers, or various species of Nymphalidae), Diptera (namely by Syrphidae) or by some other insect orders. In such type of pollination pollen exchange distance is usually less than 10 m (DE VERE 2007).

The seed transfer can reach larger distances, because thistle produce plumed achenes with a long pappus that allow far transfer (DURKA et al. 2004). As proved in *C. arvense* (the situation for other species would probably be very similar) the pappus, however, often breaks off the achene (BOSTOCK & BENTON 1979) and therefore the achenes hardly exceed the distance of 1 km. On the other hand HIGGINS and RICHARDSON (1991) and JUMP et al. (2003) declare, that the *Cirsium* species have wind-dispersible seeds, suggesting that occasional long-distance dispersal is likely.

The extent of anthropogenic transfer in the means of root fragment dispersal as a result of ploughing was studied in *C. arvense* by HETTWER & GEROWITT (2004). They found, that root fragment dispersal via soil cultivation was of minor importance. Since *C. arvense* is a ruderal species (BUREŠ 2004) and the influence of anthropogenic transfer should be therefore more significant for this species than for the other ones, we can assume that the effect of root dispersal on geographic pattern should be even more negligible in other species.

Having this prior knowledge we may assume, that intraspecific gene flow may only take place on very short distances[41]. We performed several attempts (because these attempts failed, they are not included in the thesis) to find some kind of breaking point spotting the distance in which there would be a significant drop in genetic similarity between the individuals. This point would stand for the gene flow limit. Such point was, however, not found in any of the studied species.

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takes place (ΦPT would be close to 0) or whether all populations are isolated from one another (ΦPT would be close to 1). The situation that ΦPT would be close to 1 most certainly would not become, but we could compare whether the genetic variation is rather given by long distances (among regions) indicating some kind of phylogeography or whether the genetic variation is given by short distances (among localities) indicating mosaic distribution of different genotypes in the landscape. But since the concern of our study was different and we were not aiming to do population genetics, we only had 3 samples per locality which is too little to base the analysis on.

[40] Literature research on generalist pollinators is was done by BUREŠ (unpublished data).

[41] KLEINJANS et al. (2012) however say that it is difficult to quantify the impact of pollen transfer on possible gene flow.

When we divided genetic distances between samples into groups according to respective intervals of geographic distances and plotted the data as boxplots we saw that the genetic similarity tends to decrease with increasing distance for all species (see Results **Fig. D50**). The only exception was *C. pannonicum,* where the genetic similarity was the highest at the beginning and was decreasing till 4 - 16 km and then it started increasing again. This is most likely to be an artefact given by small number of sampled individuals for this species. In the other species we may observe a pattern indicating isolation by distance. The gene flow is the strongest on short distances and gets weaker with the increasing distance.

Our results, however, were not completely agreeing with JUMP et al. (2003). The authors examined only three species – *C. acaulon, C. arvense* and *C. heterophyllum* – using microsatellites. In order to detect possible isolation by distance they performed Mantel test[42]. They found isolation by distance within *C. acaulon* and *C. arvense*, but not within *C. heterophyllum.* Our results, however, showed, that isolation by distance within *C. heterophyllum* is certainly steeper than in the case of *C. arvense.* JUMP et al. (2003) themselves are taken aback by their result and discuss possible causes of this lack of isolation by distance. They suggest that it can be caused by long-distance dispersal (which is then rejected, because within their dataset there are localities 1 500 km far apart and no such long-distance dispersal has been reported for wind-dispersed plants (CAIN et al. 2000). Another possible explanation suggested is the rapid range expansion. The last explanation they suggest is that it would be advisable to determine whether greater spatial structure of genetic variation in these *Cirsium* species might be detected by alternative molecular markers. Since our AFLP based data showed that the decrease in genetic similarity with increasing distance is steeper than in *C. arvense*, we may assume that AFLP is a more suitable marker in searching for geographic pattern for *C. heterophyllum* than the selected microsatellites.

3.2. Genetic variance and species biology

Even though we did not prove any significant genetic differences among regions, there were differences in ΦPT values among species (see Results, **Tab. D1**). When plotting the ΦPT values as boxplots according to the position of studied populations in nature range of particular species range, i. e., whether the Czech Republic is on the margin or inside, we can see that there is a difference between "range-margin" species and "intra-range" species [43](see **Fig. E18**). The maps for visualising total distribution of respective species were taken from MEUSEL & JÄGER (1992). "Range-margin" species *C. acaulon, C. canum, C. pannonicum* and *C. rivulare* are having higher ΦPT values. Each of these species has to some extent its range margin in the Czech Republic. *C. acaulon* is West European species occurring from Great Britain to the western side of Slovakia. In the Czech Republic, there is an evident decrease in quantity of localities from the west to the east. *C. canum* and *C. pannonicum* are reaching its western limit in Bohemia and *C. rivulare* is reaching its western margin even sooner. One of the most western localities of last mentioned species is in our studied region

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[42] We did not apply Mantel test for final dataset, because recently it has been criticised for its misleading and inaccuracy (GUILLOT & ROUSSET 2013). The results of pilot analysis using Mantel test for our data were also hard to interpret and not consistent with results we can see e. g. in boxplots (**Fig. D50**).

[43] The result was, however, not significant (p=0.057; Mann-Whitney test), possibly because there were too few numbers.

Žďárské vrchy Hills. Intra-range species – *C. arvense, C. oleraceum, C. palustre* and *C. vulgare* – are having lower ΦPT values. The reason why range-margin species have higher genetic variance among regions could be their ecological limitation. Populations typically become smaller and more fragmented as species approach their ecological limits near range limit (BROWN et al. 1995, THOMAS & KUNIN 1999, BRIDLE & VINES 2007). This fragmentation at range margins may result in increases in among-population (for our case regional) differentiation. ECKERT et al. (2008) reviewed 134 studies representing 115 species that were tested for increases in among-population differentiation towards range margins using nuclear molecular genetic markers. More than 70 % of studies showed increased differentiation. Our results are consistent with this prevailing trend.

JUMP et al. (2003) also showed, that there was low variation among populations within *C. arvense,* which is an intra-range species in Great Britain as well as it is in the Czech Republic. The result for *C. arvense* was also fortified by DURKA et al. (2004) who examined this species in southern Germany. As well as in our case, higher variation among populations was found in *C. acaulon,* which in Great Britain reaches its western margin (JUMP et al. 2003)*.* Results obtained for *C. heterophyllum* by these authors could not be compared, because we only had one region, where *C. heterophyllum* was sampled.

The species that showed the lowest total genetic variation among regions (ΦPT = 0.03) was *C. arvense*. Although it is a clonal species and we may therefore suppose that every locality may persist of only one large clone, when visualised as NJ tree (see Results, **Fig. D30**), we could clearly see that samples from the same localities do not tend to cluster together as much as in the other species[44]. There was also only a very narrow decrease visualised by boxplots indicating only slight effect of isolation by distance in this species. The reason why *C. arvense* is so distinct from the other species is probably that it is a widely distributed and strongly ruderal species. HETTWER & GEROWITT (2004) studied patch populations of *C. arvense* in arable fields. They found that clonal reproduction of *C. arvense* when colonising new localities was only of minor importance[45]. (Sub) dioecious[46] *C. arvense* is almost a strict outcrosser (unlike other thistle species that are either gynodioecious or even hermaphrodites). The combination of clonal reproduction with the recruitment of sexually outcrossed seedlings in the first years of colonization allows the species to perform efficient establishment even with founder effects, to undergo selection without loss of diversity. Every population then consists of many different genets.

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[44] This proves that sampling was designed well to avoid clonality.

[45] Although it has got an ability to reproduce clonally, because it can form long plagiotropic roots (BUREŠ 2004).

[46] Incompletely dioecious; male flower head could contain a few hermaphrodite florets resulting in a few achenes per capitulum (KAY 1985).
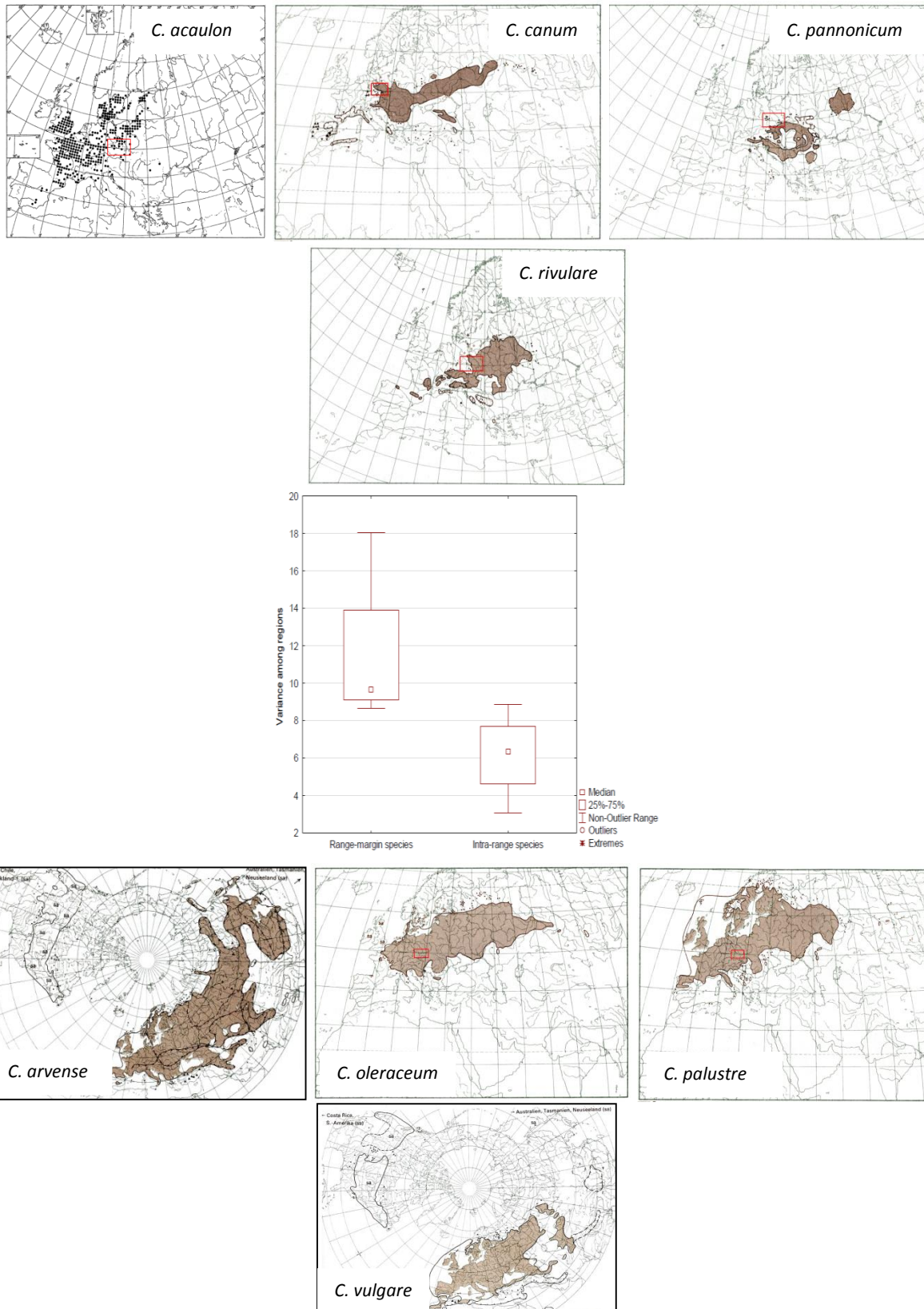
Fig. E18: Differences in ΦPT values of range-margin species (maps above) and intra-range species (maps below); STATISTICA and MEUSEL & JÄGER (1992).

C. arvense – being a C-strategist (GRIME et al. 1988) – is very successful in colonizing new, empty, ruderal habitats[47], but is not so successful in enforcing itself into natural or artificial plant communities with a dense plant cover (DURKA et al. 2004), because the seedlings are very susceptible to shading and competition (BAKKER 1960). This strategy appears to be very efficient in *C. arvense* and may have contributed to the worldwide success of this species (DURKA et al. 2004). Its success is mainly given by people, who create suitable, ruderal habitats and therefore this species can spread rapidly.

It is generally known that populations in unstable habitats tend to have a higher genotypic diversity compared with populations in stable habitats (PIQUOT et al. 1996). This might also be the reason for lower genetic variance among regions not only in *C. arvense,* but also in another ruderal species *C. vulgare*[48]. Other "intra-range" species with low genetic variance among regions – *C. oleraceum* and *C. palustre* – are found in stable habitats of wet meadows, however, recently they are successfully spreading along road ditches like ruderal species. The range-margin species are not found in ruderal habitats with the only exception – *C. canum* – which is having similar ecological preferences like *C. oleraceum* (although it never reaches so high altitudes) and spreads along the road ditches, but its genetic variance among regions was the highest.

4. Genetic diversity

Our results showed that species differ significantly in their intraspecific genetic variations (see **Results, Fig. D51** and **Tab. D2**). The only two species that did not differ significantly were *C. acaulon* and *C. pannonicum.* When visualising genetic variability (average genetic similarity) of all species as barplot (see **Fig. E19**), we can see that genetic similarity within monocarpic species (*C. eriophorum, C. palustre* and *C. vulgare*) is higher than that within polycarpic species.
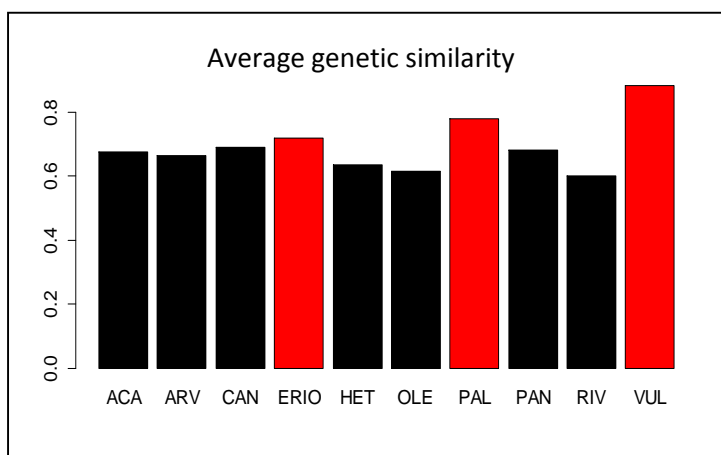
Fig. E19: Average genetic similarities of all species. In red colour there are monocarpic species and in black colour there are polycarpic species.

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[47] Like in other clonal plants (WOLF et al. 2000), the recruitment of *C. arvense* seedlings is possible only during the early phases of colonization (DURKA et al. 2004).
[48] This species also showed little locality clustering when visualised as NJ tree, but it could be also given by the fact that *C. vulgare* is a monocarpic species.

We performed Mann-Whitney test in order to see, whether this difference is significant. The test showed, that genetic variability within the monocarpic species are significantly higher (Z = 84.59501, p = 0.00) than in polycarpic species (see **Fig. E20**).
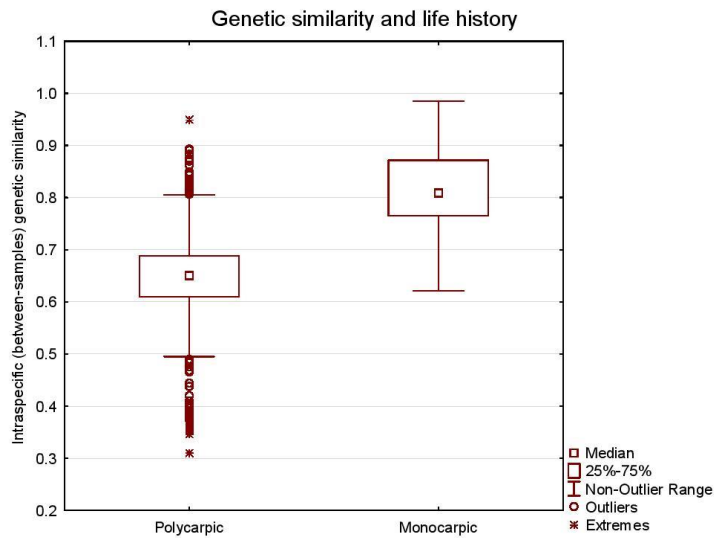
Fig. E20: Boxplots of genetic similarities within polycarpic and monocarpic species

We compared genetic variabilities of all species with variables that would possibly explain their pattern (see Materials and Methods, **Tab. C4**). The variability in average genetic similarities among species cannot be explained by the ability of a species to create hybrids (see **Fig. E21**).
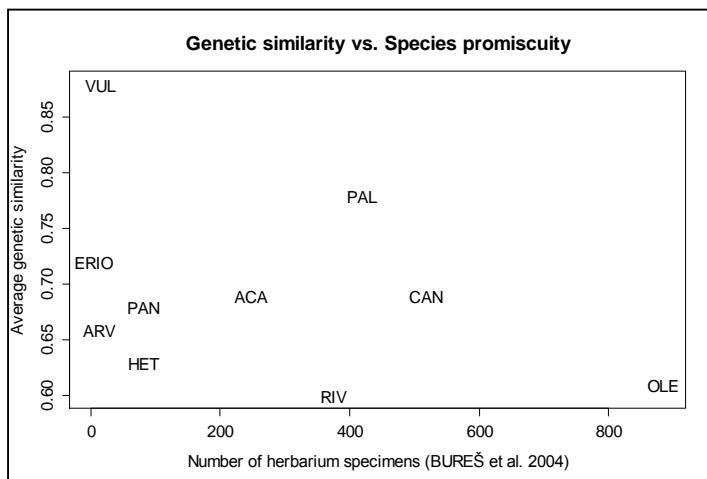
Fig. E21: Genetic variability vs. Species promiscuity; Spearman´s rho = -2.918, p-value = 0.413; (R 3.2.3)

There was a positive correlation (p = 0.0498) found between genetic variability and genome size (see **Fig. E22**). This correlation was, however, caused by outlier *C. vulgare* which is a tetraploid, whereas other taxa are diploid (BUREŠ et al. 2004). The ploidy level of the taxa under investigation may affect the amount of variability observed (ROBINSON & HARRIS 1999, JIMÉNEZ et al. 2008), which may result in higher genetic similarity within taxa with higher ploidy level. After removing *C. vulgare* from the dataset and repeating the analysis,

Spearman´s rho was 0.2437 and p = 0.1768 showing that if we exclude *C. vulgare*, there is no significant relationship between genetic variability and genome size.
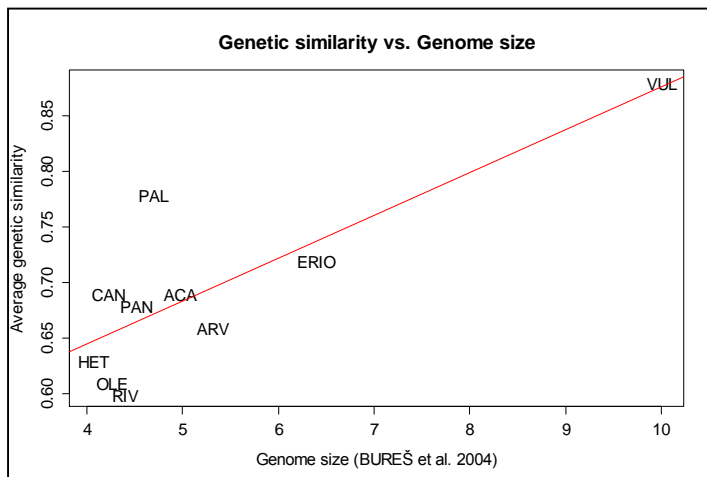
Fig. E22: Genetic variability vs. Genome size; Spearman´s rho = 0.6322, p-value = 0.0498; (R 3.2.3)

The only relationship was found when we compared genetic variability with average number of females (p = 0.024). There was a negative correlation between these two variables (see **Fig. E23**), showing that intraspecific genetic variability is lower in species that are having few females (especially in hermaphrodite species *C. vulgare* and *C. eriophorum* that are having almost no females in their populations) and is higher in species with more females (gynodioecious or dioecious species).
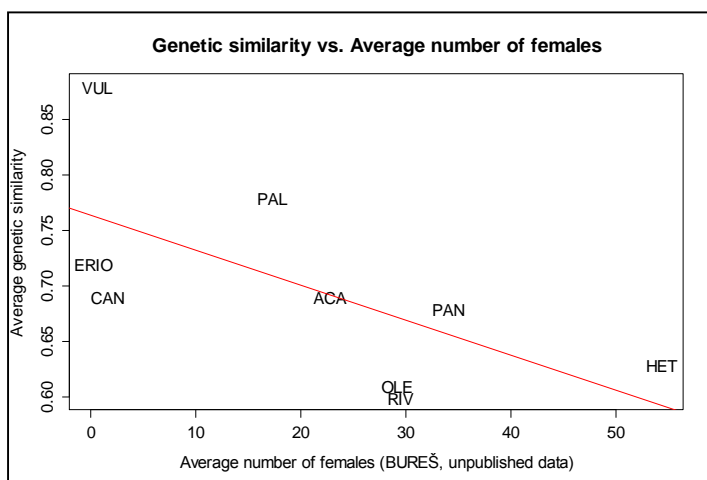
Fig. E23: Genetic similarity vs. Number of females; Spearman´s rho = - 0.733, p-value = 0.024

We suppose that significant relationship between lower genetic variability and monocarpy as well as significant decrease in genetic variability within species with fewer females could be given by the fact that both monocarpic species and species with few females[49] are obliged to reproduce via selfing more often than polycarpic taxa or taxa with more females in populations. MABLE & ADAM (2007) claim that selfing populations (in our case we apply

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[49] Almost negligible percentage of females in populations is in hermaphrodite species *C. canum, C. eriophorum* and *C. vulgare*. Two of these species (*C. eriophorum* and *C. vulgare*) are also monocarpic. Another monocarpic species is *C. palustre,* which also has a relatively low percentage of females in populations.

the term populations on the different species) have lower genetic variability than outcrossing populations. The extent of self-compatibility in thistle species has, however, never been studied and therefore we do not know exactly, to what extent are the monocarpic and hermaphrodite species actually more vulnerable to selfing than the polycarpic and gynodioecious species. We can, however suppose that the polycarpic species may afford to "wait" for the pollen till the next season rather than investing energy to self-pollination which is disadvantageous, because it may lead to inbreeding depression (CHARLESWORTH & CHARLESWORTH 1987)[50], whereas the monocarpic species are urged to reproduce during the only flowering season otherwise the genetic information of an individual will be lost forever (BUREŠ, in verb).

So far there is, however, no published support for our results. There are very few studies based on a comparison of genetic diversity and biology of two (or more) respective species[51]. Comparison of interspecific genetic diversity may be also so sparsely studied, because there are numerous biological traits that may potentially affect the species diversity. Apart from our examined variables (i. e., species promiscuity, genome size, life history and number of females in populations) the loss in genetic diversity can be also given by other aspects e. g., species rarity (GIBSON et al. 2008) or range margin effect (ECKERT et al. 2008). There are very few genera that have been so carefully studied in the means of all possible biological characteristics like it has been studied in the case of the genus *Cirsium*. A decent knowledge of thistles biology may allow us to pronounce some verdict about differences in genetic diversity among species, nevertheless the reality may be much more complex.

