



Going west – A subtropical lineage (*Vincetoxicum*, Apocynaceae: Asclepiadoideae) expanding into Europe [☆]



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ABSTRACT

Vincetoxicum sensu lato is a tropical lineage comprising two clades that have reached high northern latitudes. Of the temperate clades, one is restricted to the Far East, the other one (*Vincetoxicum s. str.* Clade) extends into Europe, but their ranges overlap in Central China and Japan. Three species invasive in North America, *V. hirundinaria*, *V. nigrum* and *V. rossicum*, are members of the *Vincetoxicum s. str.* Clade. We explore the prerequisites for the range expansion in the *Vincetoxicum s. str.* Clade performing Bayesian and Maximum Likelihood phylogenetic analyses on sequences of the nuclear internal transcribed spacer (ITS) region, the nuclear external transcribed spacer region (ETS), and five plastid markers. The resulting phylogeny is used to conduct biogeographic analysis using BioGeoBEARS to reconstruct ancestral species ranges. Moreover, we map the known occurrences of two rare characters in Asclepiadoideae, the possession of phenanthroindolizidine alkaloids and reported cases of autogamy onto our phylogeny. Finally, we have conducted ecological niche modelling using Maxent on a total of 220 spatially unique occurrences of nine *Vincetoxicum s. str.* species spanning more than 4000 km along the east–west gradient to learn about the climatic conditions along the presumed migration route. Our results indicate a north–westward migration in *Vincetoxicum s. str.* along the Asian mountain chains to Europe. Climatic preferences of the nine species sampled are dissimilar, except for the common exposure to at least one month of sub-freezing temperatures, indicating a rather wide climatic tolerance for the clade as a whole. The three species invasive in North America belong to the northern Eurasian subclade and show the rare combination of phenanthroindolizidine alkaloids and autogamy.

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1. Introduction

In Europe, the only Asclepiadoideae found naturally north of the Alps and the Pyrenees are members of the genus *Vincetoxicum* Wolf (Markgraf, 1972), except for a few occurrences of *Cynanchum acutum* L. along the seashores of southern France (Dupont, 1986). The plants are of erect, sometimes apically weakly twining herbaceous

habit with clear latex and fascicled roots. Their small flowers possess a corona of staminal lobes that are occasionally connected interstaminally, and small, round pollinia (Liede, 1996). Recently, however, Liede-Schumann et al. (2012) have shown that *Vincetoxicum* in the classic circumscription does not constitute a monophyletic genus. Instead, *Vincetoxicum* comprises at least two different lineages that are deeply nested in *Tylophora*, (sub-) tropical plants of very similar floral characters, but often forming large, woody twiners. Because *Vincetoxicum* Wolf (1776) predates *Tylophora* R. Brown (1810), the correct name for the genus is *Vincetoxicum*. In this circumscription, henceforth termed *Vincetoxicum sensu lato* (*Vincetoxicum s.l.*), the genus comprises c. 140 species, naturally distributed in Africa, Asia, and Europe, but introduced in North America, where several species are now considered noxious weeds (e.g., Douglass et al., 2009). *Vincetoxicum s.l.* is unique

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in Asclepiadeae because several of its members possess phenanthroindolizidine alkaloids otherwise not found in the tribe (Liede, 1996; Staerk et al., 2005; Burzynski et al., 2015). Furthermore, several species in the subtribe have been reported as autogamous, another extremely uncommon feature in Asclepiadoideae (Liede-Schumann et al., 2012).

Of the two lineages of temperate, more or less erect plants, one, henceforth called “Far Eastern Clade” (corresponding to clade II of Yamashiro et al., 2004) is confined to the Far East (Figs. 1 and 2A), including Japan (Honshu, Shikoku, Kiushu, Okinawa), Eastern China (provinces: Beijing, Chongqing, Guangxi, Hebei, Hunan, Jiangsu, Jiangxi, Jilin, Shandong, Yunnan) and the south-easternmost corner of Russia (Khabarovsk Krai, Primorsky Krai, Zabaykalsky Krai). The other one, henceforth called “*Vincetoxicum s. str.* Clade” (Figs. 1 and 2B) is well-supported sister to a clade of subtropically distributed (Figs. 1 and 2B), twining evergreen plants, henceforth called “Subtropical Clade”. These two clades together correspond to clade I of Yamashiro et al. (2004). The *Vincetoxicum s. str.* Clade occupies an area by far larger than the one of the Far Eastern Clade, ranging from Japan (as far north as Hokkaido), and China (Gansu, Hubei, Hunan, Ningxia, Sichuan, Xizang, Yunnan) along the Himalaya (Nepal, India, Afghanistan), to Pakistan, Iran, and then into the Mediterranean and, via the Caucasus, to southern Russia and Europe. In Europe, it reaches as far as 60° North in Sweden and Finland (Solbreck, 2000), but is naturally absent from Great Britain (Lindley, 1838). The areas of the Far Eastern Clade and *Vincetoxicum s. str.* Clade overlap in Japan and some parts of China (Fig. 2), and to date, attribution of a particular species to one of the two clades can only be accomplished safely by molecular analysis, because a morphological and taxonomical revision of *Vincetoxicum* over its whole area is still wanting. Therefore, it is difficult to estimate the number of species in these two clades. Flora of Japan (Yamazaki, 1993) lists fifteen *Vincetoxicum s.l.* species, Flora of China (Li et al., 1995) 21 species, of which seven are considered as occurring in both areas. Unfortunately, both floras erroneously (Liede, 1996) synonymize *Vincetoxicum* with *Cynanchum* L., making correct generic attribution difficult. Flora of Russia (Czerepanov, 1995) lists 25 *Vincetoxicum* species, of which four are also listed in Flora of China; and Flora Europaea (Markgraf, 1972) enumerates 11 species, of which six are also listed in Flora of Russia, provided the synonymy given is correct. Three additional species are known from Pakistan (Ali and Khaton, 1982), two from Iran (Zaeifi, 1999), and one each from Afghanistan (Podlech, 2012), and Turkey (Browicz, 1978), so that 60–70 species can be expected in the Far Eastern Clade and the *Vincetoxicum s. str.* Clade together. From the results of Yamashiro et al. (2008) that species of the Far Eastern Clade have a tendency to comparatively large, showy flowers, while those of the *Vincetoxicum s. str.* Clade tend to be small and inconspicuous, 20–25 species