Our original aim was studying genetic diversity in order to understand to what extent is genetic diversity of a species influenced by its ability to hybridize and whether it can tell us something about possible gene flow. Our results, however, indicate that there is no relationship between species promiscuity and genetic diversity and a more likely explanation for differences among species may be given by different reproduction strategies.

5. Interspecific gene flow identification

In order to detect gene flow, we compared the regional differences in average genetic diversity among chosen species (co-occurring and potentially hybridizing only in certain regions). Our assumption, that if there was gene flow between two hybridizing species, the genetic similarity between these species should be higher in the regions where they co-occur than in the regions where only one of these species is present, was not approved by our results. The results were corresponding to our assumption only in some cases (see Results, **Tab. D3**). The differences in similarities were also extremely small (of the order of

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[50] Preference of pollen of other individual may be so strong that an individual uses non-conspecific pollen of rather than using its own. This results in frequent interspecific hybridization. The individuals using foreign pollen do not have to be only females (that might not have other choice), but also hermaphrodites (e. g., *C. canum* – being a hermaphrodite species – is having an extremely low number of females, but a high number of interspecific hybrids).

[51] If the authors are dealing with genetic diversity, it is mostly comparing of genetic diverzity among populations of one species with a characteristic biology (HAMRICK 1989). E. g., there was a genetic diversity study done for a monocarpic species *Saxifraga mutata* (HOLDEREGGER 2008), but there was no comparison with other taxa of this genus.

thousandths). Our results therefore did not approve, that there is interspecific gene flow among these analysed species and since the correspondence of expected and observed genetic similarity between species was more or less random, it does not make sense to comment putative gene flow in respective species combinations. The reason, why we were not able to detect any gene flow within species represented by "pure" individuals most probably was not, that there would be no gene flow taking place, because if two species hybridize, there should be gene flow proceeding between them and our data set is certainly robust enough to spot it.

It is very complicated to find potential gene flow when examining so many species at once. Gene flow and possible introgression detection using AFLP are predominantly analysed in STRUCTURE (HIPP & WEBER 2008, KRONFORST 2008, SMISSEN et al. 2008, BLAIR & HUFBAUER 2009, BACON et al. 2011, THOMPSON et al. 2013, MARQUES et al. 2014, MARCZEWSKI et al. 2015 and others), but STRUCTURE was not applicable for our data and was not even able to identify the hybrids successfully (see chapter E. 2. 1. Two models comparison). The reason why the approach is inapplicable for our data might be, that STRUCTURE analyses in the papers above are based on comparison of only two, maximally three species, whereas we are comparing 10 species[52].

A similar yet more sophisticated approach to that we used to detect gene flow in our data set was succesfully detected by LEY & HARDY (2014), who also used AFLP. They examined different species pairs using geographic correlation in shared alleles and found gene flow between two sister species *Marantochloa monophylla* and *M. incertifolia.*

Within this pilot study we were not able to detect gene flow among our species, but the question is, whether more sophisticated statistical approach would solve our problem. Although our sampling strategy was done very carefully, the problem might be insufficient resolution of the AFLP marker. AFLP was well applicable for other problems we were solving (and we would never be able to obtain such results from, e. g., microsatellites, because we would never have so many primer pairs applicable for all species or cpDNA sequencing, because they do not have enough variability; LAJKEPOVÁ 2013), but because it suffers several methodical problems, it might not be useable for such a concrete study as detection of introgressed fragments of genomes of other species within a "pure" individual.

The most serious limitation of using AFLP for our purpose is that the marker is dominant (VOS et al. 1995), which disables us to see any heterozygous states that would carry the crucial information. Other often mentioned problem – homoplazies in fragment lengths (ROBINSON & HARRIS 1999) – should not be so grave, because our studied species are closely related, yet we should always keep also this limitation into account.

Other approach that would solve these methodical problems and would potentially be very successful in gene flow detection are the methods based on next generation sequencing, e. g., RADSeq (restriction-site associated DNA). Like AFLP, RADSeq is a genome wide marker and it is also universal and can therefore be used for any organism. RADSeq, however, does not "read" the genome in the means of presences or absences of locus in certain position, but in the means of actual sequences. This means, that homoplazies within the dataset are enormously reduced and since it is a co-dominant marker we may observe both

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[52] To be more accurate, we compare 7 species, because we do not expect any gene flow proceeding in non-hybridizing species (*C. arvense, C. eriophorum* and *C. vulgare*).

alleles of the locus, not only the dominant one (DAVEY & BLAXTER 2010)[53]. Nowadays, the use of RADSeq is still limited mostly because the molecular analyses are quite expensive and subsequent bioinformatic processing is very skill demanding and time consuming. Nevertheless, RADSeq has been successfully applied in gene flow detection by e. g., NADEAU et al. (2013) and SUPPLE et al. (2015) in butterflies or by STÖLTIONG et al. (2013) in poplars.

F. Conclusion

Within the diploma thesis we examined interspecific hybridization and gene flow from various aspects using AFLP analysis in the model genus *Cirsium*. We performed molecular approval of samples of both morphologically pure individuals and interspecific hybrids using a Bayesian approach (in STRUCTURE software) and our own script. Both methods were successful in molecular approval of "pure" samples, but the STRUCTURE failed in hybrid parents' detection. Although STRUCTURE is often used for finding putative parents, when applied for our data it showed a very bad resolution and many hybrids were determined incorrectly. Our script, on the other hand, was reliable and determined overwhelming majority of samples according to our prior morphological determination. We proved molecularly 14 out of 31 hybrid combinations already reported within the Czech flora, including a very rare hybrid of *C. palustre* × *C. pannonicum* that has been reported only from one locality in the Czech Republic. We have also detected a triple hybrid of *C. acaulon, C. canum* and *C. pannonicum.* This combination has never been reported neither within the Czech flora, nor anywhere else. Although this hybrid was proved molecularly, a careful morphological study will have to be done to describe it as a new nothotaxon.

Different approaches we used for detecting geographic pattern within our data – PCO, NJ trees and AMOVA – showed that most of intraspecific genetic variance is found within the respective regions, whereas the variance among regions is very low indicating that the regions are not genetically diversified. Based on AMOVA results we realised, that species, that occur at their range margin in the Czech Republic (*C. acaulon, C. canum, C. pannonicum* and *C. rivulare*) show more diversification between regions than "intra-range"[54] species (*C. arvense, C. oleraceum, C. palustre* and *C. vulgare*). The reason why "range-margin" species have higher genetic variance among regions could be their ecological limitation. Populations typically become smaller and more fragmented as the species approach their ecological limits near range limit. This fragmentation at range margins may result in increase of regional differentiation. Although we did not find any significant difference among regions, we detected that there is a decrease in genetic similarity between the individuals with increasing geographical distance. This trend – isolation by distance – is remarkable in all species[55].

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[53] TURNER et al. (2013) studied phylogeny of a genus that underwent rapid adaptive radiation using AFLP. The marker, however, did not provide enough resolution to reveal the phylogenetic relationships among the species. It was not until RADSeq was applied the authors were finally able to reconstruct the phylogeny (PAUN et al. 2015).
[54] Intra-range species means that the Czech Republic is within the species geographic range.
[55] Except for *C. pannonicum* which is probably the result of insufficient sampling.

The analyses revealed, that species genetic diversity is not significantly related with species promiscuity and (Spearman´s rho = -2.918, p = 0.413) nor with the genome size (Spearman´s rho = 0.2437, p = 0.1768). More probable determinant of species genetic diversity could be the reproductive strategy. Species that are more vulnerable to self-pollination (monocarpic species and species with low female frequency) had significantly lower genetic diversity than species that may afford to wait for the foreign pollen and reproduce via outcrossing (polycarpic species and species with high number of females). The result of Mann-Whitney test comparing genetic variability within monocarpic and polycarpic species approved that there is lower variability within monocarpic species (Z = 84.59501, p = 0.00). Spearmann´s correlation coefficient showed a significant negative correlation between species diversity and average number of females (Spearmann´s rho = – 0.733, p = 0.024).

Our assumption, that if there was gene flow between two hybridizing species, the genetic similarity between these species should be higher in the regions where they co-occur than in the regions where only one of them is present, was not approved by our approach. The results were corresponding to our expectations more or less randomly. The question is, whether potential use of more sophisticated statistical methods would help, or whether the gene flow within our dataset is not detectable due to AFLP methodical limitations.

G. Literature

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The photographs, if not mentioned else wise, were taken by Petr Bureš and Ester Lajkepová.

H. Supplements

1. Protocols

1. 1. DNA isolation

- homogenise a deep frozen sample in liquid nitrogen using mortar and pestle
- put 2 mg of homogenised sample into cooled 2 ml eppendorf tubes
- add 300 μl of PL2 buffer and shake
- 10 min incubation/65°C
- add 10 μl of RNase, shake gently
- 10 min incubation/56 °C
- add 75 μl of PL3 buffer, shake gently
- 5 min incubation/0°C
- centrifuge at maximal rotations (13 400 rpm) /5 min
- put supernatant to eppendorf tubes with purple filters
- centrifuge at rotations (8 000 rpm)/2 min
- pipette centrifuged supernatant into eppendorf tubes with 450 μl of PC buffer
- mix supernatant with PC buffer
- put the solution into eppendorf tubes with green filters
- centrifuge at rotations (8 000 rpm)/1 min
- pour away the solution that passed the membrane
- add 400 μl of PW1 buffer
- centrifuge at rotations (8 000 rpm)/1 min
- pour away the solution that passed the membrane
- add 600 μl of PW2 buffer
- centrifuge at rotations (8 000 rpm)/1 min
- pour away the solution that passed the membrane
- add 200 μl of PW2 buffer
- centrifuge at rotations (8 000 rpm)/2 min
- pour away the solution that passed the membrane
- put the green filters into clean eppendorf tubes
- add 40 μl of TE buffer warmed up to 65°C
- incubate in room temperature for 10 min
- centrifuge at maximal rotations (13 4000 rpm)/30 s
- repeat previous 3 steps 2 times
- pipette the whole content into 0.6 ml eppendorfs
- store at 20°C or at -80°C in case of long time storing

1. 2. AFLP

Restriction-ligation reaction (RLR)

- prepare reaction mix for RLR according to **Tab. H1**
- set PCR cycler according to **Tab. H2**
- dilute final product with TE buffer (1 : 10)

Tab. H1: Reaction mix for RLR.

	Storage concentration	Pipetted volume (1x)	Final concentration
DNA	20-80 ng/µl	6,2 µl	
10x T4 ligation buffer*	10 x	1,1 µl	1x
NaCl	0,5 M	1,1 µl	50 mM
BSA	10 mg/ml	0,055 µl	50 ng/µl
MSE I (NEB)*	10 U/µl	0,1 µl	1 U/reaction
ECOR I (NEB)*	20 U/µl	0,25 µl	5 U/reaction
T4 DNA ligase (NEB)*	400 U/µl	0,168 µl	67,2 U/reaction
MSE I ADAPTOR **	25 µM	1 µl	2,27 µM
ECOR I ADAPTOR ***	2,5 µM	1 µl	0,23 µM
ddH₂O		0,027 µl	Add to 11 µl
Total		11 µl	

***Chemicals from New England Biolabs
** MSE I ADAPTOR: 5´GAC GAT GAG TCC TGA G, 3´TAC TCA GGA CTC AT
*** ECOR I ADAPTOR: 5´CTC GTA GAC TGC GTA CC, 3´AAT TGG TAC GCA GTC TAC

Tab. H2: Cycler program for RLR reaction.

Time	Temperature	
4 h	37°C	RLR reaction
20 min	65°C	Enzyme inactivation

Preselection reaction (PES)

- prepare reaction mix for PES according to Tab. J. 3, used primers see **Tab. H3**
- set PCR cycler according to **Tab. H4**
- dilute final product with TE buffer (1 : 20)

Tab. H3: Reaction mix for PES

	Storage concentration	Pipetted volume (1x)	Final concentration
Diluted RLR (1:10)		4 µl	
Taq polymerase*	1 U/µl	1 µl	1U/ reaction
dNTP*	10 mM each	0,24 µl	120 nM each
Primer E-PES**	50 µM	0,08 µl	200 nM
Primer M-PES***	50 µM	0,08 µl	200 nM
Buffer with MgCl$_2$*	10 x	2 µl	1 x
ddH$_2$O		12,6 µl	Add to 20 µl
Total		20 µl	

* Chemicals from Top-Bio (Czech Republic)

Tab. H4: Cycle programme for PES reaction.

Time	Temperature	
2 min	70 °C	initial
20 sec	94 °C	
30 sec	56 °C	20x
2 min	72 °C	
30 min	60 °C	terminal
∞	4 °C	

Tab. H5: Overview of primer combinations used for PES and SEL (commercial primers, New England Biolabs, Great Britain)

	Primer	5´end modification	Annealing temperature	5´ - 3´ primer sequence
PES				
	E-PES		56	GAC TGC GTA CCA ATT C
	M-PES		56	GAT GAG TCC TGA GTA A
SEL				
1	E-SEL	ACT-FAM	66/55	GAC TGC GTA CCA ATT C
	M-SEL	CTC	66/55	GAT GAG TCC TGA GTA A
2	E-SEL	AGG-JOE	66/55	GAC TGC GTA CCA ATT C
	M-SEL	CAG	66/55	GAT GAG TCC TGA GTA A
3	E-SEL	AGC-NED	66/55	GAC TGC GTA CCA ATT C
	M-SEL	CAT	66/55	GAT GAG TCC TGA GTA A

6.2.3. Selection reaction (SEL)

- prepare reaction mix for SEL according to **Tab. H6**, used primers see **Tab. H5**.
- set PCR cycler according to **Tab. H7**
- after finishing last SEL, mix all three SEL reactions to one eppendorf tube and send to fragmentation analysis

Tab. H6: Reaction mix for SEL

	Storage concentration	Pipetted volume (1x)	Final concentration
Diluted PES (1:20)		4 µl	
COMBI *Taq* polymerase*	1 U/µl	1 µl	1U/ reaction
dNTP*	10 mM each	0,25 µl	125 nM
Primer E**	1 µM	1 µl	50 nM
Primer M***	4 µM	1 µl	200 nM
Buffer s MgCl$_2$*	10 x	2 µl	1x
ddH$_2$O		10,75 µl	Add to 20 µl
Total		20 µl	

* Chemicals form Top-Bio (Czech Republic)

Tab. H7: Cycle programme for SEL.

Time	Temperature		
2 min	94 °C	initial	
20 sec	94 °C		
30 sec	66 °C; Drop - 1 °C/cycle*		10x
2 min	72 °C		
20 sec	94 °C		
30 sec	56 °C		20x
2 min	72 °C		
30 min	60 °C	terminal	

* Gradual dropping of temperature by 1 °C per each cycle set.

2. R- script

```
# 2.1. Map construction
library(sp)
library(rworldmap) # Basic world maps
library(rworldxtra)
pal<-read.Tab.("clipboard", header=F, sep="\t", row.names=1)
# pal - matrix of two columns [ ,1] = latitude, [ ,2] = longitude
windows(width = c(48.5, 51), height =c(12, 19))
plot(x=getMap(resolution="high"), xlim=c(12, 19), ylim=c(48.5, 51), asp=2,
lwd=1.5)
points(x=pal[ ,2], pal[ ,1], pch=4, col="red", cex=0.7)

# 2.2. NJ construction
library(vegan)
library(ape)
library(phangorn)
ALL_SAMPLES<-read.Tab.(file="clipboard", header=T, sep="\t", row.names=1)
# ALL_SAMPLES - binary matrix from original data
ALL_SAMPLES_dist<-vegdist(ALL_SAMPLES, method = "jaccard")
windows(width=11,height=11)
par(mfrow=c(1,2));
treeNJ <- NJ(ALL_SAMPLES_dist)
plot.phylo(x=treeNJ, type="unrooted", main= "Neighbour joining tree - all
samples", show.tip.label = F)
groups<-factor(gsub('(.).', '\\1', abbreviate(row.names(ALL_SAMPLES), 3,
strict=T)))
cols<-c("deepskyblue1", "deeppink","darkorchid4", "goldenrod2", "yellow",
"darkgray", "darkorange3", "darksalmon", "forestgreen", "red")[groups]
tiplabels(pch=21, cex=0.5, bg=cols)

#2.3. Sample membership identification - species identity identification
spec_ident<-read.Tab.("clipboard", header=T, sep="\t", row.names=1)
# spec_ident: values of STRUCTURE/script by Jakub Šmerda, every column
represents one species for the values see Tab. H33-H36.
plot(as.factor(colnames(spec_ident)), spec_ident[1,], type="n",
ylim=c(min(spec_ident),max(spec_ident)), axes=F,
main="C. vulgare", ylab="Species identity %", xlab="Species")
axis(1, at=1:10, lab=c("ACA","ARV","CAN", 'ERIO', "HET", "OLE", "PAL",
"PAN", "RIV", "VUL"))
axis(2)
for(i in 1:ncol(spec_ident))
{
lines(as.factor(colnames(spec_ident)), spec_ident[i,], col=i);
points(as.factor(colnames(spec_ident)), spec_ident[i,], col=i);
}

#2.4. Geography pattern identification - 3D PCO
library(scatterplot3d)
library(vegan)
ACA<-read.Tab.(file="clipboard", header=F, sep="\t", row.names=1)
ACA_dist<-vegdist(ACA, method = "jaccard")
pcoa_ACA<-cmdscale(ACA_dist)
#Divission of samples according to their geographical region
groups<-factor(gsub('(.).', '\\1', abbreviate(row.names(ACA), 5,
strict=T)))
#Colours of geographical regions; tomato=Brno, yellow=White Carpathians,
#cornflowerblue=Zdarske vrchy
cols<-c("tomato", "yellow", "cornflowerblue")[groups]
scatterplot3d(pcoa_ACA, pch=21, bg= cols, type="h",
        main="C. vulgare")
```

```
#To visualise eigenvalues
ACA_eig<-pcoa(ACA_dist)
ACA_eig$values

#2.5. Spearman´s coefficient calculation
spearman.test.data<-read.Tab.("clipboard", header=T, sep="\t", row.names=1)
# spearman.test.data (see Materials and methods Tab. 3)
library(Hmisc)
spearman(spearman.test.data[ ,1], spearman.test.data[ ,2])
spearman.test(spearman.test.data[ ,1], spearman.test.data[ ,2])
fit <- lm(y ~ x, data=spearman.test.data)
summary(fit) # show results
reg1 <- lm(spearman.test.data[ ,1]~spearman.test.data[ ,2])
plot(spearman.test.data[ ,1]~spearman.test.data[ ,2], cex=1.5, type= "n",
ylab="Average genetic distance", xlab="Number of hybrids per species",
main="Genetic distance vs. Species promiscuity")
text(x =spearman.test.data[ ,2],spearman.test.data[ ,1], labels =
row.names(spearman.test.data))
abline(reg1, col="red")
```

3. Sampling localities

3.1. Sampling localities of respective species

Tab. H8: Localities of *C. acaulon*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K6	Březová: dry grassy vegetation, natural reserve of Cestiska, N of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'42.455"N, 17°43'47.895"E	3	ARV
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	2	CAN, VUL
K21	Suchov: E slopes, W side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'1.561"N, 17°34'37.882"E	3	VUL
K24	Nedašov: spring meadow at the neutral reserve, E side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'4.898"N, 18°5'58.190"E	3	PAL, RIV
K25	Nedašov: S slopes of natural reserve, SE side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'53.921"N, 18°6'18.073"E	4	PAL, RIV
Z3	Chrast: dry slopes in natural reserve Altán, P. Bureš & J. Bureš 19. 5. 2011	49°54'19.000"N, 15°56'54.968"E	2	
Z4	Střemošice: dry marlite slopes, W side of the road, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°53'11.795"N, 16°4'6.777"E	3	
Z5	Široký Důl: marlite slopes, N of the village, P. Bureš & J. Bureš 20. 5. 2011	49°44'51.350"N, 16°13'15.179"E	3	
Z10	Libice nad Doubravou: dry marlite slopes above the road, NW side of the village, E. Lajkepová & P. Bureš 3. 7. 2011	49°45'6.078"N, 15°41'52.133"E	2	
Z11	Běstvina, E. Lajkepová & P. Bureš 3. 7. 2011	49°50'32.643"N, 15°35'41.298"E	3	VUL
Z27	Chotěnov: slopes, S side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°48'54.953"N, 16°11'9.631"E	3	CAN, OLE
Total			31	
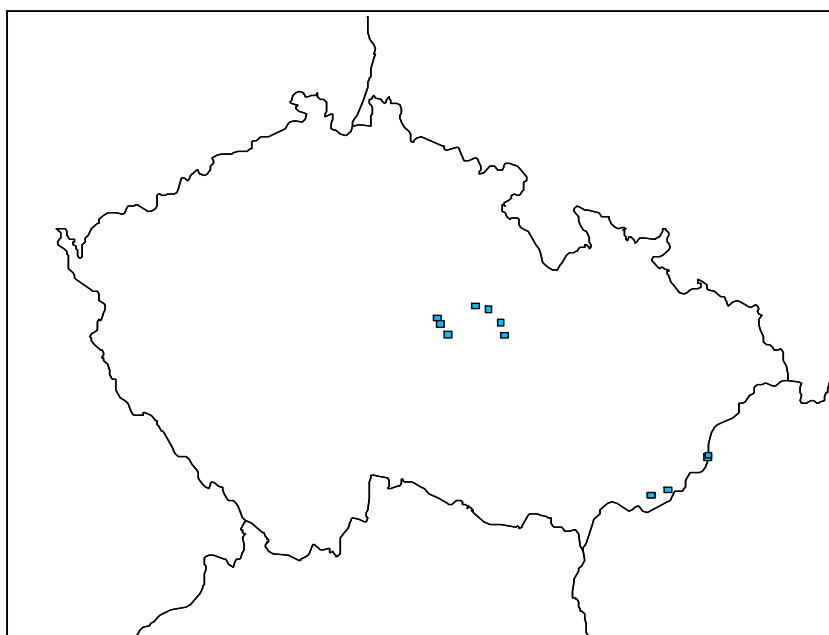
Fig. H1: Map of localities of *C. acaulon*

Tab. H9: Localities of *C. arvense*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B4	Lelekovice: ruderal margins of field path, NW of village, P. Bureš & J. Výhodová 22. 5. 2011	49°17'10.459"N, 16°34'6.843"E	3	
B5	Střelice: wet meadows in Bobrava brook valley, P. Bureš & J. Výhodová 22. 5. 2011	49°8'6.350"N, 16°30'56.217"E	2	CAN, OLE
B6	Ostopovice: ditch at the road to Moravany, P. Bureš & J. Výhodová 22. 5. 2011	49°9'3.256"N, 16°33'38.655"E	2	CAN
B7	Popůvky: meadows above the upper of the two ponds at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'20.032"N, 16°28'48.556"E	2	PAL, RIV, VUL
B8	Střelice: grassy road bench close to Střelická bažina, E. Lajkepová & P. Bureš 2. 7. 2011	49°8'41.132"N, 16°28'5.810"E	3	CAN, OLE
B9	Křtiny: wet grassy road bench to Babice, W side of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'38.854"N, 16°44'9.540"E	1	CAN, RIV
B10	Lhotky: clearing, E side of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'19.636"N, 16°47'42.349"E	1	VUL

B11	Olšany, wet meadows in brook valley at the crossroads Malá Říčka, NW of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'33.939"N, 16°48'49.862"E	1	PAL, RIV
B12	Křtiny: wet meadows close to the Arboretum entrance, E. Lajkepová & P. Bureš 4. 7. 2011	49°19'2.189"N, 16°44'41.202"E	2	RIV
B13	Křtiny: ruderal ditches at the road close to gamekeeper´s lodge, E of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'32.095"N, 16°45'23.919"E	1	VUL
B14	Bukovinka: grassy ditches of the road to Račice , E. Lajkepová & P. Bureš 4. 7. 2011	49°17'14.445"N, 16°50'28.582"E	1	PAL
B15	Bukovinka: clearing at the road to Račice, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'44.577"N, 16°51'32.835"E	1	PAL
B20	Lesní Hluboké: grassy edge of forest pathway at Devět Křížů, E. Lajkepová & P. Bureš 6. 7. 2011	49°15'41.325"N, 16°17'58.696"E	2	PAL, VUL
K1	Louka: ruderal places between road and railway, E. Lajkepová & P. Bureš 1. 7. 2011	48°54'31.495"N, 17°30'7.848"E	2	ERIO
K2	Boršice u Blatnice: dry ruderal bushy slopes od southern edge of the village, E. Lajkepová & P. Bureš 1. 7. 2011	48°55'35.702"N, 17°34'34.913"E	2	ERIO, VUL
K3	Korytná: dry, bushy pasture 1,5 km NNW from the church , E. Lajkepová & P. Bureš 1. 7. 2011	48°57'11.640"N, 17°39'31.493"E	2	CAN, ERIO, OLE, VUL
K4	Komňa: grassy, wet areas between planted trees, N road at the quarry of Rasová, 2,2 km SSE from the church in the village, E. Lajkepová & P. Bureš 1.7. 2011	48°58'39.4"N, 17°48'43.2"E	3	CAN, PAL, RIV
K5	Lopeník: wet meadows on NE slopes of Vysoký vrch hill, NE of the village, E. Lajkepová & P. Bureš 1. 7. 2011	48°57'24.4"N, 17°47'44.8"E	3	OLE, PAL, RIV
K6	Březová: dry grassy vegetation, natural reserve of Cestiska, N of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'42.455"N, 17°43'47.895"E	1	ACA
K7,1	Březová: pasture SE of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'34.501"N, 17°44'53.401"E	1	VUL
K8	Březová : the pasture at the cowshed, NNE from the village church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'12.649"N, 17°44'19.656"E	1	ERIO, VUL

K9	Březová: grassy road ditch in serpentines, W of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'32.185"N, 17°43'43.894"E	2	CAN
K10	Strání: abandoned orchard under the road to Březová, near a dump, 1.2 km NNE from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°54'50.520"N, 17°42'24.715"E	1	ERIO
K12	Suchá Loz: mesophilic meadow at W oriented slope facing the, Lubná 3.1 km WSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°57'8.324"N, 17°40'53.151"E	2	ERIO, OLE
K13	Suchá Loz: meadow with scatter trees, 3 km SSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'36.169"N, 17°42'22.884"E	1	OLE, VUL
Z1	Louka: reuderalised places at the pond in the village, P. Bureš & J. Bureš 19. 5. 2011	49°50'46.392"N, 15°55'24.754"E	1	CAN, VUL
Z2	Horka: grassy road ditch at the railway crossing, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°52'54.484"N, 15°54'40.193"E	1	CAN
Z4,1	Střemošice: road ditches, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°53'16.101"N, 16°4'6.822"E	1	OLE, VUL
Z6	Polička: grassy places at the parking place in front of Masokombinát, P. Bureš & J. Bureš 20. 5. 2011	49°43'34.452"N, 16°13'56.495"E	2	PAL, VUL
Z8,1	Vortová: wasteland, S side of Lhoty village, P. Bureš & J. Bureš 21. 5. 2011	49°42'44.312"N, 15°56'44.863"E	3	
Z9	Karlov: grassy road ditches at the crossroad Karlov - Radostín - Vojnův Městec, E. Lajkepová & P. Bureš 6. 7. 2011	49°39'22.028"N, 15°54'14.772"E	1	HET
Z10,1	Libice nad Doubravou: grassy margins of a field path above the road, NW of the village, E. Lajkepová & P. Bureš 3. 7. 2011	49°44'58.659"N, 15°41'58.061"E	1	CAN
Z13	Hluboká: calcareous meadows and dryer meadows in the valley of Doubrava, above the Řeka pond E. Lajkepová & P. Bureš 6. 7. 2011	49°40'15.929"N, 15°51'13.210"E	2	OLE, PAL , RIV
Z14	Krucemburk: wetter meadow at the road Krucemburk - Zalíbené, E. Lajkepová & P. Bureš 6. 7. 2011	49°41'58.963"N, 15°52'52.864"E	2	OLE, PAL, RIV
Z15	Košinov: grassy road ditch, S side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°42'25.810"N, 15°53'17.916"E	2	HET, PAL

Z16	Hamry: grassy sides of the road to Vortová, SE side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'10.726"N, 15°55'46.215"E	3	HET, PAL
Z17	Mariánská Huť: wetter meadows and ditches at the road, E. Lajkepová & P. Bureš 6. 7. 2011	49°40'53.559"N, 15°57'41.127"E	2	HET, PAL, RIV
Z18	Herálec: wetter meadow under the road to gamekeeper´s lodge "Na Svobodě", E. Lajkepová & P. Bureš 6. 7. 2011	49°41'41.766"N, 15°58'5.490"E	3	HET, PAL
Z19	Chlumětín: wetter meadows at the road to Svratka, E. Lajkepová & P. Bureš 6. 7. 2011	49°43'11.415"N, 15°59'53.903"E	2	HET, PAL
Z20	Čachnov: wetter pasture in brook valley, SW of the villagev, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'44.130"N, 16°3'35.275"E	3	OLE, PAL, RIV
Z22	Borová u Poličky: grassy edges of the road Hlinsko - Polička, E. Lajkepová & P. Bureš 6. 7. 2011	49°45'5.063"N, 16°8'13.801"E	2	HET
Z23	Cikháj: wetter meadow in the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°38'44.469"N, 15°57'55.016"E	1	HET, PAL
Total			75	
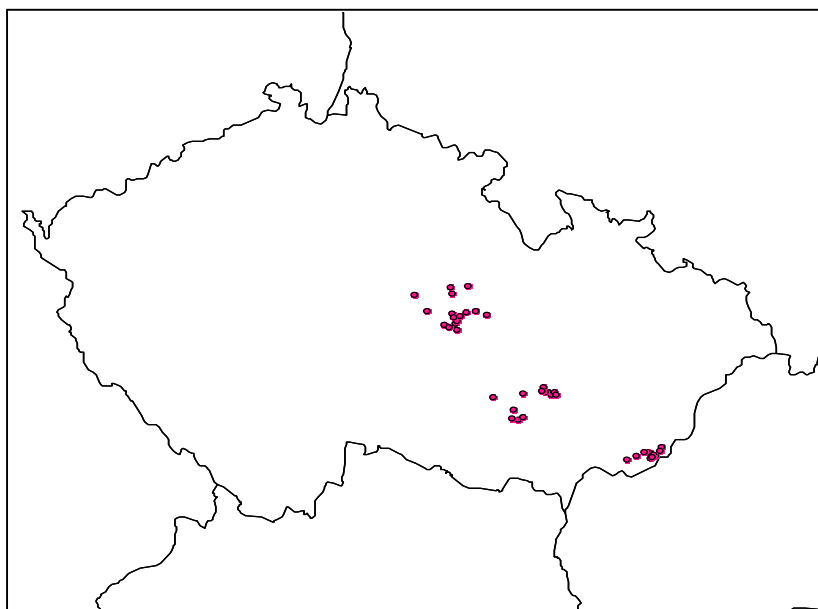
Fig. H2: Map of localities of *C. arvense*

Tab. H10: Localities of *C. canum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B3	Lelekovice: wet meadows in Ponávka brook valley, P. Bureš & J. Výhodová 22. 5. 2011	49°17'0.496"N, 16°34'43.419"E	3	RIV
B5	Střelice: wet meadows in Bobrava brook valley, P. Bureš & J. Výhodová 22. 5. 2011	49°8'6.350"N, 16°30'56.217"E	3	ARV, OLE
B6	Ostopovice: ditch at the road to Moravany, P. Bureš & J. Výhodová 22. 5. 2011	49°9'3.256"N, 16°33'38.655"E	2	ARV
B7,1	Popůvky: meadows at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'44.941"N, 16°28'15.277"E	3	OLE
B8	Střelice: grassy road bench close to Střelická bažina, E. Lajkepová & P. Bureš 2. 7. 2011	49°8'41.132"N, 16°28'5.810"E	3	ARV, OLE
B9	Křtiny: wet grassy road bench to Babice, W side of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'38.854"N, 16°44'9.540"E	3	ARV, RIV
B11,1	Olšany, wet meadows in brook valley at the crossroads Malá Říčka, NW of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'32.991"N, 16°49'1.731"E	3	
B13,1	Křtiny: wetter meadows at the road near gamekeeper´s lodge, E of the villageE. Lajkepová & P. Bureš 4. 7. 2011	49°17'31.746"N, 16°45'27.719"E	2	RIV
B23	Popůvky: wet meadow along the brook, W side of the village, E. Lajkepová & P. Bureš 19.7.2013	49°10'40.729"N, 16°28'33.726"E	2	RIV
K3	Korytná: dry, bushy pasture 1,5 km NNWfrom the church , E. Lajkepová & P. Bureš 1. 7. 2011	48°57'11.640"N, 17°39'31.493"E	3	ARV, ERIO, OLE, VUL
K4	Komňa: grassy, wet areas between planted trees, N road at the quarry of Rasová, 2,2 km SSE from the church in the village, E. Lajkepová & P. Bureš 1.7. 2011; hybr pal x riv	48°58'39.4"N, 17°48'43.2"E	3	ARV, PAL, RIV
K9	Březová: grassy road ditch in serpentines, W of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'32.185"N, 17°43'43.894"E	3	ARV

K11	Suchá Loz: bushy orchards 0.5 km NNE from the crossroad of Strání-Březová-Suchá Loz, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'53.567"N, 17°43'0.497"E	3	ERIO, OLE
K16	Velká nad Veličkou: W edge of the natural reserve Zahrady pod Hájem, E side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'36.852"N, 17°31'51.039"E	3	VUL
K18	Suchovské mlýny: wet meadows along the road to Javorník, SW of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'51.711"N, 17°34'7.526"E	3	OLE
K19	Nová Lhota-Vápenky: along a field path, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'5.868"N, 17°36'53.676"E	2	OLE, VUL
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	4	VUL, PAN
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	3	OLE, PAL, RIV
K23	Rokytnice: meadow at the road to Jestřabí, a bend at the FVE areal, EES of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°4'10.696"N, 17°55'47.135"E	2	OLE
Z1	Louka: reuderalised places at the pond in the village, P. Bureš & J. Bureš 19. 5. 2011	49°50'46.392"N, 15°55'24.754"E	3	ARV, VUL
Z2	Horka: grassy road ditch at the railway crossing, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°52'54.484"N, 15°54'40.193"E	1	ARV
Z3,1	Chrast: wet meadows at the brook under the natural reserve Altán, P. Bureš & J. Bureš 19. 5. 2011	49°54'13.159"N, 15°56'58.438"E	2	OLE
Z10,1	Libice nad Doubravou: grassy margins of a field path above the road, NW of the village, E. Lajkepová & P. Bureš 3. 7. 2011	49°44'58.659"N, 15°41'58.061"E	3	ARV
Z27	Chotěnov: slopes, S side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°48'54.953"N, 16°11'9.631"E	3	ACA, OLE
Z28	Nové Hrady: road ditches at the cementary, E of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°50'59.763"N, 16°9'22.362"E	3	OLE
Z30	Střemošice: wet meadow at the road to Domanice, W side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°53'36.631"N, 16°3'52.639"E	3	OLE
Total			71	
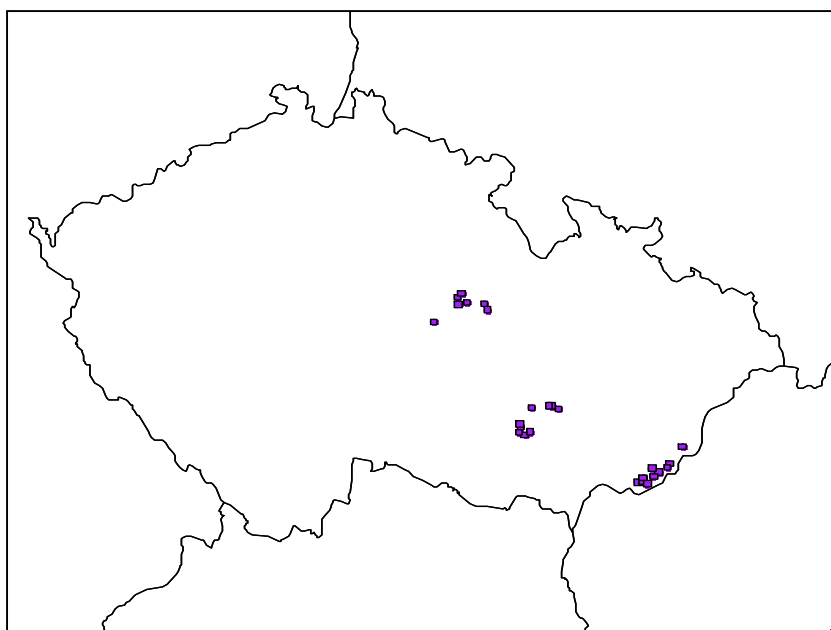
Fig. H3: Map of localities of *C. canum*

Tab. H11: Localities of *C. eriophorum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B2	Vilémovice: grassy cover in the amphitheater above Suchý žleb, P. Bureš & J. Výhodová 22. 5. 2011	49°22'11.061"N, 16°44'34.396"E	3	
K1	Louka: ruderal places between road and railway, E. Lajkepová & P. Bureš 1. 7. 2011	48°54'31.495"N, 17°30'7.848"E	3	ARV
K2	Boršice u Blatnice: dry ruderal bushy slopes od southern edge of the village, E. Lajkepová & P. Bureš 1. 7. 2011	48°55'35.702"N, 17°34'34.913"E	3	ARV, VUL
K3	Korytná: dry, bushy pasture 1,5 km NNWfrom the church , E. Lajkepová & P. Bureš 1. 7. 2011	48°57'11.640"N, 17°39'31.493"E	3	ARV, CAN, OLE, VUL
K8	Březová : the pasture at the cowshed, NNE from the village church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'12.649"N, 17°44'19.656"E	2	ARV, VUL
K10	Strání: abandoned orchard under the road to Březová, near a dump, 1.2 km NNE from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°54'50.520"N, 17°42'24.715"E	3	ARV
K11	Suchá Loz: bushy orchards 0.5 km NNE from the crossroad of Strání-Březová-Suchá Loz, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'53.567"N, 17°43'0.497"E	2	CAN, OLE

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K12	Suchá Loz: mesophilic meadow at W oriented slope facing the, Lubná 3.1 km WSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°57'8.324"N, 17°40'53.151"E	2	ARV, OLE
Total			21	
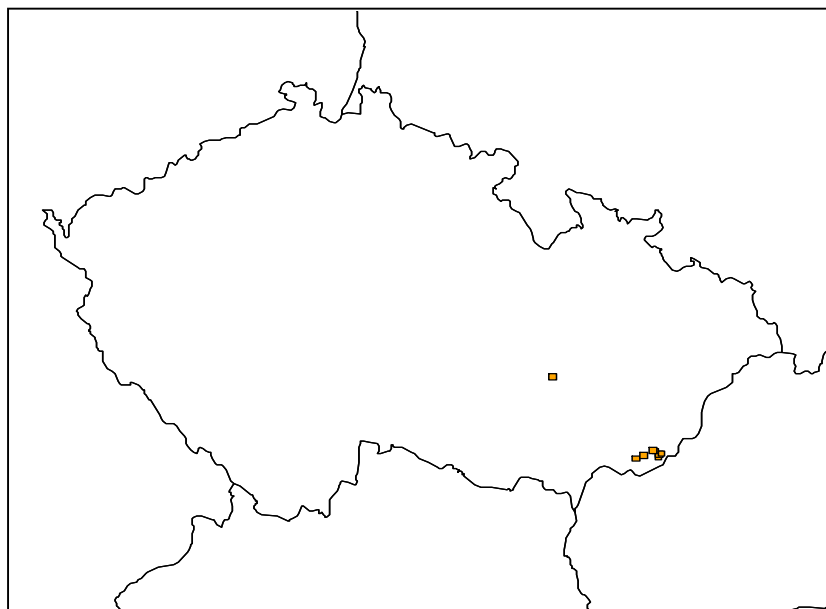
Fig. H4: Map of localities of *C. eriophorum*

Tab. H12: Localities of *C. heterophyllum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
Z9	Karlov: grassy road ditches at the crossroad Karlov - Radostín - Vojnův Městec, E. Lajkepová & P. Bureš 6. 7. 2011	49°39'22.028"N, 15°54'14.772"E	3	ARV
Z15	Košinov: grassy road ditch, S side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°42'25.810"N, 15°53'17.916"E	3	ARV, PAL
Z16	Hamry: grassy sides of the road to Vortová, SE side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'10.726"N, 15°55'46.215"E	3	ARV, PAL
Z17	Mariánská Huť: wetter meadows and ditches at the road, E. Lajkepová & P. Bureš 6. 7. 2011	49°40'53.559"N, 15°57'41.127"E	3	ARV, PAL, RIV
Z18	Herálec: wetter meadow under the road to gamekeeper´s lodge "Na Svobodě", E. Lajkepová & P. Bureš 6. 7. 2011	49°41'41.766"N, 15°58'5.490"E	3	ARV, PAL

Z19	Chlumětín: wetter meadows at the road to Svratka, E. Lajkepová & P. Bureš 6. 7. 2011	49°43'11.415"N, 15°59'53.903"E	2	ARV, PAL
Z22	Borová u Poličky: grassy edges of the road Hlinsko - Polička, E. Lajkepová & P. Bureš 6. 7. 2011	49°45'5.063"N, 16°8'13.801"E	3	ARV
Z23	Cikháj: wetter meadow in the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°38'44.469"N, 15°57'55.016"E	2	ARV, PAL
Z33	Dlouhý: wet meadow at the road Trhová Kamenice - Ždírec, E of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°45'11.108"N, 15°49'3.549"E	3	
Z34	Radostín: along the forest path, W of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°38'56.639"N, 15°51'33.567"E	3	
Z36	Samotín-Blatky: mountainous meadow at Blatky, W of the village, P. Bureš & J. Bureš 22. 9. 2013	49°39'0.816"N, 16°2'57.193"E	2	
Z37	Kadov: mountainous meadow under the road at SW village edge, P. Bureš & J. Bureš 22. 9. 2013	49°37'46.375"N, 16°5'4.909"E	3	
Z38	Pohledec: grassy margins of the road to Koníkov, NE of the village, P. Bureš & J. Bureš 22. 9. 2013	49°35'14.411"N, 16°6'55.351"E	3	
Z39	Studnice-Paseky: mountainous meadow at the road Kuklík - Rokytno, P. Bureš & J. Bureš 22. 9. 2013	49°36'50.439"N, 16°5'14.930"E	3	
Z40	Vříšť: wet meadows at the Fryšávka brook valley, E of the village, P. Bureš & J. Bureš 22. 9. 2013	49°38'11.563"N, 16°8'16.054"E	3	OLE
Z41	Podlesí: grassy margins of the road Milovy - Sněžné, P. Bureš & J. Bureš 22. 9. 2013	49°39'52.735"N, 16°6'21.202"E	3	
Total			45	
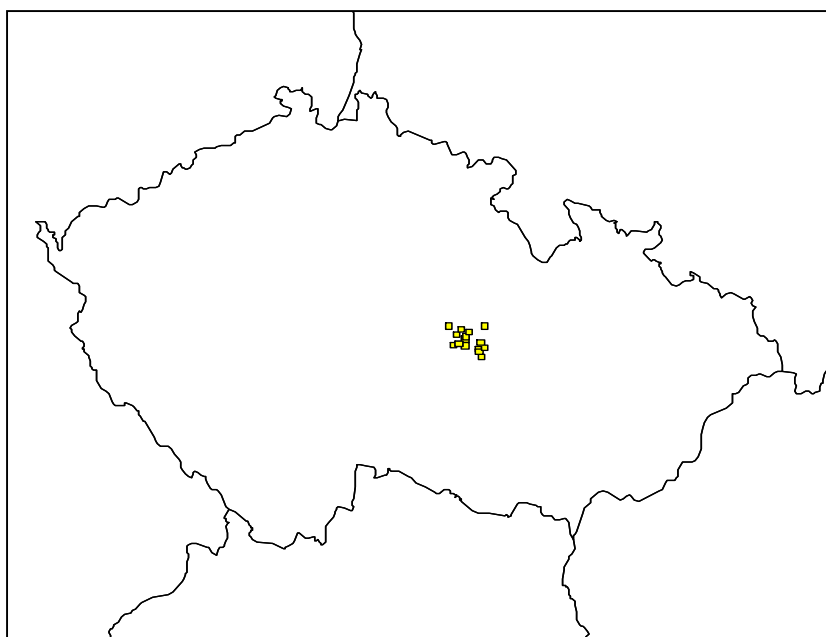
Fig. H5: Map of localities of *C. heterophyllum*

Tab. H13: Localities of *C. oleraceum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B5	Střelice: wet meadows in Bobrava brook valley, P. Bureš & J. Výhodová 22. 5. 2011	49°8'6.350"N, 16°30'56.217"E	2	ARV, CAN
B7,1	Popůvky: meadows at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'44.941"N, 16°28'15.277"E	3	CAN
B8	Střelice: grassy road bench close to Střelická bažina, E. Lajkepová & P. Bureš 2. 7. 2011	49°8'41.132"N, 16°28'5.810"E	3	ARV, CAN
B12,1	Křtiny: wet meadows close to the Arboretum entrance, E. Lajkepová & P. Bureš 4. 7. 2011	49°19'13.610"N, 16°44'39.772"E	3	
B16	Račice, grassy ditches at the pond, N of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'11.354"N, 16°52'31.270"E	2	
B21	Velká Bíteš: forest edge at Býtýška brook, SE of the town, E. Lajkepová & P. Bureš 19.7.2013	49°17'51.497"N, 16°14'38.259"E	2	PAL, VUL
K3	Korytná: dry, bushy pasture 1,5 km NNWfrom the church , E. Lajkepová & P. Bureš 1. 7. 2011	48°57'11.640"N, 17°39'31.493"E	2	ARV, CAN, ERIO, VUL

K5	Lopeník: wet meadows on NE slopes of Vysoký vrch hill, NE of the village, E. Lajkepová & P. Bureš 1. 7. 2011; hybr pal x riv, ole x riv	48°57'24.4"N, 17°47'44.8"E	3	PAL, RIV
K9,1	Březová: grassy road ditch in serpentines, W of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'36.242"N, 17°43'41.485"E	3	
K11	Suchá Loz: bushy orchards 0.5 km NNE from the crossroad of Strání-Březová-Suchá Loz, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'53.567"N, 17°43'0.497"E	2	CAN, ERIO
K12	Suchá Loz: mesophilic meadow at W oriented slope facing the, Lubná 3.1 km WSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°57'8.324"N, 17°40'53.151"E	3	ARV, ERIO
K13	Suchá Loz: meadow with scatter trees, 3 km SSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'36.169"N, 17°42'22.884"E	2	ARV, VUL
K17	Javorník: wet meadow, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°49'59.969"N, 17°32'25.911"E	3	OLE, RIV
K18	Suchovské mlýny: wet meadows along the road to Javorník, SW of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'51.711"N, 17°34'7.526"E	3	CAN
K19	Nová Lhota-Vápenky: along a field path, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'5.868"N, 17°36'53.676"E	3	CAN, VUL
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	2	CAN, PAL, RIV
K23	Rokytnice: meadow at the road to Jestřabí, a bend at the FVE areal, EES of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°4'10.696"N, 17°55'47.135"E	3	
Z3,1	Chrast: wet meadows at the brook under the natural reserve Altán, P. Bureš & J. Bureš 19. 5. 2011	49°54'13.159"N, 15°56'58.438"E	3	
Z4,1	Střemošice: road ditches, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°53'16.101"N, 16°4'6.822"E	3	ARV, VUL
Z7	Babka: grassy wet ditches of the road Polička - Hlinsko, P. Bureš & J. Bureš 20. 5. 2011	49°43'47.970"N, 16°13'16.099"E	2	
Z10,2	Libice nad Doubravou: road ditches, NW of the village, E. Lajkepová & P. Bureš 3. 7. 2011	49°44'56.615"N, 15°41'38.630"E	3	
Z13	Hluboká: calcareous meadows and dryer meadows in the valley of Doubrava, above the Řeka pond E. Lajkepová & P. Bureš 6. 7. 2011	49°40'15.929"N, 15°51'13.210"E	3	PAL, RIV

Z14	Krucemburk: wetter meadow at the road Krucemburk - Zalíbené, E. Lajkepová & P. Bureš 6. 7. 2011	49°41'58.963"N, 15°52'52.864"E	2	PAL, RIV
Z20	Čachnov: wetter pasture in brook valley, SW of the villagev, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'44.130"N, 16°3'35.275"E	2	PAL, RIV
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	3	PAL, RIV
Z27	Chotěnov: slopes, S side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°48'54.953"N, 16°11'9.631"E	3	ACA, CAN
Z28	Nové Hrady: road ditches at the cementary, E of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°50'59.763"N, 16°9'22.362"E	3	CAN
Z30	Střemošice: wet meadow at the road to Domanice, W side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°53'36.631"N, 16°3'52.639"E	3	CAN
Z40	Vříšť: wet meadows at the Fryšávka brook valley, E of the village, P. Bureš & J. Bureš 22. 9. 2013	49°38'11.563"N, 16°8'16.054"E	3	HET
Total			77	
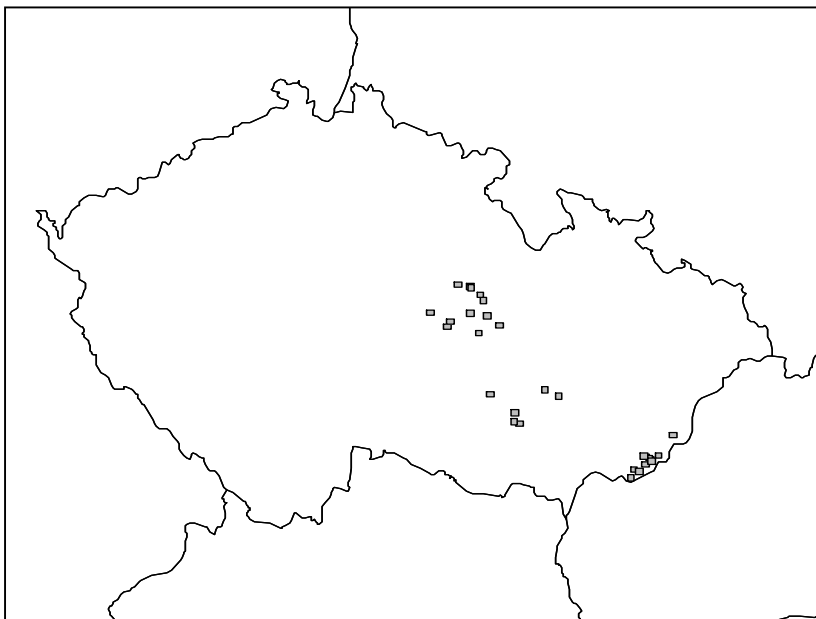
Fig. H6: Map of localities of *C. oleraceum*

Tab. H14: Localities of *C. palustre*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B7	Popůvky: meadows above the upper of the two ponds at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'20.032"N, 16°28'48.556"E	3	ARV, RIV, VUL
B11	Olšany, wet meadows in brook valley at the crossroads Malá Říčka, NW of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'33.939"N, 16°48'49.862"E	1	ARV, RIV
B14	Bukovinka: grassy ditches of the road to Račice , E. Lajkepová & P. Bureš 4. 7. 2011	49°17'14.445"N, 16°50'28.582"E	2	ARV
B15	Bukovinka: clearing at the road to Račice, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'44.577"N, 16°51'32.835"E	2	ARV
B20	Lesní Hluboké: grassy edge of forest pathway at Devět Křížů, E. Lajkepová & P. Bureš 6. 7. 2011	49°15'41.325"N, 16°17'58.696"E	2	ARV, VUL
B21	Velká Bíteš: forest edge at Býtýška brook, SE of the town, E. Lajkepová & P. Bureš 19.7.2013	49°17'51.497"N, 16°14'38.259"E	3	OLE, VUL
K4	Komňa: grassy, wet areas between planted trees, N road at the quarry of Rasová, 2,2 km SSE from the church in the village, E. Lajkepová & P. Bureš 1.7. 2011; hybr pal x riv	48°58'39.4"N, 17°48'43.2"E	2	ARV, CAN, RIV
K5	Lopeník: wet meadows on NE slopes of Vysoký vrch hill, NE of the village, E. Lajkepová & P. Bureš 1. 7. 2011; hybr pal x riv, ole x riv	48°57'24.4"N, 17°47'44.8"E	3	ARV, OLE, RIV
K7	Březová: spring fen SE of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'36.030"N, 17°45'2.628"E	1	RIV
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	3	CAN, OLE, RIV
K24	Nedašov: spring meadow at the neutral reserve, E side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'4.898"N, 18°5'58.190"E	2	ACA, RIV
K25	Nedašov: S slopes of natural reserve, SE side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'53.921"N, 18°6'18.073"E	2	ACA, RIV, PAN

K26	Návojná: at the forest path on the slope of the Cikán hill, SE of the village JV obce, E. Lajkepová & P. Bureš 21. 7. 2013	49°05'30.6"N, 18°05'36.1"E	3	
Z6	Polička: grassy places at the parking place in front of Masokombinát, P. Bureš & J. Bureš 20. 5. 2011	49°43'34.452"N, 16°13'56.495"E	3	ARV, VUL
Z8	Vortová: grassy wet forest path, S of Lhoty, P. Bureš & J. Bureš 21. 5. 2011	49°42'32.369"N, 15°56'48.127"E	2	
Z12	Radostín: wetter meadow of the slope of Radostínský hill, S of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°38'37.605"N, 15°52'50.225"E	3	RIV
Z13	Hluboká: calcareous meadows and dryer meadows in the valley of Doubrava, above the Řeka pond E. Lajkepová & P. Bureš 6. 7. 2011	49°40'15.929"N, 15°51'13.210"E	3	ARV, OLE, RIV
Z14	Krucemburk: wetter meadow at the road Krucemburk - Zalíbené, E. Lajkepová & P. Bureš 6. 7. 2011	49°41'58.963"N, 15°52'52.864"E	3	ARV, OLE, RIV
Z15	Košinov: grassy road ditch, S side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°42'25.810"N, 15°53'17.916"E	3	ARV, HET
Z16	Hamry: grassy sides of the road to Vortová, SE side of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'10.726"N, 15°55'46.215"E	3	ARV, HET
Z17	Mariánská Huť: wetter meadows and ditches at the road, E. Lajkepová & P. Bureš 6. 7. 2011	49°40'53.559"N, 15°57'41.127"E	3	ARV, HET, RIV
Z18	Herálec: wetter meadow under the road to gamekeeper´s lodge "Na Svobodě", E. Lajkepová & P. Bureš 6. 7. 2011	49°41'41.766"N, 15°58'5.490"E	3	ARV, HET
Z19	Chlumětín: wetter meadows at the road to Svratka, E. Lajkepová & P. Bureš 6. 7. 2011	49°43'11.415"N, 15°59'53.903"E	2	ARV, HET
Z20	Čachnov: wetter pasture in brook valley, SW of the villagev, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'44.130"N, 16°3'35.275"E	1	ARV, OLE, RIV
Z23	Cikháj: wetter meadow in the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°38'44.469"N, 15°57'55.016"E	2	ARV, HET
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	3	OLE, RIV
Z26	Zrnětín: wetter forest margin, S of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°46'54.207"N, 16°11'56.590"E	1	

Total		64	
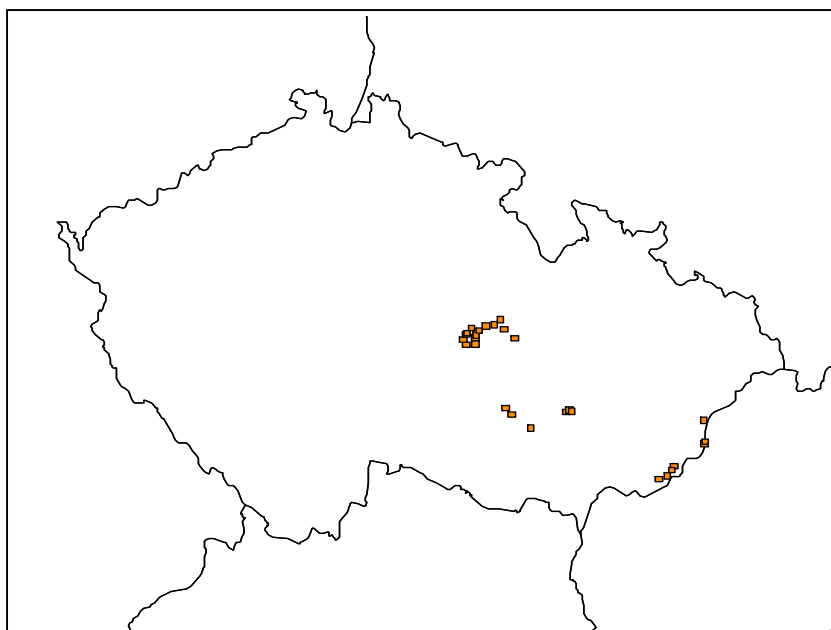
Fig. H7: Map of localities of *C. palustre*

Tab. H16: Localities of *C. pannonicum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B30	Kurdějov: hillside above the road, 800 m E from the village, E. Lajkepová & O. Knápek, 15. 7. 2014	48°57'54.284"N, 16°46'25.899"E	3	
B31	Letonice: hillside at natural reserve Větrníky, 16 km SSE from the village, E. Lajkepová & O. Knápek, 15. 7. 2014	49°11'49.682"N, 16°59'1.497"E	3	
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	3	ACA, CAN, VUL
K21	Suchov: E slopes, W side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'1.561"N, 17°34'37.882"E	3	VUL
K25	Nedašov: S slopes of natural reserve, SE side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'53.921"N, 18°6'18.073"E	2	PAL, RIV
K30	Čertoryje: S slope of the natural reserve, E. Lajkepová & O. Knápek, 19. 7. 2014	48°50'57"N, 17°23'50.9"	3	
K31	Javorník -Machová: slope 250 m NEE from the railway station, E. Lajkepová & O. Knápek, 19. 7. 2014	48°49'29.100"N 17°31'13.000"E	4	

K32	Velká nad Veličkou: W side of the natural reserve Zahrady pod Hájem, E. Lajkepová & O. Knápek, 19. 7. 2014	48°53'2"N, 17°31'36.9"	3
K33b	Korytná: slope above the road, 2.4 km NNE from the village side, E. Lajkepová & O. Knápek, 19. 7. 2014	48°45'44.6'N', 17°40'34.8"E	1
K35	Komňa: the quarry of Rasová, 1.5 km NNE from the village side, E. Lajkepová & O. Knápek, 20. 7. 2014	48°58'32.944"N 17°48'39.498"E	5
Total			30
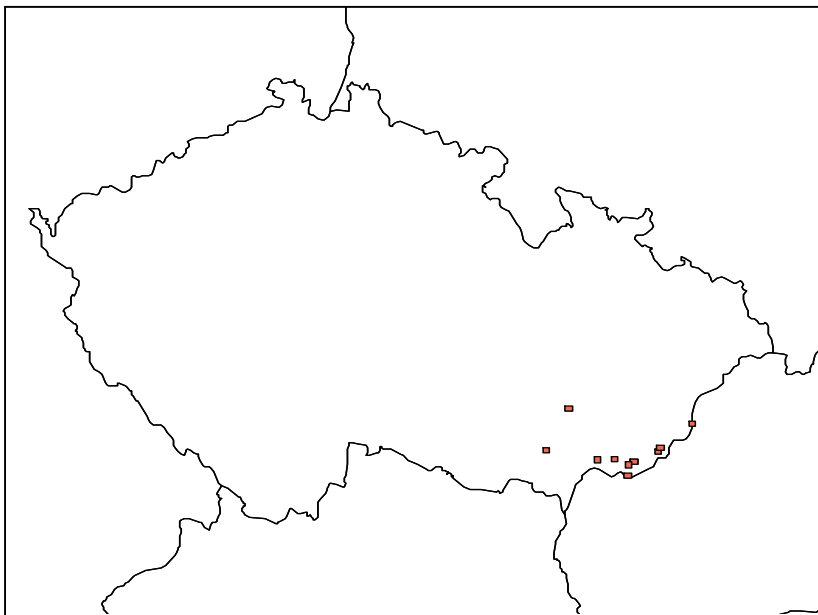
Fig. H8: Map of localities of *C. pannonicum*

Tab. H17: Localities of *C. rivulare*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B3	Lelekovice: wet meadows in Ponávka brook valley, P. Bureš & J. Výhodová 22. 5. 2011	49°17'0.496"N, 16°34'43.419"E	3	CAN
B7	Popůvky: meadows above the upper of the two ponds at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'20.032"N, 16°28'48.556"E	3	ARV, PAL, VUL
B9	Křtiny: wet grassy road bench to Babice, W side of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'38.854"N, 16°44'9.540"E	3	ARV, CAN

B11	Olšany, wet meadows in brook valley at the crossroads Malá Říčka, NW of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°16'33.939"N, 16°48'49.862"E	3	ARV, PAL
B12	Křtiny: wet meadows close to the Arboretum entrance, E. Lajkepová & P. Bureš 4. 7. 2011	49°19'2.189"N, 16°44'41.202"E	2	ARV
B13,1	Křtiny: wetter meadows at the road near gamekeeper´s lodge, E of the villageE. Lajkepová & P. Bureš 4. 7. 2011	49°17'31.746"N, 16°45'27.719"E	3	CAN
B23	Popůvky: wet meadow along the brook, W side of the village, E. Lajkepová & P. Bureš 19.7.2013	49°10'40.729"N, 16°28'33.726"E	2	CAN
K4	Komňa: grassy, wet areas between planted trees, N road at the quarry of Rasová, 2,2 km SSE from the church in the village, E. Lajkepová & P. Bureš 1.7. 2011; hybr pal x riv	48°58'39.4"N, 17°48'43.2"E	1	ARV, CAN, PAL
K5	Lopeník: wet meadows on NE slopes of Vysoký vrch hill, NE of the village, E. Lajkepová & P. Bureš 1. 7. 2011; hybr pal x riv, ole x riv	48°57'24.4"N, 17°47'44.8"E	2	ARV, OLE, PAL
K7	Březová: spring fen SE of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'36.030"N, 17°45'2.628"E	3	PAL
K17	Javorník: wet meadow, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°49'59.969"N, 17°32'25.911"E	3	OLE
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	3	CAN, OLE, PAL
K24	Nedašov: spring meadow at the neutral reserve, E side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'4.898"N, 18°5'58.190"E	3	ACA, PAL
K25	Nedašov: S slopes of natural reserve, SE side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'53.921"N, 18°6'18.073"E	2	ACA, PAL, PAN
Z12	Radostín: wetter meadow of the slope of Radostínský hill, S of the village, E. Lajkepová & P. Bureš 6. 7. 2011	49°38'37.605"N, 15°52'50.225"E	3	PAL
Z13	Hluboká: calcareous meadows and dryer meadows in the valley of Doubrava, above the Řeka pond E. Lajkepová & P. Bureš 6. 7. 2011	49°40'15.929"N, 15°51'13.210"E	3	ARV, OLE, PAL
Z14	Krucemburk: wetter meadow at the road Krucemburk - Zalíbené, E. Lajkepová & P. Bureš 6. 7. 2011	49°41'58.963"N, 15°52'52.864"E	3	ARV, OLE, PAL

Z17	Mariánská Huť: wetter meadows and ditches at the road, E. Lajkepová & P. Bureš 6. 7. 2011	49°40'53.559"N, 15°57'41.127"E	3	ARV, HET, PAL
Z20	Čachnov: wetter pasture in brook valley, SW of the villagev, E. Lajkepová & P. Bureš 6. 7. 2011	49°44'44.130"N, 16°3'35.275"E	3	ARV, OLE, PAL
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	3	OLE, PAL
Total			54	
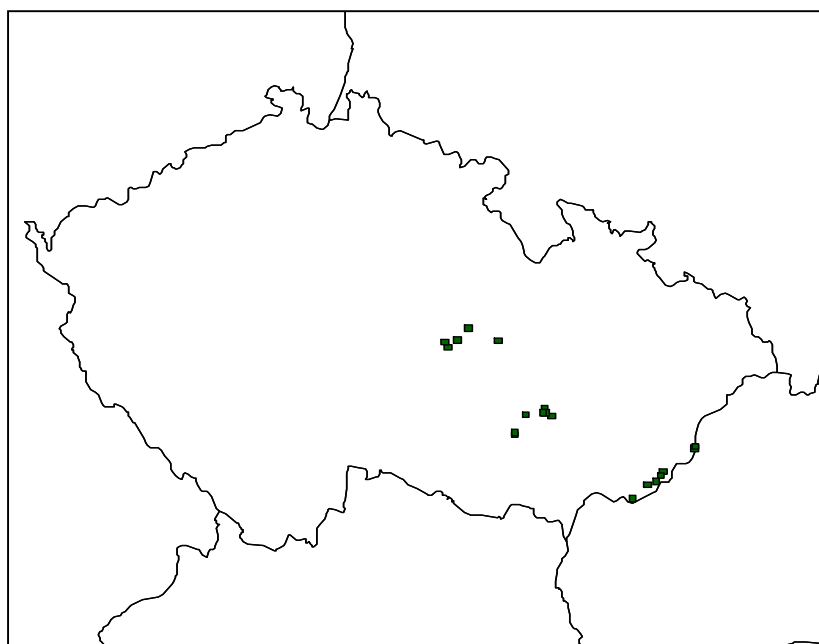
Fig. H9: Map of localities of *C. rivulare*

Tab. H18: Localities of *C. vulgare*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B1	Jedovnice: grassy bench of Olšovec pond, P. Bureš & J. Výhodová 22. 5. 2011	49°20'3.924"N, 16°45'38.192"E	3	
B7	Popůvky: meadows above the upper of the two ponds at the Augšperský pond valley, E. Lajkepová & P. Bureš 2. 7. 2011	49°11'20.032"N, 16°28'48.556"E	2	ARV, PAL, RIV
B10	Lhotky: clearing, E side of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'19.636"N, 16°47'42.349"E	3	ARV

B13	Křtiny: ruderal ditches at the road close to gamekeeper´s lodge, E of the village, E. Lajkepová & P. Bureš 4. 7. 2011	49°17'32.095"N, 16°45'23.919"E	3	ARV
B20	Lesní Hluboké: grassy edge of forest pathway at Devět Křížů, E. Lajkepová & P. Bureš 6. 7. 2011	49°15'41.325"N, 16°17'58.696"E	3	ARV, PAL
B21	Velká Bíteš: forest edge at Býtýška brook, SE of the town, E. Lajkepová & P. Bureš 19.7.2013	49°17'51.497"N, 16°14'38.259"E	2	OLE, PAL
K2	Boršice u Blatnice: dry ruderal bushy slopes od southern edge of the village, E. Lajkepová & P. Bureš 1. 7. 2011	48°55'35.702"N, 17°34'34.913"E	3	ARV, ERIO
K3	Korytná: dry, bushy pasture 1,5 km NNW from the church , E. Lajkepová & P. Bureš 1. 7. 2011	48°57'11.640"N, 17°39'31.493"E	3	ARV, CAN, ERIO, OLE
K7,1	Březová: pasture SE of the village, E. Lajkepová & P. Bureš 5. 7. 2011	48°55'34.501"N, 17°44'53.401"E	2	ARV
K8	Březová : the pasture at the cowshed, NNE from the village church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'12.649"N, 17°44'19.656"E	3	ARV, ERIO
K13	Suchá Loz: meadow with scatter trees, 3 km SSW from the church, E. Lajkepová & P. Bureš 5. 7. 2011	48°56'36.169"N, 17°42'22.884"E	3	ARV, OLE
K16	Velká nad Veličkou: W edge of the natural reserve Zahrady pod Hájem, E side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'36.852"N, 17°31'51.039"E	3	CAN
K19	Nová Lhota-Vápenky: along a field path, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'5.868"N, 17°36'53.676"E	3	CAN, OLE
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	3	ACA. CAN, PAN
K21	Suchov: E slopes, W side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'1.561"N, 17°34'37.882"E	3	ACA, PAN
Z1	Louka: reuderalised places at the pond in the village, P. Bureš & J. Bureš 19. 5. 2011	49°50'46.392"N, 15°55'24.754"E	1	ARV, CAN
Z4,1	Střemošice: road ditches, S of the village, P. Bureš & J. Bureš 19. 5. 2011	49°53'16.101"N, 16°4'6.822"E	2	ARV, OLE
Z5,1	Široký Důl: ruderal places at the brook at the village square, P. Bureš & J. Bureš 20. 5. 2011	49°44'45.228"N, 16°13'17.686"E	2	
Z6	Polička: grassy places at the parking place in front of Masokombinát, P. Bureš & J. Bureš 20. 5. 2011	49°43'34.452"N, 16°13'56.495"E	3	ARV, PAL

Z11	Běstvina, E. Lajkepová & P. Bureš 3. 7. 2011	49°50'32.643"N, 15°35'41.298"E	3	ACA
Z21	Františky: pasture at the road Hlinsko - Polička, E. Lajkepová & P. Bureš 6. 7. 2011	49°45'50.809"N, 16°6'7.297"E	2	
Total			55	
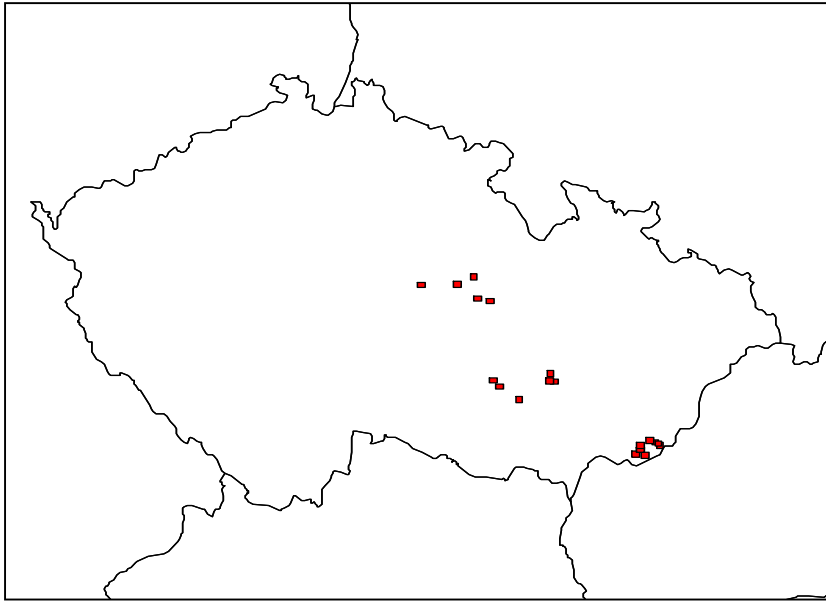
Fig. H10: Map of localities of *C. vulgare*

3.1. Localities of hybrids

For maps of respective hybrids see maps in Discussion, **Fig. E3 – E16**. The localities where we performed the sampling are marked as a red cross.

Tab. H19: Localities of *C. acaulon × C. canum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K21	Suchov: E slopes, W side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'1.561"N, 17°34'37.882"E	1	ACA, VUL, PAN
Z27	Chotěnov: slopes, S side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°48'54.953"N, 16°11'9.631"E	1	ACA, CAN, OLE
Total			2	

Tab. H20: Localities of *C. acaulon × C. oleraceum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
Z27	Chotěnov: slopes, S side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°48'54.953"N, 16°11'9.631"E	1	ACA, CAN, OLE
Z29	Střemošice: dry marlite slopes, W side of the road, S side of the vilage, E. Lajkepová aP. Bureš 18. 7. 2013	49°53'11.795"N, 16°4'6.777"E	3	
Z35	Radostín: grassy S slopes of Radostínský hill, S of the village, P. Bureš & J. Bureš 22. 9. 2013	49°38'44.278"N, 15°52'2.722"E	3	
Total			7	

Tab. H21: Localities of *C. acaulon × C. pannonicum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	2	ACA, CAN, VUL, PAN
K25	Nedašov: S slopes of natural reserve, SE side of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°6'53.921"N, 18°6'18.073"E	3	ACA, PAL, RIV, PAN
Total			5	

Tab. H22: Localities of *C. canum* × *C. oleraceum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B22	Střelice: meadow between the road and Bobrava brook, W of the village, E. Lajkepová & P. Bureš 19.7.2013	49°8'56.802"N, 16°27'50.846"E	1	
K17	Javorník: wet meadow, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°49'59.969"N, 17°32'25.911"E	1	OLE, RIV
K19	Nová Lhota-Vápenky: along a field path, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°52'5.868"N, 17°36'53.676"E	2	CAN, OLE, VUL
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	2	CAN, OLE, PAL, RIV
K23	Rokytnice: meadow at the road to Jestřabí, a bend at the FVE areal, EES of the village, E. Lajkepová & P. Bureš 21. 7. 2013	49°4'10.696"N, 17°55'47.135"E	4	CAN, OLE
K33a	Korytná: at the Dubina crossroad, 2 km NNE from the village side, E. Lajkepová & O. Knápek, 19. 7. 2014	48°55'00.1"N, 17°41'04"E	3	
Z28	Nové Hrady: road ditches at the cementary, E of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°50'59.763"N, 16°9'22.362"E	3	CAN, OLE
Z30	Střemošice: wet meadow at the road to Domanice, W side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°53'36.631"N, 16°3'52.639"E	2	CAN, OLE
Z31	Štěpánov: ditches of the road to Skuteč, S of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°51'17.408"N, 15°59'51.034"E	1	
Z32	Louka: wet meadow at the pond, SE of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°50'43.476"N, 15°55'24.998"E	2	
Total			21	

Tab. H23: Localities of *C. canum* × *C. palustre*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	3	CAN, OLE, PAL, RIV

Code	Locality	Geographical coordinates	Nr. of samples	
K33a	Korytná: at the Dubina crossroad, 2 km NNE from the village side, E. Lajkepová & O. Knápek, 19. 7. 2014	48°55'00.1"N, 17°41'04"E	3	
Total			6	

Tab. H24: Map of localities of *C. canum × C. pannonicum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K21	Suchov: E slopes, W side of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'1.561"N, 17°34'37.882"E	2	ACA, PAN, VUL

Tab. H25: Localities of *C. canum × C. rivulare*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B23	Popůvky: wet meadow along the brook, W side of the village, E. Lajkepová & P. Bureš 19.7.2013	49°10'40.729"N, 16°28'33.726"E	1	CAN, RIV

Tab. H26: Localities of *C. heterophyllum × C. oleraceum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
Z34	Radostín: along the forest path, W of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°38'56.639"N, 15°51'33.567"E	1	HET
Z40	Vříšť: wet meadows at the Fryšávka brook valley, E of the village, P. Bureš & J. Bureš 22. 9. 2013	49°38'11.563"N, 16°8'16.054"E	1	HET, OLE
Total			2	

Tab. H27: Localities of *C. heterophyllum × C. palustre*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
Z34	Radostín: along the forest path, W of the illage, E. Lajkepová & P. Bureš 18. 7. 2013	49°38'56.639"N, 15°51'33.567"E	1	HET
Z37	Kadov: mountainous meadow under the road at SW village edge, P. Bureš & J. Bureš 22. 9. 2013	49°37'46.375"N, 16°5'4.909"E	1	HET

			Total	
			2	

Tab. H28: Localities of *C. heterophyllum* × *C. rivulare*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	1	OLE, PAL, RIV

Tab. H29: Localities of *C. oleraceum* × *C. rivulare*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
B23	Popůvky: wet meadow along the brook, Z obce, E. Lajkepová & P. Bureš 19.7.2013	49°10'40.729"N, 16°28'33.726"E	1	CAN, RIV
K17	Javorník: wet meadow, S of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°49'59.969"N, 17°32'25.911"E	3	OLE, RIV
K34	Lopeník: wet meadows on NE slopes of Vysoký vrch hill, NE of the village, E. Lajkepová & O. Knápek, 20. 7. 2014	48°57'24.4"N, 17°47'44.8"E	1	
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	5	OLE, PAL, RIV
Z26	Zrnětín: wetter forest margin, S of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°46'54.207"N, 16°11'56.590"E	4	PAL
Total			10	

Tab. H30: Localities of *C. oleraceum* × *C. palustre*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K22	Strání: W bank of a pond, natural reserve of Hrnčárky, W of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'24.117"N, 17°40'22.147"E	1	CAN, OLE, PAL, RIV
Z25	Jedlová: wet meadow in the forest of Balda, N side of the village, E. Lajkepová & P. Bureš 18. 7. 2013	49°40'46.298"N, 16°19'44.611"E	1	OLE, PAL, RIV
Total			2	

Tab. H31: Localities of *C. palustre* × *C. pannonicum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K35	Komňa: the quarry of Rasová, 1.5 km NNE from the village side, E. Lajkepová & O. Knápek, 20. 7. 2014	48°58'32.944"N 17°48'39.498"E	1	PAN

Tab. H32: Localities of hybrid of species *C. acaulon, C. canum, C. pannonicum*

Code	Locality	Geographical coordinates	Nr. of samples	Other species
K20	Suchov: E slopes, E of the village, E. Lajkepová & P. Bureš 20. 7. 2013	48°54'5.133"N, 17°34'38.213"E	1	ACA, CAN, VUL, PAN

4. Results of molecular approval of samples

4.1. STRUCTURE results

Tab. H33: Species identity according to STRUCTURE

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
ACA_K20b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K20c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K21a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K21b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K21c	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K24a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K24b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K24c	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
ACA_K25a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K25b	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K25c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K25d	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K6a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K6b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_K6c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z10b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z10c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z11a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z11b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z11c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z27a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z27b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z27c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z3a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z3b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z4a	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z4b	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z4c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z5a	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z5b	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_Z5c	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B10a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B11b	0.00	0.98	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
ARV_B12a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B12c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B13b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B14a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B15b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B20b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

ARV_B20c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B4a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B4b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B4c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B5b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B5c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B6b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B6c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B7b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B7c	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B8a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B8b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B8c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_B9a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K10c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K12a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K12b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K13b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K1b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K1c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K2b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K2c	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K3a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K3b	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K4a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K4b	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K4c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K5a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K5b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K5c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K6c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K7b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K8b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K9a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_K9b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z10	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z13a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z13b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z14b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z14c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z15a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z15c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z16a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z16b	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z16c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

ARV_Z17b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z17c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z18a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z18b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z18c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z19a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z19c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z1b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z20a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z20b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z20c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z22a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z22c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z23b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z2c	0.08	0.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z4a	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z6b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z6c	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z8a	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z8b	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z8c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ARV_Z9c	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B11a	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B11b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B11c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B13b	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B13c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B23a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B23b	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B3a	0.00	0.00	0.96	0.00	0.00	0.01	0.00	0.02	0.00	0.00
CAN_B3b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B3c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B5a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B5b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B5c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B6a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B6c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B7a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B7b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B7c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B8a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B8b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B8c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B9a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_B9b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CAN_B9c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K11a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K11b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K11c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K16a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K16b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K16c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K18a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K18b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K18c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K19a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K19b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K20a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K20b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K20c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K20d	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K22a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K22b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K22c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K23a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K23b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K3a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K3b	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K3c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K4a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K4b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K4c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K9a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K9b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_K9c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z10a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z10b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z10c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z1a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z1b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z1c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z27a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z27b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z27c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z28a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z28b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z28c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z2b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z30a	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z30b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

CAN_Z30c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z3b	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAN_Z3c	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_B2a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_B2b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_B2c	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K10a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K10b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K10c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K11b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K11c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K12b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K12c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K1a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K1b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K1c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K2a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K2b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K2c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K3a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K3b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K3c	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K8a	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ERI_K8b	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
HET_Z15a	0.00	0.01	0.00	0.01	0.95	0.00	0.01	0.00	0.01	0.00
HET_Z15b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z15c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z16a	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00
HET_Z16b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z16c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z17a	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00
HET_Z17b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z17c	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00
HET_Z18a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z18b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z18c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z19b	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.16	0.00	0.00
HET_Z19c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z22a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z22b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z22c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z23b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z23c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z33a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z33b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00

HET_Z33c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z34a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z34b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z34c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z36a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z36b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z37a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z37b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z37c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z38a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z38b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z38c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z39a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z39b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z39c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z40a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z40b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z40c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z41a	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z41b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z41c	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z9b	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00
HET_Z9c	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00
OLE_B12a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B12b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B12c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B16b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B16c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B21a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_B21c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B5b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B5c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B7a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_B7b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B7c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B8a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_B8b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_B8c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K11a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K11c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K12a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K12b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K12c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K13a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K13b	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00

OLE_K17a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_K17b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K17c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K18a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K18b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K18c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K19a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K19b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K19c	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_K22a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_K22b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K23a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K23b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K23c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K3b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K3c	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_K5a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K5b	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_K5c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K9a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_K9b	0.00	0.00	0.12	0.00	0.00	0.88	0.00	0.00	0.00	0.00
OLE_K9c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z10a	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z10b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z10c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z13a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z13b	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z13c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z14b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z14c	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z20b	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z20c	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z21b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z25a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z25b	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z25c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z27a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z27b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z27c	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00
OLE_Z28a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z28b	0.00	0.00	0.13	0.00	0.00	0.86	0.00	0.00	0.00	0.00
OLE_Z28c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z30a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z30b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z30c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00

OLE_Z3a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z3b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z3c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z40a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z40b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z40c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z4a	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z4b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z4c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z7b	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
OLE_Z7c	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
PAL_B11b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B14a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B14c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B15a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B15c	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
PAL_B20b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B20c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B21a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B21b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B21c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B7a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B7b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_B7c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K22a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K22b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K22c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K24a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K24b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K25a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K25c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K26a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K26b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K26c	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
PAL_K4a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K4c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K5a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K5b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K5c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_K7b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z12a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z12c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z13a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z13b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z13c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00

PAL_Z14a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z14b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z14c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z15a	0.00	0.01	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
PAL_Z15b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z15c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z16a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z16b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z16c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z17a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z17b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z17c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z18a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z18b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z18c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z19b	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
PAL_Z19c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z20c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z23a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z23c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z25a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z25b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z25c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z26c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z6a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z6b	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z6c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z8a	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAL_Z8c	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
PAN_B30a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_B30b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_B30c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_B31a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_B31b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_B31c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K20a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00
PAN_K20b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K20c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K21a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K21b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K21c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K25b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K25c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K30a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K30b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00

PAN_K30c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K31a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K31b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K31c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K31d	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K32a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K32b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K32c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K33a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K34a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K34b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K34c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K35a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K35e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K35b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
PAN_K35c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00
PAN_K35d	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
RIV_B11a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B11b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_B11c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_B12b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B12c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_B13a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B13b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B13c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B23a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B23c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B3a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B3b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B3c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B7a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B7b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B7c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_B9a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_B9b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_B9c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K17a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_K17b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K17c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K22a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.99	0.00
RIV_K22b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K22c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_K24a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K24b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K24c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00

RIV_K25b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K25c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K2c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K4a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K5a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K5b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K7a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K7b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_K7c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z12a	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.92	0.00
RIV_Z12b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z12c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z13a	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.86	0.00
RIV_Z13b	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.98	0.00
RIV_Z13c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z17a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z17b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z17c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z20a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z20b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z20c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
RIV_Z25a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_Z25b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00
RIV_Z25c	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.97	0.00
VUL_B10a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B10b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B10c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B13a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B13b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B13c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B1a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B1b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B1c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B20a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B20b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B20c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B21b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B21c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_B7b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99
VUL_B7c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K13a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K13b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K13c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K16a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K16b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

VUL_K16c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K19a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K19b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K19c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K20a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K20b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K20c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K21a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K21b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K21c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K2a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K2b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K2c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K3a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K3b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K3c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K7a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K7c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K8a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K8b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_K8c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z11a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z11b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z11c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z1b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z21a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z21b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z4b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z4c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z5a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z5c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z6a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z6b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
VUL_Z6c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00

Tab. H34: Hybrids identity to species according to STRUCTURE

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
ACA_x_CAN_Z27a	0.59	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_x_CAN_K21a	0.60	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ACA_x_OLE_Z25b	0.55	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.01	0.00
ACA_x_OLE_Z27a	0.58	0.00	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.00
ACA_x_OLE_Z29a	0.59	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00
ACA_x_OLE_Z29b	0.60	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.00	0.00
ACA_x_OLE_Z29c	0.64	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00
ACA_x_OLE_Z35a	0.49	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00

ACA_x_OLE_Z35c	0.37	0.00	0.00	0.00	0.00	0.61	0.00	0.00	0.00	0.00
ACA_x_PAN_K20a	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00
ACA_x_PAN_K20c	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
ACA_x_PAN_K25a	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.00	0.00
ACA_x_PAN_K25b	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00
ACA_x_PAN_K25c	0.66	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00
CAN_x_OLE_K23a	0.01	0.00	0.33	0.00	0.03	0.63	0.00	0.00	0.00	0.00
CAN_x_OLE_B22b	0.01	0.00	0.47	0.00	0.00	0.00	0.00	0.51	0.00	0.00
CAN_x_OLE_K19a	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.61	0.00
CAN_x_OLE_K19b	0.01	0.00	0.41	0.00	0.00	0.57	0.00	0.00	0.01	0.00
CAN_x_OLE_K22a	0.00	0.00	0.54	0.00	0.00	0.46	0.00	0.00	0.00	0.00
CAN_x_OLE_K22c	0.00	0.00	0.91	0.00	0.00	0.08	0.00	0.00	0.00	0.00
CAN_x_OLE_K23a	0.00	0.00	0.44	0.00	0.00	0.56	0.00	0.00	0.00	0.00
CAN_x_OLE_K23b	0.00	0.00	0.49	0.00	0.00	0.50	0.00	0.00	0.00	0.00
CAN_x_OLE_K23c	0.00	0.00	0.52	0.00	0.00	0.47	0.00	0.00	0.00	0.00
CAN_x_OLE_K33a	0.00	0.00	0.49	0.00	0.00	0.50	0.00	0.00	0.00	0.00
CAN_x_OLE_K33b	0.00	0.00	0.49	0.00	0.00	0.34	0.12	0.03	0.01	0.00
CAN_x_OLE_K33c	0.00	0.01	0.54	0.00	0.00	0.38	0.00	0.02	0.04	0.00
CAN_x_OLE_Z23b	0.00	0.00	0.50	0.00	0.00	0.34	0.00	0.12	0.03	0.00
CAN_x_OLE_Z28a	0.00	0.00	0.48	0.00	0.00	0.52	0.00	0.00	0.00	0.00
CAN_x_OLE_Z28b	0.00	0.00	0.48	0.00	0.00	0.51	0.00	0.00	0.00	0.00
CAN_x_OLE_Z28c	0.00	0.00	0.43	0.00	0.00	0.56	0.00	0.00	0.00	0.00
CAN_x_OLE_Z30a	0.00	0.00	0.47	0.00	0.00	0.52	0.00	0.00	0.00	0.00
CAN_x_OLE_Z30b	0.00	0.00	0.37	0.00	0.00	0.62	0.00	0.00	0.00	0.00
CAN_x_OLE_Z31a	0.00	0.02	0.03	0.00	0.04	0.90	0.01	0.00	0.00	0.00
CAN_x_OLE_Z32a	0.00	0.00	0.41	0.00	0.00	0.59	0.00	0.00	0.00	0.00
CAN_x_OLE_Z32c	0.00	0.00	0.38	0.00	0.00	0.61	0.00	0.00	0.00	0.00
CAN_x_OLE_K17a	0.00	0.00	0.49	0.00	0.00	0.00	0.03	0.00	0.47	0.00
CAN_x_PAL_K22b	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.43	0.00
PAL_x_CAN_K22c	0.00	0.00	0.52	0.00	0.00	0.47	0.00	0.00	0.00	0.00
CAN_x_PAL_K33a	0.00	0.00	0.42	0.00	0.00	0.00	0.58	0.00	0.00	0.00
CAN_x_PAL_K33b	0.00	0.00	0.49	0.01	0.00	0.00	0.48	0.01	0.00	0.01
CAN_x_PAL_K33c	0.00	0.00	0.42	0.00	0.00	0.00	0.54	0.02	0.01	0.00
PAL_x_CAN_K22a	0.00	0.00	0.49	0.01	0.00	0.00	0.48	0.01	0.00	0.01
CAN_x_PAN_K21b	0.00	0.00	0.53	0.00	0.00	0.00	0.36	0.09	0.00	0.00
CAN__x_PAN_K21a	0.01	0.00	0.54	0.00	0.00	0.00	0.00	0.44	0.00	0.00
CAN_x_RIV_B23a	0.01	0.00	0.54	0.00	0.00	0.00	0.00	0.44	0.00	0.00
HET_x_OLE_Z34a	0.00	0.00	0.51	0.00	0.00	0.38	0.00	0.00	0.11	0.00
HET_x_OLE_Z40a	0.01	0.00	0.00	0.00	0.44	0.55	0.00	0.00	0.00	0.00
HET_x_PAL_Z34a	0.00	0.00	0.00	0.00	0.48	0.51	0.00	0.00	0.00	0.00
HET_x_PAL_Z37a	0.00	0.00	0.00	0.00	0.42	0.00	0.57	0.00	0.00	0.00
HET_x_RIV_Z25a	0.00	0.00	0.00	0.00	0.43	0.00	0.56	0.00	0.00	0.00
OLE_x_PAL_K22a	0.08	0.00	0.00	0.00	0.48	0.01	0.00	0.02	0.41	0.00
PAL(xOLE)_Z25a	0.01	0.00	0.00	0.00	0.03	0.38	0.02	0.00	0.55	0.00
OLE_x_RIV_Z25d	0.00	0.00	0.00	0.00	0.00	0.44	0.55	0.00	0.00	0.00
OLE_x_RIV_Z25e	0.00	0.00	0.00	0.00	0.00	0.43	0.02	0.01	0.53	0.00

OLE_x_RIV_K17a	0.00	0.00	0.00	0.00	0.00	0.57	0.00	0.00	0.42	0.00
OLE_x_RIV_K17b	0.00	0.00	0.00	0.00	0.00	0.59	0.01	0.00	0.39	0.00
OLE_x_RIV_K17c	0.00	0.00	0.00	0.00	0.01	0.42	0.00	0.00	0.56	0.00
OLE_x_RIV_Z25a	0.00	0.00	0.01	0.00	0.00	0.21	0.46	0.30	0.01	0.00
OLE_x_RIV_Z25b	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00	0.61	0.00
OLE_x_RIV_Z25c	0.00	0.00	0.00	0.00	0.01	0.57	0.00	0.00	0.41	0.00
OLE_x_RIV_B23a	0.00	0.00	0.00	0.00	0.00	0.39	0.06	0.12	0.42	0.00
RIV_x_OLE_Z26a	0.07	0.01	0.01	0.00	0.00	0.31	0.01	0.01	0.58	0.00
RIV_(xOLE)_Z26a	0.01	0.00	0.00	0.00	0.03	0.38	0.02	0.00	0.55	0.00
RIV_(xOLE)_Z26b	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.60	0.00	0.00
RIV_(xOLE)_Z26c	0.15	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.58	0.00
PAL_x_RIV_B23a	0.00	0.00	0.52	0.00	0.00	0.47	0.00	0.00	0.00	0.00
PAL_x_RIV_K34a	0.00	0.00	0.38	0.00	0.00	0.00	0.61	0.00	0.00	0.00
PAL_x_RIV_Z25a	0.00	0.00	0.00	0.00	0.00	0.39	0.06	0.12	0.42	0.00
PAN_x_PAL_K35a	0.00	0.00	0.00	0.00	0.00	0.00	0.51	0.15	0.34	0.00
ACAxCANxPAN_K20b	0.31	0.00	0.50	0.00	0.00	0.00	0.00	0.18	0.00	0.00

4.2. Our script results

Tab. H35: Species identity (in %) according to scirpt

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
ACA_K20b	14.76	9.08	9.33	9.42	9.67	10.13	9.35	9.92	9.64	8.70
ACA_K20c	15.51	9.13	9.33	9.32	9.40	9.95	9.29	9.88	9.60	8.58
ACA_K21a	15.05	9.20	9.32	9.18	9.72	9.78	9.29	9.92	9.74	8.79
ACA_K21b	15.02	9.09	9.50	9.50	9.69	9.85	9.17	9.89	9.74	8.56
ACA_K21c	14.91	9.09	9.44	9.23	9.71	9.91	9.10	10.08	9.91	8.62
ACA_K24a	15.54	9.06	9.22	9.33	9.58	9.80	9.23	9.95	9.69	8.60
ACA_K24b	15.45	9.05	9.40	9.20	9.79	9.83	9.37	9.86	9.69	8.37
ACA_K24c	15.03	9.13	9.42	9.10	9.85	9.76	9.35	9.94	9.70	8.72
ACA_K25a	15.89	9.17	9.36	9.02	9.38	9.67	9.16	9.78	9.86	8.71
ACA_K25b	15.42	9.06	9.29	9.08	9.71	9.70	9.35	9.90	9.72	8.78
ACA_K25c	15.64	9.24	9.34	9.17	9.61	9.64	9.23	9.65	9.72	8.76
ACA_K25d	15.73	9.32	9.25	9.04	9.52	9.81	9.05	9.72	9.77	8.80
ACA_K6a	15.43	9.52	9.37	8.90	9.42	9.78	9.12	9.88	9.93	8.64
ACA_K6b	15.43	9.34	9.42	9.18	9.63	9.81	9.10	9.71	9.70	8.66
ACA_K6c	15.13	9.39	9.40	9.17	9.58	9.83	9.36	9.88	9.74	8.52
ACA_Z10b	15.03	9.31	9.38	8.86	9.50	9.99	9.14	10.07	10.02	8.69
ACA_Z10c	15.38	9.18	9.25	9.61	9.62	9.96	9.15	9.67	9.56	8.64
ACA_Z11a	14.88	9.26	9.48	9.14	9.69	9.85	9.37	9.92	9.72	8.69
ACA_Z11b	15.39	9.00	9.13	9.37	9.44	10.01	9.19	9.77	9.80	8.91
ACA_Z11c	15.03	9.14	9.48	9.41	9.74	9.80	9.31	9.69	9.65	8.75
ACA_Z27a	15.29	9.18	9.41	8.91	9.50	9.89	9.19	9.92	9.91	8.81
ACA_Z27b	15.51	9.08	9.25	9.16	9.54	9.94	9.07	9.90	9.75	8.80
ACA_Z27c	15.32	9.03	9.28	9.25	9.65	9.86	9.06	10.06	9.60	8.89
ACA_Z3a	15.13	9.15	9.21	8.93	9.81	9.90	9.45	10.08	9.73	8.61

ACA_Z3b	15.57	9.03	9.42	9.13	9.57	10.04	9.24	9.73	9.65	8.62
ACA_Z4a	14.85	9.04	9.27	8.77	9.67	9.94	9.57	10.23	9.80	8.87
ACA_Z4b	15.09	9.46	9.45	9.06	9.69	9.62	9.16	10.13	9.75	8.59
ACA_Z4c	15.93	9.22	9.16	8.99	9.57	10.14	9.01	9.85	9.69	8.43
ACA_Z5a	15.26	9.21	9.24	9.15	9.45	9.97	9.15	9.98	9.84	8.76
ACA_Z5b	15.22	9.20	9.43	9.04	9.37	10.15	9.30	9.86	9.78	8.65
ACA_Z5c	15.26	9.28	9.31	9.29	9.52	9.86	9.33	9.93	9.75	8.46
ARV_B10a	9.34	16.30	9.41	9.22	9.01	9.26	9.54	9.63	9.20	9.09
ARV_B11b	9.28	15.74	9.54	9.34	9.28	9.23	9.57	9.59	9.33	9.10
ARV_B12a	9.02	16.76	9.37	8.99	9.21	9.33	9.26	9.63	9.20	9.24
ARV_B12c	9.37	16.62	9.49	9.14	9.10	9.20	9.28	9.63	9.03	9.13
ARV_B13b	9.00	16.78	9.36	9.57	9.20	9.24	9.17	9.45	9.14	9.09
ARV_B14a	9.06	16.37	9.59	9.19	9.05	9.26	9.33	9.60	9.10	9.47
ARV_B15b	9.06	16.93	9.34	9.25	9.09	9.18	9.30	9.66	8.98	9.20
ARV_B20b	9.31	16.55	9.59	9.29	9.00	9.00	9.41	9.56	9.29	9.01
ARV_B20c	9.38	16.48	9.36	9.22	9.11	9.30	9.39	9.61	9.07	9.09
ARV_B4a	9.19	16.62	9.41	9.00	9.14	9.24	9.34	9.57	9.33	9.16
ARV_B4b	9.23	16.90	9.46	8.97	9.23	9.32	9.40	9.54	9.12	8.82
ARV_B4c	9.18	16.70	9.48	9.21	9.07	9.12	9.32	9.59	8.98	9.34
ARV_B5b	9.38	16.43	9.69	9.23	9.20	9.21	9.22	9.53	9.12	8.98
ARV_B5c	9.21	16.50	9.72	9.25	9.19	9.17	9.26	9.64	9.01	9.05
ARV_B6b	9.05	16.99	9.66	9.46	8.96	9.05	9.04	9.43	9.07	9.28
ARV_B6c	9.15	17.04	9.29	9.34	8.97	9.35	9.16	9.31	9.08	9.29
ARV_B7b	9.19	16.54	9.52	9.28	8.95	9.45	9.33	9.82	9.13	8.79
ARV_B7c	9.22	16.39	9.56	9.16	9.11	9.48	9.34	9.46	9.21	9.05
ARV_B8a	9.10	16.49	9.61	9.32	9.13	9.25	9.10	9.62	9.26	9.13
ARV_B8b	9.14	16.49	9.61	9.30	9.17	9.07	9.24	9.49	9.17	9.32
ARV_B8c	9.16	16.64	9.53	9.24	9.03	9.04	9.47	9.46	9.28	9.16
ARV_B9a	9.17	16.23	9.49	9.40	9.20	9.27	9.13	9.82	9.10	9.17
ARV_K10c	8.93	16.57	9.69	9.23	9.15	9.27	9.24	9.51	9.24	9.18
ARV_K12a	9.20	16.67	9.39	9.26	8.88	9.16	9.35	9.74	9.06	9.28
ARV_K12b	9.17	16.59	9.49	9.03	9.12	9.47	9.21	9.53	9.26	9.12
ARV_K13b	9.10	16.40	9.53	9.33	9.12	9.40	9.26	9.54	9.16	9.15
ARV_K1b	9.06	16.80	9.50	9.16	9.02	9.16	9.36	9.53	9.15	9.26
ARV_K1c	9.35	16.81	9.54	9.28	9.06	9.20	9.19	9.30	9.06	9.20
ARV_K2b	9.35	16.16	9.35	9.29	9.17	9.33	9.20	9.63	9.33	9.18
ARV_K2c	9.19	16.21	9.61	9.12	9.26	9.42	9.40	9.54	9.15	9.11
ARV_K3a	9.26	16.06	9.57	9.21	9.06	9.51	9.46	9.61	9.14	9.11
ARV_K3b	9.28	16.01	9.51	9.28	9.34	9.12	9.42	9.59	9.23	9.22
ARV_K4a	9.10	16.52	9.53	9.15	9.15	9.36	9.17	9.62	9.29	9.11
ARV_K4b	9.12	15.76	9.73	9.19	9.16	9.57	9.24	9.70	9.22	9.32
ARV_K4c	9.01	16.45	9.59	9.42	9.26	9.30	9.07	9.55	9.05	9.30
ARV_K5a	9.20	16.44	9.56	9.22	9.03	9.19	9.37	9.52	9.14	9.32
ARV_K5b	9.20	16.64	9.50	9.44	9.06	9.26	9.38	9.55	9.04	8.92
ARV_K5c	9.12	16.55	9.67	9.44	9.11	9.24	9.29	9.49	9.13	8.97

ARV_K6c	9.31	16.36	9.52	9.10	9.12	9.32	9.26	9.57	9.18	9.26
ARV_K7b	9.17	16.76	9.63	9.19	9.20	9.40	9.23	9.35	9.11	8.96
ARV_K8b	9.28	16.64	9.40	9.20	9.05	9.22	9.27	9.63	9.14	9.18
ARV_K9a	9.02	16.91	9.56	9.21	9.04	9.36	9.11	9.67	9.10	9.02
ARV_K9b	9.21	17.11	9.21	9.09	9.08	9.28	9.30	9.29	9.25	9.18
ARV_Z10	9.34	16.58	9.44	9.15	9.02	9.46	9.22	9.61	9.04	9.13
ARV_Z13a	9.15	16.50	9.70	9.34	8.99	9.21	9.21	9.52	9.21	9.17
ARV_Z13b	9.12	16.74	9.36	9.23	9.19	9.31	9.21	9.46	9.27	9.10
ARV_Z14b	9.06	16.56	9.61	9.62	9.05	9.18	9.42	9.47	9.05	8.97
ARV_Z14c	9.13	16.74	9.41	9.25	9.18	9.28	9.34	9.38	9.17	9.11
ARV_Z15a	9.29	16.37	9.60	9.10	9.15	9.04	9.28	9.67	9.12	9.38
ARV_Z15c	9.50	16.65	9.68	9.18	9.11	9.07	9.26	9.50	9.15	8.90
ARV_Z16a	8.94	17.30	9.53	9.09	8.95	9.29	8.89	9.60	9.12	9.28
ARV_Z16b	9.17	16.17	10.03	8.90	9.09	9.36	9.34	9.51	9.33	9.10
ARV_Z16c	8.95	16.95	9.58	9.08	9.13	9.19	9.13	9.61	9.17	9.21
ARV_Z17b	9.23	17.03	9.37	9.15	9.09	9.20	9.20	9.37	9.16	9.21
ARV_Z17c	9.12	16.82	9.40	9.29	9.12	9.18	9.23	9.69	9.10	9.04
ARV_Z18a	9.11	16.64	9.41	8.90	9.30	9.19	9.20	9.53	9.40	9.32
ARV_Z18b	9.13	16.57	9.32	9.24	9.18	9.43	9.13	9.58	9.11	9.30
ARV_Z18c	8.95	16.54	9.76	9.06	9.00	9.21	9.16	9.56	9.22	9.55
ARV_Z19a	9.18	16.26	9.38	9.11	9.25	9.36	9.31	9.72	9.23	9.20
ARV_Z19c	9.06	16.99	9.34	9.26	9.05	9.40	9.23	9.38	9.31	8.98
ARV_Z1b	9.06	16.89	9.66	9.21	9.02	9.26	9.15	9.42	9.23	9.10
ARV_Z20a	9.12	16.87	9.54	8.97	9.13	9.00	9.21	9.70	9.17	9.30
ARV_Z20b	9.23	16.85	9.51	8.96	9.13	9.10	9.13	9.70	9.24	9.15
ARV_Z20c	9.16	16.67	9.40	9.14	8.94	9.07	9.56	9.52	9.14	9.39
ARV_Z22a	9.10	16.38	9.62	9.15	9.09	9.31	9.37	9.60	9.16	9.22
ARV_Z22c	9.06	16.79	9.44	9.32	9.41	9.48	8.90	9.40	9.33	8.88
ARV_Z23b	9.07	16.68	9.65	8.97	9.09	9.24	9.15	9.59	9.17	9.39
ARV_Z2c	9.71	15.69	9.59	9.30	9.27	9.48	9.17	9.65	9.22	8.92
ARV_Z4a	9.19	16.70	9.32	9.21	9.09	9.37	8.99	9.56	9.40	9.15
ARV_Z6b	9.12	17.26	9.22	9.07	9.10	9.18	9.28	9.61	8.97	9.18
ARV_Z6c	9.21	16.46	9.41	9.13	9.23	9.24	9.40	9.71	9.14	9.06
ARV_Z8a	9.31	16.05	9.54	9.38	8.90	9.41	9.39	9.68	9.14	9.19
ARV_Z8b	9.26	16.94	9.51	9.34	9.09	9.08	9.41	9.32	8.99	9.04
ARV_Z8c	9.17	16.01	9.56	9.71	9.00	9.17	9.34	9.70	9.06	9.28
ARV_Z9c	9.07	16.59	9.41	9.18	8.98	9.57	9.28	9.70	9.08	9.14
CAN_B11a	9.35	9.55	15.48	8.87	9.79	9.30	9.52	9.81	9.77	8.56
CAN_B11b	9.49	9.45	15.88	8.88	9.79	9.49	9.50	9.47	9.64	8.41
CAN_B11c	9.44	9.57	15.70	8.85	9.75	9.60	9.41	9.61	9.70	8.37
CAN_B13b	9.52	9.82	14.90	9.05	9.75	9.64	9.37	9.75	9.55	8.65
CAN_B13c	9.20	9.42	16.12	8.77	9.82	9.56	9.23	9.59	9.82	8.47
CAN_B23a	9.28	9.30	16.07	8.90	9.74	9.48	9.42	9.68	9.73	8.41
CAN_B23b	9.44	9.46	15.64	9.23	9.66	9.44	9.36	9.56	9.52	8.69
CAN_B3a	9.42	9.68	14.53	9.13	9.65	9.70	9.34	9.91	9.83	8.80

CAN_B3b	9.50	9.37	15.01	9.32	9.79	9.61	9.42	9.81	9.55	8.63
CAN_B3c	9.34	9.37	15.74	9.31	9.75	9.41	9.43	9.63	9.44	8.58
CAN_B5a	9.21	9.51	15.45	8.90	9.81	9.52	9.46	9.66	9.68	8.79
CAN_B5b	9.40	9.69	15.81	8.98	9.65	9.52	9.31	9.65	9.54	8.45
CAN_B5c	9.28	9.63	15.92	8.99	9.76	9.43	9.36	9.63	9.66	8.35
CAN_B6a	9.17	9.61	15.39	8.87	9.78	9.55	9.38	9.82	9.66	8.75
CAN_B6c	9.35	9.56	14.89	9.39	9.53	9.54	9.51	9.81	9.72	8.71
CAN_B7a	9.51	9.51	15.65	8.78	9.75	9.44	9.27	9.60	9.76	8.72
CAN_B7b	9.71	9.31	15.72	8.96	9.75	9.27	9.45	9.66	9.52	8.65
CAN_B7c	9.34	9.37	16.11	9.22	9.72	9.47	9.17	9.62	9.49	8.50
CAN_B8a	9.23	9.52	15.39	9.18	9.83	9.40	9.73	9.67	9.45	8.61
CAN_B8b	9.30	9.62	15.27	8.94	9.79	9.46	9.25	9.75	9.80	8.80
CAN_B8c	9.39	9.36	15.43	8.97	9.66	9.38	9.58	9.88	9.53	8.82
CAN_B9a	9.18	9.66	15.97	8.75	9.76	9.53	9.41	9.62	9.69	8.43
CAN_B9b	9.47	9.38	15.91	9.04	9.65	9.49	9.40	9.62	9.46	8.59
CAN_B9c	9.20	9.54	15.55	9.12	9.85	9.39	9.37	9.77	9.57	8.65
CAN_K11a	9.32	9.57	15.83	9.11	9.59	9.40	9.34	9.69	9.71	8.46
CAN_K11b	9.27	9.45	15.54	8.89	9.79	9.47	9.59	9.69	9.68	8.64
CAN_K11c	9.30	9.41	16.37	9.27	9.44	9.44	9.27	9.54	9.59	8.38
CAN_K16a	9.21	9.53	15.98	8.85	9.58	9.42	9.35	9.62	9.69	8.76
CAN_K16b	9.14	9.52	15.40	8.99	9.68	9.47	9.63	9.71	9.78	8.68
CAN_K16c	9.58	9.29	16.06	8.87	9.72	9.32	9.52	9.55	9.67	8.43
CAN_K18a	9.25	9.44	16.03	8.92	9.68	9.49	9.56	9.56	9.58	8.49
CAN_K18b	9.06	9.61	15.80	8.92	9.72	9.40	9.44	9.73	9.64	8.68
CAN_K18c	9.20	9.70	15.67	8.87	9.66	9.38	9.47	9.65	9.60	8.80
CAN_K19a	9.35	9.47	15.75	8.78	9.76	9.37	9.78	9.70	9.53	8.51
CAN_K19b	9.22	9.27	15.88	8.85	9.91	9.45	9.46	9.71	9.85	8.38
CAN_K20a	9.36	9.51	15.68	9.07	9.72	9.35	9.31	9.73	9.60	8.68
CAN_K20b	9.44	9.54	15.59	8.67	9.73	9.56	9.49	9.83	9.60	8.55
CAN_K20c	9.21	9.54	15.67	8.96	9.66	9.49	9.58	9.77	9.57	8.55
CAN_K20d	9.22	9.51	15.81	9.12	9.73	9.41	9.53	9.74	9.43	8.51
CAN_K22a	9.31	9.53	15.29	9.09	9.86	9.55	9.39	9.89	9.75	8.35
CAN_K22b	9.34	9.60	15.10	9.18	9.82	9.40	9.45	9.79	9.56	8.75
CAN_K22c	9.21	9.55	15.73	8.91	9.63	9.58	9.42	9.62	9.72	8.62
CAN_K23a	9.39	9.45	15.64	8.98	9.84	9.54	9.31	9.57	9.75	8.54
CAN_K23b	9.23	9.34	16.12	8.73	9.75	9.52	9.61	9.68	9.73	8.29
CAN_K3a	9.30	9.28	15.70	9.14	9.55	9.44	9.58	9.65	9.62	8.73
CAN_K3b	9.19	9.63	15.29	8.95	9.58	9.43	9.63	9.80	9.75	8.75
CAN_K3c	9.22	9.43	15.53	8.98	9.67	9.47	9.36	9.69	9.73	8.92
CAN_K4a	9.44	9.55	15.57	8.77	9.88	9.58	9.36	9.70	9.67	8.49
CAN_K4b	9.57	9.47	15.78	9.10	9.65	9.47	9.30	9.60	9.60	8.47
CAN_K4c	9.19	9.55	15.90	9.17	9.64	9.42	9.25	9.67	9.59	8.61
CAN_K9a	9.38	9.77	15.54	9.02	9.66	9.45	9.28	9.65	9.56	8.68
CAN_K9b	9.34	9.51	15.33	9.15	9.66	9.36	9.47	9.75	9.74	8.69
CAN_K9c	9.44	9.52	15.89	8.82	9.63	9.64	9.35	9.52	9.72	8.46

CAN_Z10a	9.38	9.77	14.97	8.87	9.79	9.55	9.49	9.81	9.75	8.62
CAN_Z10b	9.28	9.44	15.51	9.04	9.79	9.48	9.43	9.70	9.72	8.62
CAN_Z10c	9.22	9.41	15.52	9.05	9.94	9.47	9.41	9.67	9.62	8.69
CAN_Z1a	9.32	9.59	15.51	9.05	9.73	9.40	9.34	9.67	9.65	8.75
CAN_Z1b	9.39	9.57	15.12	8.89	9.89	9.56	9.51	9.78	9.69	8.61
CAN_Z1c	9.33	9.43	15.12	9.07	9.70	9.61	9.39	9.83	9.82	8.70
CAN_Z27a	9.20	9.55	15.67	8.71	9.80	9.49	9.41	9.70	9.83	8.65
CAN_Z27b	9.35	9.43	15.61	8.62	9.75	9.60	9.25	9.76	9.89	8.74
CAN_Z27c	9.46	9.51	15.53	8.85	9.78	9.48	9.35	9.70	9.76	8.59
CAN_Z28a	9.49	9.78	14.81	8.75	9.87	9.52	9.45	9.85	9.69	8.78
CAN_Z28b	9.29	9.69	15.43	8.91	9.77	9.53	9.34	9.77	9.57	8.71
CAN_Z28c	9.60	9.57	14.70	8.95	9.77	9.60	9.55	9.92	9.67	8.68
CAN_Z2b	9.35	9.64	15.08	8.86	9.89	9.59	9.38	9.87	9.73	8.61
CAN_Z30a	9.45	9.51	15.15	9.06	9.68	9.49	9.57	9.80	9.67	8.63
CAN_Z30b	9.55	9.47	15.32	9.08	9.73	9.34	9.33	9.75	9.69	8.74
CAN_Z30c	9.26	9.51	15.63	8.95	9.80	9.50	9.34	9.66	9.78	8.56
CAN_Z3b	9.44	9.57	15.34	8.82	9.73	9.51	9.33	9.82	9.87	8.57
CAN_Z3c	9.22	9.34	15.87	8.99	9.84	9.41	9.39	9.65	9.76	8.54
ERI_B2a	9.29	9.25	8.80	18.74	8.99	8.62	8.78	9.24	8.39	9.90
ERI_B2b	9.18	9.24	8.98	18.70	8.99	8.59	8.93	9.21	8.53	9.65
ERI_B2c	9.28	9.17	9.04	18.39	9.03	8.65	8.88	9.23	8.50	9.81
ERI_K10a	9.24	9.26	9.06	18.15	9.09	8.66	8.94	9.26	8.59	9.75
ERI_K10b	9.22	8.97	8.86	19.26	8.99	8.64	8.60	9.07	8.42	9.98
ERI_K10c	8.86	9.15	9.07	19.34	8.92	8.68	8.89	9.07	8.49	9.53
ERI_K11b	8.94	9.15	9.16	19.25	8.98	8.65	8.87	8.95	8.49	9.55
ERI_K11c	9.16	9.19	8.88	18.98	8.95	8.59	8.85	9.24	8.65	9.52
ERI_K12b	9.11	9.15	9.03	18.24	9.17	8.85	8.97	9.23	8.65	9.59
ERI_K12c	8.94	9.49	8.99	18.18	9.09	8.77	8.91	9.29	8.69	9.65
ERI_K1a	8.99	9.55	8.90	19.20	8.96	8.62	8.70	9.08	8.50	9.52
ERI_K1b	8.95	9.41	9.01	18.83	8.98	8.69	8.87	9.17	8.57	9.52
ERI_K1c	9.18	9.11	8.97	19.23	9.03	8.58	8.51	9.07	8.50	9.81
ERI_K2a	9.21	9.40	8.98	18.81	8.93	8.62	8.77	9.15	8.45	9.68
ERI_K2b	9.19	9.34	9.09	18.89	8.88	8.53	8.80	9.01	8.57	9.70
ERI_K2c	9.32	9.21	9.08	18.79	8.90	8.58	8.58	9.07	8.58	9.90
ERI_K3a	9.18	9.10	9.08	18.56	9.00	8.53	8.81	9.26	8.72	9.75
ERI_K3b	8.93	9.27	8.94	19.34	8.97	8.62	8.59	9.29	8.38	9.66
ERI_K3c	9.30	8.89	8.80	19.28	8.80	8.59	8.59	9.19	8.56	10.01
ERI_K8a	9.20	9.05	9.06	18.79	8.79	8.62	8.70	9.12	8.49	10.19
ERI_K8b	9.20	8.98	8.79	19.40	8.87	8.64	8.57	9.20	8.45	9.90
HET_Z15a	9.53	9.27	9.66	9.49	14.15	9.56	9.64	9.73	9.95	9.02
HET_Z15b	9.68	9.09	9.78	9.07	14.40	9.90	9.39	9.74	10.23	8.71
HET_Z15c	9.76	9.02	9.80	8.77	14.53	9.96	9.31	9.84	10.18	8.82
HET_Z16a	9.61	9.28	9.71	9.18	14.38	9.89	9.32	9.96	9.87	8.80
HET_Z16b	9.40	9.28	9.79	8.86	15.15	9.64	9.39	9.67	9.84	8.98
HET_Z16c	9.85	8.89	9.83	8.78	15.07	9.79	9.19	9.89	9.89	8.82

HET_Z17a	9.69	9.17	9.91	9.02	14.34	9.91	9.33	9.77	10.19	8.68
HET_Z17b	9.79	9.14	9.62	8.66	14.89	9.76	9.56	9.97	10.02	8.60
HET_Z17c	9.50	9.17	9.73	9.23	14.50	9.70	9.49	9.52	10.07	9.07
HET_Z18a	9.64	9.18	9.63	8.88	14.55	10.03	9.30	9.87	10.01	8.92
HET_Z18b	9.64	8.88	9.98	8.96	14.80	9.74	9.40	9.68	10.04	8.89
HET_Z18c	9.44	8.99	9.98	8.87	14.61	9.77	9.30	9.76	10.33	8.95
HET_Z19b	9.67	9.47	9.98	9.38	13.21	9.55	9.51	10.20	9.92	9.10
HET_Z19c	9.61	9.17	9.80	8.82	14.92	9.69	9.15	9.66	10.15	9.03
HET_Z22a	9.50	9.01	9.79	8.77	15.20	9.83	9.38	9.90	10.00	8.62
HET_Z22b	9.63	9.25	9.67	8.82	15.34	9.72	9.29	9.53	9.88	8.87
HET_Z22c	9.72	9.28	9.66	8.86	15.29	9.64	9.27	9.57	9.87	8.84
HET_Z23b	9.81	9.19	10.06	8.83	14.97	9.52	9.39	9.78	9.81	8.64
HET_Z23c	9.65	9.01	9.76	9.04	15.20	9.86	9.23	9.87	9.86	8.51
HET_Z33a	9.72	8.97	9.88	9.39	14.60	9.78	9.37	9.78	9.91	8.60
HET_Z33b	9.53	8.79	10.00	8.83	15.21	9.94	9.42	9.78	9.84	8.65
HET_Z33c	9.57	8.69	9.86	9.05	15.18	9.95	9.28	9.83	9.83	8.77
HET_Z34a	9.66	8.86	9.84	8.93	14.91	9.99	9.42	10.07	9.91	8.41
HET_Z34b	9.62	8.93	9.72	8.91	14.90	9.98	9.41	9.90	10.08	8.54
HET_Z34c	9.77	9.14	9.79	8.92	14.58	9.78	9.61	9.91	9.93	8.57
HET_Z36a	9.42	9.31	9.48	8.90	14.79	9.97	9.60	9.78	10.00	8.74
HET_Z36b	9.26	9.10	9.38	8.82	15.17	9.94	9.42	9.88	9.98	9.07
HET_Z37a	9.59	8.86	9.71	9.32	14.77	9.86	9.45	9.89	9.95	8.62
HET_Z37b	9.50	9.04	9.74	8.73	14.75	9.96	9.79	9.87	10.02	8.59
HET_Z37c	9.64	8.82	9.83	8.89	14.97	10.01	9.47	10.01	9.77	8.58
HET_Z38a	9.37	9.08	9.74	8.91	15.02	10.06	9.40	9.75	10.01	8.66
HET_Z38b	9.48	9.45	9.68	8.86	14.82	9.99	9.48	9.75	9.89	8.60
HET_Z38c	9.67	9.09	9.76	8.83	14.70	9.90	9.38	9.71	10.09	8.85
HET_Z39a	9.57	9.08	9.79	9.14	14.98	9.72	9.33	9.97	9.88	8.56
HET_Z39b	9.66	9.15	9.69	9.02	14.77	9.84	9.48	9.90	9.72	8.76
HET_Z39c	9.64	9.16	9.65	9.04	14.83	9.60	9.31	9.72	10.06	8.98
HET_Z40a	9.70	8.96	9.56	8.80	14.82	9.99	9.16	9.82	10.09	9.09
HET_Z40b	9.42	9.22	9.57	8.95	14.91	9.95	9.05	9.87	10.02	9.02
HET_Z40c	9.28	9.39	9.78	8.92	15.03	9.92	8.94	10.05	9.88	8.82
HET_Z41a	9.51	8.98	9.63	8.89	15.05	10.04	9.08	9.79	10.10	8.93
HET_Z41b	9.53	9.11	9.58	8.56	15.15	10.05	9.39	9.75	9.97	8.90
HET_Z41c	9.54	9.07	9.34	8.89	15.09	10.07	9.21	9.93	9.84	9.02
HET_Z9a	9.39	9.47	9.55	10.31	13.00	9.74	9.88	10.02	9.76	8.87
HET_Z9b	9.58	9.18	9.68	8.88	15.20	9.98	9.28	9.73	9.90	8.60
HET_Z9c	9.89	9.35	9.68	8.99	14.02	9.86	9.29	10.05	9.90	8.96
OLE_B12a	9.95	9.29	9.53	8.57	9.78	14.65	8.98	9.85	10.34	9.05
OLE_B12b	9.53	9.35	9.45	8.53	9.79	15.41	9.05	9.59	10.38	8.91
OLE_B12c	9.81	9.13	9.46	8.58	9.94	14.83	9.04	9.77	10.46	8.99
OLE_B16b	9.78	9.63	9.56	8.34	9.91	14.49	9.10	9.84	10.52	8.83
OLE_B16c	9.94	9.09	9.46	8.55	9.90	14.51	9.14	9.88	10.56	8.97
OLE_B21a	10.01	9.10	9.53	8.82	9.75	14.13	9.33	10.20	10.27	8.87

OLE_B21c	9.98	9.15	9.28	8.72	9.98	14.08	9.35	10.02	10.41	9.03
OLE_B5b	9.92	9.32	9.45	8.54	9.83	14.75	9.04	9.80	10.59	8.77
OLE_B5c	9.84	9.38	9.45	8.28	9.76	14.60	8.96	9.88	10.57	9.28
OLE_B7a	10.06	9.55	9.40	8.78	9.81	14.32	9.01	9.76	10.43	8.88
OLE_B7b	9.86	9.45	9.28	8.76	9.79	15.17	8.79	9.69	10.57	8.64
OLE_B7c	10.04	9.42	9.40	8.74	9.92	14.29	8.99	9.81	10.53	8.86
OLE_B8a	9.87	9.41	9.46	8.69	9.90	14.08	9.30	9.86	10.25	9.19
OLE_B8b	9.60	9.33	9.63	8.72	9.89	14.62	9.15	9.71	10.55	8.82
OLE_B8c	9.75	9.34	9.64	8.61	9.70	14.39	9.08	9.84	10.66	8.99
OLE_K11a	9.99	9.24	9.49	8.72	9.67	14.31	9.31	10.01	10.48	8.78
OLE_K11c	9.94	9.31	9.57	8.41	9.81	14.37	9.40	9.91	10.44	8.84
OLE_K12a	9.76	9.53	9.57	8.87	9.56	14.30	9.42	9.81	10.23	8.97
OLE_K12b	9.75	9.17	9.33	8.92	9.65	14.82	9.47	9.58	10.31	8.99
OLE_K12c	9.80	9.25	9.40	8.70	9.78	14.63	9.33	9.76	10.44	8.92
OLE_K13a	9.83	9.29	9.51	8.61	9.80	14.32	9.02	10.10	10.50	9.01
OLE_K13b	10.04	9.27	9.72	8.89	9.84	13.62	9.15	9.95	10.25	9.26
OLE_K17a	9.91	9.02	9.40	8.68	9.82	14.31	9.45	10.12	10.38	8.90
OLE_K17b	9.84	9.35	9.60	8.82	9.88	14.34	9.32	9.81	10.41	8.63
OLE_K17c	9.99	9.23	9.35	8.49	9.74	14.48	9.46	10.10	10.22	8.95
OLE_K18a	9.65	9.14	9.42	8.79	9.88	14.34	9.13	10.09	10.33	9.22
OLE_K18b	9.73	9.05	9.38	8.57	10.08	14.94	9.37	9.89	10.28	8.70
OLE_K18c	9.67	9.27	9.55	8.75	9.95	14.09	8.91	10.03	10.34	9.44
OLE_K19a	9.80	9.40	9.24	8.55	10.12	14.36	9.30	9.93	10.22	9.08
OLE_K19b	9.63	8.97	9.34	8.68	10.31	14.56	9.15	9.99	10.46	8.92
OLE_K19c	9.94	9.05	9.31	8.78	9.92	13.99	9.31	10.12	10.58	9.00
OLE_K22a	9.90	9.23	9.54	8.76	10.19	13.74	9.55	10.01	10.28	8.80
OLE_K22b	10.02	8.88	9.66	8.64	9.97	14.19	9.14	9.99	10.43	9.08
OLE_K23a	9.89	8.85	9.54	8.59	10.25	14.33	9.29	9.83	10.53	8.91
OLE_K23b	9.62	9.12	9.26	8.61	10.18	14.66	9.14	9.73	10.49	9.19
OLE_K23c	9.99	8.88	9.43	8.39	10.36	14.90	9.12	9.73	10.41	8.78
OLE_K3b	9.92	9.12	9.27	8.70	9.79	14.36	9.49	9.90	10.46	9.01
OLE_K3c	9.88	9.65	9.54	8.47	9.77	14.36	9.04	10.07	10.26	8.95
OLE_K5a	10.11	9.22	9.25	8.54	9.69	14.57	9.15	9.99	10.54	8.94
OLE_K5b	9.65	9.79	9.63	8.94	9.48	14.06	9.29	9.73	10.35	9.10
OLE_K5c	9.63	9.62	9.70	8.88	9.53	14.19	9.07	9.78	10.42	9.18
OLE_K9a	10.04	9.17	9.34	8.73	9.87	14.22	9.39	9.73	10.36	9.14
OLE_K9b	9.79	9.22	10.31	8.61	9.90	13.76	9.18	9.75	10.45	9.02
OLE_K9c	9.90	9.50	9.53	8.74	9.72	14.45	8.98	9.81	10.44	8.93
OLE_Z10a	10.20	9.22	9.39	8.48	9.70	14.53	9.15	10.09	10.66	8.58
OLE_Z10b	9.98	9.20	9.50	8.49	9.79	14.61	9.08	10.00	10.37	8.97
OLE_Z10c	9.90	9.11	9.19	8.68	9.73	14.44	9.51	10.08	10.50	8.87
OLE_Z13a	9.99	8.91	9.55	8.61	9.80	14.69	8.99	9.87	10.60	8.99
OLE_Z13b	10.02	9.24	9.56	8.51	9.84	14.33	9.21	9.84	10.57	8.86
OLE_Z13c	9.96	9.55	9.48	8.60	9.59	14.30	9.15	9.87	10.52	8.97
OLE_Z14b	9.92	9.48	9.47	8.57	9.83	14.22	9.26	9.97	10.39	8.90

OLE_Z14c	10.07	9.51	9.49	8.64	9.78	14.22	9.14	9.73	10.58	8.83
OLE_Z20b	9.98	9.49	9.58	8.53	9.86	14.04	9.12	9.92	10.50	8.97
OLE_Z20c	10.11	9.29	9.28	8.98	9.84	13.98	9.04	9.83	10.53	9.12
OLE_Z21b	10.05	8.97	9.29	8.52	9.92	14.96	9.15	9.84	10.56	8.74
OLE_Z25a	9.88	9.10	9.17	8.74	9.81	14.53	9.28	9.79	10.59	9.12
OLE_Z25b	9.90	9.19	9.41	8.83	9.59	14.17	9.30	9.73	10.86	9.01
OLE_Z25c	9.85	9.03	9.61	8.65	9.91	14.10	9.19	9.90	10.65	9.11
OLE_Z27a	10.01	9.18	9.56	8.58	10.06	14.62	9.21	9.75	10.27	8.76
OLE_Z27b	9.95	9.17	9.28	8.58	9.82	14.52	9.47	9.78	10.44	8.98
OLE_Z27c	9.92	9.32	9.43	8.64	9.90	13.88	9.24	9.82	10.48	9.37
OLE_Z28a	9.95	9.02	9.56	8.48	10.03	14.73	9.28	9.68	10.80	8.47
OLE_Z28b	9.68	9.21	10.82	8.47	9.81	13.74	9.31	9.77	10.31	8.88
OLE_Z28c	9.75	9.28	9.49	8.57	9.44	14.60	9.26	9.72	10.77	9.12
OLE_Z30a	9.64	9.26	9.24	8.44	9.88	15.35	9.01	9.64	10.57	8.97
OLE_Z30b	9.94	9.10	9.50	8.44	9.94	14.90	9.16	9.70	10.43	8.89
OLE_Z30c	9.83	9.21	9.45	8.55	9.86	14.99	8.85	9.85	10.60	8.81
OLE_Z3a	9.95	9.22	9.43	8.33	9.85	14.81	9.02	9.84	10.48	9.09
OLE_Z3b	9.82	9.53	9.35	8.54	9.87	14.49	9.03	9.84	10.36	9.17
OLE_Z3c	10.01	9.59	9.66	8.53	9.54	14.42	9.18	9.95	10.37	8.76
OLE_Z40a	9.80	9.42	9.12	8.49	10.26	14.55	9.30	9.97	10.26	8.82
OLE_Z40b	9.99	9.22	9.29	8.56	10.06	14.51	8.99	10.08	10.39	8.91
OLE_Z40c	9.73	9.24	9.48	8.68	9.95	14.60	9.04	9.85	10.36	9.07
OLE_Z4a	10.21	9.39	9.67	8.73	9.79	14.14	8.94	9.87	10.43	8.83
OLE_Z4b	10.16	9.09	9.49	8.72	9.63	14.70	8.75	9.81	10.49	9.17
OLE_Z4c	10.09	9.39	9.45	8.71	9.81	14.79	9.02	9.73	10.30	8.71
OLE_Z7b	10.03	9.61	9.65	8.62	10.00	14.32	8.88	9.82	10.52	8.55
OLE_Z7c	9.89	9.45	9.39	8.46	9.97	14.49	9.11	9.80	10.64	8.79
PAL_B11b	9.26	9.09	9.28	8.89	9.43	9.10	17.31	9.55	9.38	8.71
PAL_B14a	9.10	9.30	9.37	8.60	9.47	9.26	16.85	9.62	9.43	9.01
PAL_B14c	9.38	9.13	9.43	8.72	9.32	9.41	17.13	9.57	9.31	8.59
PAL_B15a	9.27	9.34	9.51	8.58	9.40	9.09	17.32	9.44	9.27	8.78
PAL_B15c	9.27	9.23	9.54	8.94	9.29	9.05	16.86	9.65	9.44	8.72
PAL_B20b	9.08	9.21	9.42	8.79	9.35	9.04	17.63	9.46	9.14	8.87
PAL_B20c	9.25	9.12	9.31	8.72	9.35	9.12	17.65	9.48	9.19	8.81
PAL_B21a	9.35	9.35	9.47	8.54	9.51	9.02	17.41	9.57	9.11	8.68
PAL_B21b	9.27	9.79	9.44	8.55	9.40	9.03	17.02	9.49	9.30	8.71
PAL_B21c	9.20	9.40	9.48	8.71	9.34	9.11	17.35	9.48	9.26	8.67
PAL_B7a	9.40	9.24	9.44	8.74	9.38	9.15	17.21	9.56	9.22	8.66
PAL_B7b	9.12	9.32	9.48	8.61	9.29	9.08	17.37	9.61	9.33	8.78
PAL_B7c	9.12	9.18	9.49	8.68	9.33	9.14	17.49	9.49	9.30	8.79
PAL_K22a	9.31	9.21	9.36	8.77	9.37	9.08	17.47	9.49	9.24	8.70
PAL_K22b	9.22	9.22	9.22	8.71	9.34	9.04	17.87	9.40	9.17	8.80
PAL_K22c	9.36	9.46	9.49	8.69	9.32	9.14	17.11	9.48	9.33	8.63
PAL_K24a	9.18	9.52	9.24	8.80	9.50	9.12	17.45	9.40	9.08	8.72
PAL_K24b	9.11	9.23	9.35	8.73	9.33	9.06	17.69	9.38	9.25	8.86

PAL_K25a	8.98	9.37	9.35	8.72	9.41	9.00	17.48	9.54	9.17	8.97
PAL_K25c	9.15	9.29	9.50	9.00	9.58	9.13	16.96	9.54	9.27	8.58
PAL_K26a	9.22	9.31	9.24	8.76	9.46	9.34	16.93	9.48	9.27	8.98
PAL_K26b	9.16	9.32	9.51	8.82	9.37	9.23	16.97	9.51	9.19	8.91
PAL_K26c	9.46	9.15	9.58	8.81	9.30	9.11	16.91	9.56	9.36	8.75
PAL_K4a	9.17	9.27	9.49	8.78	9.30	9.28	17.54	9.60	9.12	8.46
PAL_K4c	9.25	9.19	9.37	8.75	9.49	9.24	17.42	9.53	9.25	8.50
PAL_K5a	9.30	9.07	9.45	8.70	9.48	9.29	17.08	9.55	9.44	8.64
PAL_K5b	9.30	9.08	9.53	8.83	9.35	9.31	16.82	9.73	9.40	8.65
PAL_K5c	9.28	9.11	9.48	8.71	9.30	9.34	17.15	9.64	9.37	8.63
PAL_K7b	9.29	9.31	9.38	8.78	9.17	9.24	17.02	9.60	9.42	8.79
PAL_Z12a	9.20	9.09	9.49	8.68	9.47	9.16	17.31	9.53	9.35	8.72
PAL_Z12c	9.16	9.24	9.35	8.82	9.30	9.14	17.45	9.58	9.27	8.70
PAL_Z13a	9.16	9.33	9.39	8.63	9.27	9.47	17.22	9.56	9.30	8.68
PAL_Z13b	9.18	9.37	9.33	8.71	9.67	9.46	16.69	9.67	9.17	8.76
PAL_Z13c	9.19	9.11	9.31	8.76	9.31	9.14	17.72	9.46	9.22	8.78
PAL_Z14a	9.31	9.03	9.41	8.55	9.32	9.33	17.20	9.68	9.45	8.73
PAL_Z14b	9.17	9.13	9.39	8.92	9.32	8.95	17.57	9.55	9.08	8.93
PAL_Z14c	9.10	9.21	9.41	8.79	9.26	9.03	17.75	9.51	9.17	8.78
PAL_Z15a	9.32	9.86	9.36	8.71	9.31	9.03	17.11	9.36	9.11	8.84
PAL_Z15b	9.29	9.24	9.32	8.65	9.39	9.16	17.46	9.52	9.27	8.69
PAL_Z15c	9.29	9.30	9.33	8.64	9.62	9.22	17.36	9.47	9.14	8.63
PAL_Z16a	9.32	9.14	9.42	8.57	9.24	9.31	17.31	9.58	9.31	8.80
PAL_Z16b	9.23	9.35	9.41	8.77	9.41	9.08	17.36	9.61	9.15	8.63
PAL_Z16c	9.24	9.13	9.18	8.78	9.30	9.21	17.81	9.44	9.18	8.73
PAL_Z17a	9.36	9.19	9.31	8.57	9.37	9.21	17.32	9.62	9.38	8.66
PAL_Z17b	9.21	9.26	9.43	8.86	9.35	9.02	17.83	9.38	9.05	8.60
PAL_Z17c	9.25	9.27	9.21	8.80	9.41	9.27	17.40	9.35	9.22	8.82
PAL_Z18a	9.28	9.11	9.50	8.70	9.20	9.37	17.29	9.71	9.34	8.49
PAL_Z18b	9.41	9.16	9.31	8.85	9.25	9.18	17.32	9.53	9.23	8.77
PAL_Z18c	9.27	9.25	9.44	8.84	9.31	9.14	17.01	9.67	9.31	8.77
PAL_Z19b	9.34	9.15	9.49	8.77	9.35	9.27	17.17	9.53	9.34	8.59
PAL_Z19c	8.97	9.30	9.28	8.84	9.43	9.10	17.57	9.49	9.09	8.93
PAL_Z20c	9.27	9.04	9.39	8.78	9.28	9.16	17.68	9.42	9.24	8.75
PAL_Z23a	9.31	9.09	9.13	8.73	9.32	9.25	17.42	9.54	9.34	8.87
PAL_Z23c	9.18	9.13	9.34	8.72	9.38	9.08	18.03	9.41	9.17	8.55
PAL_Z25a	9.15	9.26	9.39	8.55	9.35	9.10	17.75	9.48	9.23	8.73
PAL_Z25b	9.23	9.30	9.49	8.56	9.49	9.15	17.30	9.56	9.19	8.73
PAL_Z25c	9.12	9.35	9.44	8.68	9.50	8.97	17.28	9.62	9.31	8.76
PAL_Z26c	9.14	8.92	9.45	8.75	9.39	9.09	17.95	9.43	9.21	8.68
PAL_Z6a	9.12	9.13	9.55	8.53	9.43	9.11	17.42	9.49	9.35	8.87
PAL_Z6b	9.06	9.21	9.58	8.75	9.33	9.04	17.50	9.57	9.14	8.82
PAL_Z6c	9.16	9.26	9.62	8.42	9.24	9.19	17.65	9.47	9.29	8.69
PAL_Z8a	9.24	9.11	9.55	8.58	9.21	9.23	17.39	9.57	9.28	8.83
PAL_Z8c	9.20	9.44	9.45	8.59	9.36	9.05	17.29	9.68	9.17	8.78

PAN_B30a	9.84	9.60	9.65	9.06	9.78	9.84	9.57	13.58	9.85	9.24
PAN_B30b	9.87	9.44	9.64	9.10	9.76	9.77	9.51	13.89	9.89	9.13
PAN_B30c	10.01	9.39	9.67	8.99	9.78	9.87	9.48	13.93	9.94	8.95
PAN_B31a	9.90	9.61	9.62	9.12	9.78	9.80	9.38	13.87	9.86	9.06
PAN_B31b	9.94	9.72	9.61	9.02	9.78	9.88	9.59	13.66	9.90	8.91
PAN_B31c	9.89	9.61	9.68	9.01	9.84	9.89	9.54	13.60	9.84	9.10
PAN_K20a	9.83	9.43	9.64	9.39	9.98	10.06	9.55	13.29	9.87	8.97
PAN_K20b	10.18	9.54	9.66	9.17	9.77	10.03	9.38	13.61	9.88	8.78
PAN_K20c	9.92	9.55	9.69	9.04	9.94	9.67	9.62	13.43	9.95	9.18
PAN_K21a	9.92	9.48	9.72	9.15	9.86	9.94	9.64	13.62	9.83	8.84
PAN_K21b	9.92	9.44	9.74	9.23	9.97	9.90	9.64	13.29	9.84	9.03
PAN_K21c	9.87	9.53	9.83	9.08	9.74	9.87	9.39	13.93	9.92	8.83
PAN_K25b	9.93	9.46	9.89	8.83	9.95	9.87	9.55	13.72	9.94	8.87
PAN_K25c	10.10	9.69	9.67	8.94	9.89	9.93	9.65	13.35	9.71	9.06
PAN_K30a	9.82	9.66	9.62	9.39	9.86	9.69	9.49	13.55	9.77	9.15
PAN_K30b	9.78	9.44	9.65	9.15	9.97	9.84	9.51	13.92	9.86	8.86
PAN_K30c	9.82	9.61	9.67	9.14	9.71	9.94	9.73	13.32	9.78	9.28
PAN_K31a	9.74	9.64	9.76	9.25	9.89	9.86	9.70	13.31	9.69	9.15
PAN_K31b	9.90	9.56	9.77	9.14	9.82	9.84	9.57	13.41	9.82	9.17
PAN_K31c	9.82	9.44	9.78	8.95	9.95	9.81	9.50	13.72	9.97	9.08
PAN_K31d	9.84	9.48	9.77	8.97	9.82	10.02	9.48	13.79	9.97	8.87
PAN_K32a	10.01	9.70	9.76	9.41	9.85	9.91	9.52	12.81	9.88	9.15
PAN_K32b	9.65	9.61	9.70	9.16	9.82	10.01	9.75	13.61	9.72	8.96
PAN_K32c	9.87	9.60	9.66	9.12	9.84	9.75	9.63	13.28	10.09	9.15
PAN_K33a	9.91	9.54	9.74	9.42	9.83	9.83	9.48	13.20	9.85	9.21
PAN_K34a	9.95	9.50	9.67	9.13	9.69	9.92	9.40	13.61	10.05	9.06
PAN_K34b	9.68	9.86	9.81	9.52	9.60	9.86	9.66	12.87	9.90	9.23
PAN_K34c	9.98	9.64	9.69	9.39	9.78	9.81	9.62	13.21	10.03	8.87
PAN_K35a	9.82	9.60	9.74	9.40	9.91	9.87	9.56	13.16	9.80	9.15
PAN_K35e	9.94	9.59	9.62	9.00	9.80	10.12	9.38	13.71	9.95	8.89
PAN_K35b	9.80	9.64	9.68	9.18	9.76	9.80	9.56	13.30	10.03	9.27
PAN_K35c	9.71	9.79	9.67	9.33	9.74	9.67	9.65	13.37	9.80	9.27
PAN_K35d	9.90	9.65	9.63	9.13	9.93	9.69	9.33	13.63	9.97	9.14
RIV_B11a	9.74	9.04	9.81	8.84	10.07	10.32	9.32	9.93	14.00	8.94
RIV_B11b	9.85	9.20	9.45	8.76	9.91	10.10	9.24	10.16	14.20	9.13
RIV_B11c	10.21	9.11	9.42	8.65	10.05	10.50	9.29	10.01	13.53	9.24
RIV_B12b	9.76	9.21	9.64	8.28	10.18	10.41	9.05	10.02	14.08	9.36
RIV_B12c	9.91	8.99	9.64	8.51	10.06	10.52	9.43	9.87	13.76	9.31
RIV_B13a	10.00	8.90	9.68	8.38	9.96	10.78	9.23	9.76	14.21	9.11
RIV_B13b	9.69	9.21	9.58	8.28	10.04	10.73	9.13	9.89	14.40	9.06
RIV_B13c	9.60	8.96	9.56	8.66	10.11	10.50	9.48	9.91	13.95	9.28
RIV_B23a	9.96	9.08	9.70	8.42	10.03	10.36	9.12	9.95	14.25	9.13
RIV_B23c	9.83	9.34	9.93	8.96	10.10	10.20	9.41	10.21	12.95	9.07
RIV_B3a	9.86	9.04	9.46	8.39	9.84	10.62	9.08	9.93	14.56	9.22
RIV_B3b	9.72	9.22	9.66	8.33	9.96	10.25	9.17	9.84	14.62	9.23

RIV_B3c	9.60	9.07	9.89	8.42	9.99	10.46	9.10	9.82	14.54	9.12
RIV_B7a	9.87	9.24	9.86	8.92	9.71	10.37	9.11	9.93	13.68	9.31
RIV_B7b	9.74	9.23	9.82	8.66	9.99	10.13	9.40	9.94	14.23	8.87
RIV_B7c	9.70	9.29	9.80	8.43	10.11	10.36	9.37	9.82	14.08	9.05
RIV_B9a	9.86	9.08	9.76	8.64	10.11	10.54	9.32	9.82	13.72	9.14
RIV_B9b	9.72	9.04	9.67	8.52	9.89	10.53	9.28	9.93	14.09	9.34
RIV_B9c	9.79	8.70	9.64	8.60	10.22	10.69	9.20	9.82	14.25	9.09
RIV_K17a	9.56	9.38	9.66	8.84	9.80	10.25	9.25	9.93	13.73	9.59
RIV_K17b	9.77	9.42	9.78	8.78	10.00	10.32	9.23	9.69	14.14	8.87
RIV_K17c	9.90	9.24	9.75	8.66	10.07	10.41	9.17	9.77	13.65	9.39
RIV_K22a	9.87	9.37	9.69	8.45	9.86	10.39	9.00	10.28	13.99	9.12
RIV_K22b	9.92	9.41	9.82	8.16	10.03	10.48	8.94	9.95	14.20	9.09
RIV_K22c	10.05	9.21	9.94	8.37	10.04	10.27	9.48	10.01	13.67	8.96
RIV_K24a	9.98	8.99	9.59	8.34	9.84	10.31	9.73	9.86	14.24	9.10
RIV_K24b	10.02	9.13	9.42	8.35	9.62	10.73	9.49	9.83	14.10	9.30
RIV_K24c	9.44	9.21	9.74	8.49	9.90	10.43	9.34	9.94	14.20	9.30
RIV_K25b	9.53	9.35	9.69	8.49	9.72	10.39	9.13	9.81	14.44	9.45
RIV_K25c	9.83	9.20	9.75	8.30	9.71	10.60	9.06	9.74	14.49	9.32
RIV_K2c	9.73	9.05	9.39	8.80	9.80	10.22	9.49	9.64	14.58	9.30
RIV_K4a	9.77	9.33	9.76	8.75	9.84	10.37	9.13	9.73	14.19	9.12
RIV_K5a	9.62	9.13	9.54	8.31	10.06	10.16	9.33	10.01	14.39	9.45
RIV_K5b	9.60	8.98	9.59	8.29	9.90	10.72	9.59	9.75	14.39	9.19
RIV_K7a	9.77	9.03	9.75	8.91	10.15	10.37	9.11	9.92	13.77	9.22
RIV_K7b	9.58	9.00	9.70	8.39	10.24	10.24	9.21	9.77	14.53	9.34
RIV_K7c	9.79	9.15	9.63	8.37	10.22	10.42	8.96	9.90	14.55	8.99
RIV_Z12a	9.90	8.97	9.76	8.22	10.00	10.22	10.39	9.65	13.81	9.08
RIV_Z12b	9.62	9.19	9.73	8.49	9.88	10.64	9.18	9.81	14.35	9.12
RIV_Z12c	9.57	9.35	9.83	8.56	10.03	10.24	9.13	9.84	14.22	9.23
RIV_Z13a	9.69	8.89	9.53	8.47	10.25	11.41	9.46	9.79	13.38	9.11
RIV_Z13b	9.86	9.22	9.45	8.74	9.92	11.05	9.33	9.86	13.55	9.01
RIV_Z13c	9.73	9.18	9.64	8.58	9.82	10.39	9.21	9.98	14.22	9.26
RIV_Z17a	9.81	9.36	9.56	8.42	9.61	10.35	9.27	9.81	14.23	9.59
RIV_Z17b	9.84	9.01	9.56	8.36	9.74	10.58	9.13	9.83	14.54	9.41
RIV_Z17c	9.68	9.24	9.58	8.49	9.91	10.09	9.27	9.69	14.49	9.55
RIV_Z20a	9.50	9.39	9.82	8.74	10.05	10.26	9.24	9.78	14.45	8.76
RIV_Z20b	9.70	9.27	9.40	8.34	10.17	10.46	8.96	9.62	14.85	9.22
RIV_Z20c	9.51	9.31	9.96	8.57	10.16	10.36	9.08	9.74	14.29	9.02
RIV_Z25a	9.75	9.18	9.58	8.70	9.86	10.48	9.22	10.03	13.85	9.37
RIV_Z25b	9.75	9.11	9.39	8.55	9.83	10.82	9.23	10.11	14.04	9.17
RIV_Z25c	9.49	9.23	9.75	8.47	10.15	10.73	9.24	10.19	13.26	9.49
VUL_B10a	8.69	9.02	8.66	9.90	8.79	9.00	8.69	8.97	9.28	19.00
VUL_B10b	8.58	9.03	8.52	9.73	8.67	8.87	8.77	9.09	9.15	19.59
VUL_B10c	8.68	9.13	8.59	9.73	8.75	9.17	8.69	8.99	9.24	19.02
VUL_B13a	8.61	9.23	8.56	9.79	8.72	8.91	8.55	8.97	9.15	19.50
VUL_B13b	8.64	9.24	8.58	9.87	8.68	8.94	8.66	9.00	9.14	19.26

VUL_B13c	8.55	9.09	8.58	9.88	8.72	8.93	8.68	8.82	9.12	19.63
VUL_B1a	8.72	9.05	8.68	9.68	8.85	8.97	8.74	9.08	9.17	19.06
VUL_B1b	8.65	9.09	8.60	9.61	8.80	8.97	8.86	9.08	9.25	19.10
VUL_B1c	8.64	9.10	8.65	9.76	8.86	8.99	8.85	9.13	9.20	18.81
VUL_B20a	8.65	9.18	8.62	9.77	8.75	8.96	8.65	9.08	9.19	19.15
VUL_B20b	8.69	9.15	8.50	9.75	8.81	8.95	8.66	9.06	9.20	19.24
VUL_B20c	8.79	9.08	8.60	9.61	8.75	9.00	8.61	8.98	9.22	19.36
VUL_B21b	8.74	9.22	8.60	9.87	8.79	8.91	8.67	9.11	9.21	18.88
VUL_B21c	8.77	9.40	8.58	9.95	8.77	8.96	8.70	9.05	9.32	18.50
VUL_B7b	8.72	9.30	8.58	9.94	8.93	8.98	8.91	9.33	9.29	18.03
VUL_B7c	8.64	9.30	8.52	9.75	8.68	8.91	8.66	9.00	9.18	19.37
VUL_K13a	8.63	9.06	8.61	9.72	8.87	8.98	8.62	8.95	9.17	19.41
VUL_K13b	8.75	9.06	8.71	9.73	8.79	8.92	8.74	9.12	9.41	18.76
VUL_K13c	8.63	9.16	8.58	9.71	8.71	8.89	8.73	8.97	9.23	19.38
VUL_K16a	8.67	9.18	8.52	9.65	8.81	8.96	8.83	9.16	9.20	19.03
VUL_K16b	8.60	9.30	8.53	9.69	8.87	8.97	8.71	9.02	9.16	19.17
VUL_K16c	8.77	9.13	8.65	9.63	8.82	8.93	8.73	9.07	9.09	19.18
VUL_K19a	8.71	9.16	8.75	9.69	8.64	9.06	8.75	9.08	9.24	18.93
VUL_K19b	8.53	9.30	8.71	9.72	8.86	8.98	8.63	9.03	9.23	19.02
VUL_K19c	8.54	9.10	8.71	9.72	8.86	8.95	8.95	9.22	9.28	18.67
VUL_K20a	8.64	9.36	8.64	9.78	8.95	9.02	8.77	9.00	9.25	18.58
VUL_K20b	8.72	9.15	8.70	9.94	8.88	9.00	8.80	9.06	9.24	18.51
VUL_K20c	8.70	9.15	8.57	9.64	8.73	8.93	8.92	9.16	9.11	19.10
VUL_K21a	8.56	9.32	8.63	9.69	8.83	8.99	8.74	9.08	9.30	18.86
VUL_K21b	8.54	9.18	8.56	9.80	8.88	8.90	8.83	9.14	9.13	19.04
VUL_K21c	8.63	9.35	8.63	9.55	8.98	9.04	8.98	9.20	9.27	18.37
VUL_K2a	8.81	9.32	8.57	9.51	8.72	8.96	8.72	8.88	9.25	19.27
VUL_K2b	8.68	9.27	8.57	9.71	8.86	8.87	8.65	8.95	9.22	19.21
VUL_K2c	8.83	9.15	8.58	9.53	8.73	8.91	8.73	8.90	9.24	19.40
VUL_K3a	8.69	9.17	8.59	9.73	8.79	8.97	8.68	9.00	9.22	19.15
VUL_K3b	8.78	9.19	8.60	9.58	8.83	9.01	8.79	9.09	9.16	18.96
VUL_K3c	8.66	9.16	8.59	9.77	8.80	8.92	8.73	9.02	9.11	19.24
VUL_K7a	8.69	9.15	8.62	9.68	8.75	8.97	8.76	9.06	9.17	19.16
VUL_K7c	8.61	9.20	8.69	9.80	8.79	8.80	8.86	8.95	9.23	19.06
VUL_K8a	8.62	9.22	8.59	9.60	8.87	8.99	8.71	9.03	9.25	19.12
VUL_K8b	8.65	9.07	8.59	9.78	8.87	8.78	8.79	8.97	9.19	19.32
VUL_K8c	8.63	9.25	8.57	9.76	8.77	8.92	8.72	9.02	9.15	19.21
VUL_Z11a	8.67	9.06	8.61	9.69	8.89	8.96	8.74	9.02	9.32	19.04
VUL_Z11b	8.80	8.99	8.62	9.63	8.80	9.01	8.71	9.11	9.19	19.15
VUL_Z11c	8.69	9.11	8.51	9.74	8.74	8.96	8.79	8.97	9.21	19.27
VUL_Z1b	8.77	9.12	8.65	9.77	8.76	8.87	8.82	9.01	9.10	19.12
VUL_Z21a	8.78	8.94	8.65	9.85	8.77	8.92	8.74	8.88	9.12	19.35
VUL_Z21b	8.74	8.96	8.62	9.68	8.73	8.97	8.70	9.03	9.16	19.41
VUL_Z4b	8.69	9.10	8.63	9.63	8.77	9.03	8.68	8.98	9.14	19.36
VUL_Z4c	8.70	9.07	8.56	9.62	8.76	8.95	8.64	9.00	9.13	19.55

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
VUL_Z5a	8.75	9.07	8.65	9.82	8.79	8.92	8.64	9.14	9.23	18.98
VUL_Z5c	8.64	8.99	8.53	9.85	8.79	8.97	8.58	9.08	9.16	19.42
VUL_Z6a	8.64	9.17	8.65	9.68	8.73	8.90	8.80	9.01	9.28	19.14
VUL_Z6b	8.62	9.06	8.58	9.76	8.70	8.85	8.90	9.08	9.23	19.23
VUL_Z6c	8.59	9.09	8.60	9.81	8.72	8.85	8.96	9.06	9.27	19.05

Tab. H36: Hybrids identity to species (in %) according to scirpt

	ACA	ARV	CAN	ERIO	HET	OLE	PAL	PAN	RIV	VUL
ACA_x_CAN_Z27a	12.94	9.22	12.36	8.52	9.84	10.12	9.21	9.44	9.76	8.59
ACA_x_PAN_K21a	13.57	9.08	11.58	8.76	9.46	9.70	9.56	9.98	9.87	8.46
ACA_x_OLE_Z35b	12.84	9.20	9.55	8.69	9.91	12.12	8.99	9.32	10.53	8.84
ACA_x_OLE_Z27a	13.74	8.95	9.21	8.27	9.84	12.29	9.19	9.55	10.64	8.33
ACA_x_OLE_Z29a	13.24	9.18	9.37	8.36	9.93	12.30	9.03	9.58	10.60	8.40
ACA_x_OLE_Z29b	13.44	9.03	9.04	8.52	10.11	12.35	9.02	9.57	10.45	8.47
ACA_x_OLE_Z29c	13.89	9.08	9.26	8.63	9.82	12.16	8.75	9.58	10.38	8.45
ACA_x_OLE_Z35a	12.63	8.94	9.10	8.60	9.98	12.10	9.18	9.70	10.64	9.13
ACA_x_OLE_Z35c	11.52	9.12	9.29	8.87	10.27	12.52	9.46	9.82	10.42	8.72
ACA_x_PAN_K20a	13.30	9.31	9.38	9.24	9.39	10.27	9.09	11.71	9.92	8.39
ACA_x_PAN_K20c	13.29	9.28	9.52	8.90	9.61	9.99	9.16	11.96	9.81	8.47
ACA_x_PAN_K25a	13.68	9.18	9.08	9.21	9.52	9.83	9.34	11.97	9.80	8.39
ACA_x_PAN_K25b	13.99	9.41	9.18	8.89	9.44	9.98	9.08	11.48	10.09	8.48
ACA_x_PAN_K25c	13.85	9.22	9.22	9.13	9.42	9.83	9.05	11.55	10.01	8.74
CAN_x_OLE_K23a	9.66	9.58	11.67	8.32	10.17	12.59	9.50	9.64	10.14	8.74
CAN_x_OLE_B22b	9.96	9.60	11.51	8.62	9.82	12.99	9.24	9.19	10.27	8.80
CAN_x_OLE_K19a	9.65	9.19	12.26	8.10	10.04	12.56	9.32	9.47	10.47	8.93
CAN_x_OLE_K19b	9.49	9.22	13.33	8.73	9.59	11.99	9.51	9.21	10.27	8.65
CAN_x_OLE_K22a	9.30	9.07	13.14	8.22	10.11	12.32	9.35	9.41	10.32	8.75
CAN_x_OLE_K22c	9.50	9.31	12.74	8.35	9.79	13.10	9.11	9.25	10.26	8.60
CAN_x_OLE_K23a	9.44	9.19	13.44	8.02	10.11	12.45	9.12	9.39	10.20	8.65
CAN_x_OLE_K23b	9.46	9.42	13.33	8.58	9.95	12.23	9.26	9.13	9.91	8.74
CAN_x_OLE_K23c	9.37	9.11	13.24	8.07	10.06	13.09	8.95	9.41	10.16	8.54
CAN_x_OLE_K33a	9.44	9.63	12.12	8.76	9.94	10.91	9.96	9.93	10.23	9.09
CAN_x_OLE_K33b	9.37	9.71	11.93	8.87	9.86	11.00	9.75	9.96	10.45	9.12
CAN_x_OLE_K33c	9.61	9.45	11.95	9.04	9.85	10.90	9.64	10.03	10.31	9.22
CAN_x_OLE_Z23b	9.28	9.13	12.82	8.53	10.22	12.27	9.33	9.24	10.30	8.89
CAN_x_OLE_Z28a	9.55	9.50	12.79	8.10	10.03	12.36	9.05	9.63	10.46	8.51
CAN_x_OLE_Z28b	9.58	9.08	12.22	8.20	10.24	12.56	9.32	9.42	10.33	9.04
CAN_x_OLE_Z28c	9.61	9.08	12.55	8.28	10.22	12.49	9.00	9.44	10.54	8.80
CAN_x_OLE_Z30a	9.16	9.32	12.28	8.60	9.85	13.13	9.14	9.07	10.63	8.83
CAN_x_OLE_Z30b	9.91	9.10	11.84	8.37	10.18	12.81	9.28	9.53	10.49	8.48
CAN_x_OLE_Z31a	9.70	9.02	12.36	8.41	10.03	12.94	9.23	9.42	10.17	8.73
CAN_x_OLE_Z32a	9.65	9.19	12.04	8.48	9.83	12.93	9.08	9.67	10.24	8.89
CAN_x_OLE_K17a	9.25	8.98	13.07	8.63	10.09	11.35	9.52	9.51	10.56	9.05

CAN_x_OLE_Z32c	9.40	9.16	13.19	8.20	10.13	11.94	9.40	9.73	10.34	8.52
CAN_x_PAL_K22b	9.38	9.31	12.68	8.38	9.53	8.94	14.93	9.13	9.29	8.43
CAN_x_PAL_K22c	9.34	9.16	12.63	8.18	9.22	9.23	14.88	9.13	9.49	8.74
CAN_x_PAL_K33a	9.12	9.24	12.04	9.14	9.54	9.34	12.90	9.85	9.63	9.19
CAN_x_PAL_K33b	9.35	9.51	11.53	9.09	9.33	9.33	13.24	9.90	9.67	9.05
CAN_x_PAL_K33c	9.24	9.64	12.27	9.14	9.84	9.59	11.76	10.01	9.59	8.93
CAN_x_PAL_K22a	9.33	9.20	12.25	8.35	9.37	8.98	15.35	9.44	9.30	8.43
CAN_x_PAN_K21a	9.74	9.53	12.50	9.40	9.69	9.74	9.53	11.47	9.73	8.68
CAN_x_PAN_K21b	9.82	9.53	13.37	9.00	9.62	9.61	9.27	11.47	9.55	8.78
CAN_x_RIV_B23a	9.68	9.50	12.82	8.30	9.90	9.96	9.70	9.56	11.65	8.93
HET_x_OLE_Z34a	10.15	8.97	9.13	8.29	12.57	12.78	9.25	9.55	10.49	8.84
HET_x_OLE_Z40a	9.65	9.53	9.55	8.48	12.60	12.52	9.37	9.25	10.39	8.64
HET_x_PAL_Z34a	9.49	8.97	9.32	8.61	12.42	9.42	14.58	9.28	9.52	8.39
HET_x_PAL_Z37a	9.52	9.00	9.52	8.74	12.49	9.02	14.71	8.92	9.58	8.48
HET_x_RIV_Z25a	10.00	9.08	9.68	8.75	12.37	10.16	9.54	9.85	11.53	9.06
OLE_x_PAL_K22a	9.39	9.30	9.26	8.52	9.72	11.74	13.72	9.37	10.16	8.82
OLE_x_PAL_Z25a	9.59	8.44	9.23	8.18	9.43	12.51	14.49	9.51	9.94	8.68
OLE_x_RIV_B23a	9.87	9.33	9.69	8.53	9.81	12.19	9.20	9.65	12.66	9.07
OLE_x_RIV_K34a	9.64	9.35	9.71	8.79	9.82	11.62	9.98	10.26	11.69	9.15
OLE_x_RIV_Z25d	9.67	9.37	9.46	8.48	9.81	12.26	9.63	9.92	12.36	9.04
OLE_x_RIV_Z25e	9.89	9.12	9.31	8.75	9.93	12.59	9.43	9.72	11.87	9.40
OLE_x_RIV_K17a	9.64	9.50	9.21	8.86	10.13	12.84	9.67	9.71	11.95	8.49
OLE_x_RIV_K17b	10.00	9.09	9.60	8.33	10.56	12.16	9.19	9.68	12.83	8.56
OLE_x_RIV_K17c	9.66	9.23	9.22	8.59	10.09	12.71	9.86	9.95	11.88	8.81
OLE_x_RIV_Z25a	9.81	8.99	9.45	8.28	10.13	12.33	9.59	9.65	12.82	8.94
OLE_x_RIV_Z25b	9.71	9.00	9.42	8.70	10.43	12.87	9.26	9.83	11.95	8.83
OLE_x_RIV_Z25c	10.15	9.37	9.67	8.28	10.35	11.91	9.67	9.73	12.29	8.58
OLE_x_RIV_Z26a	11.07	9.02	9.25	8.28	10.37	12.44	9.40	9.75	11.33	9.09
OLE_x_RIV_Z26a	10.75	9.36	9.51	8.63	10.02	11.71	9.47	9.76	12.22	8.58
OLE_x_RIV_Z26b	10.28	9.10	9.39	8.83	10.33	11.73	9.60	10.10	11.93	8.72
OLE_x_RIV_Z26c	10.07	9.45	9.60	8.86	9.83	11.56	9.72	9.78	12.29	8.84
PAL_x_RIV_B23a	9.90	9.16	9.81	8.77	9.47	9.65	13.67	9.97	10.94	8.68
PAL_x_RIV_K34a	9.61	9.21	9.74	8.93	9.71	9.61	13.03	10.33	11.15	8.68
PAL_x_RIV_Z25a	9.80	9.32	9.28	8.61	9.23	10.22	15.01	9.30	10.87	8.38
PAN_x_PAL_K35a	9.43	9.62	9.60	9.17	9.51	9.54	12.56	11.85	9.69	9.04
ACA_x_CAN_x_PAN_K20b	11.85	9.51	12.51	8.50	9.67	9.88	9.25	11.02	9.77	8.02

4. Supplements on CD

Panels_GeneMarker: contains panels designed and applied on the results of fragmentation analyses, requires GeneMarker USB key

All species and error rate: binary matrix of all samples of morphologically pure individuals and hybrids and estimation of error rate

Gen_similarity_vs_Geo_distance: genetic similarity matrix and geographic distance matrix of all species respectively

Script for hybrid determination: our script made for molecular approval of samples and parental species of a hybrid. Created by Jakub Šmerda.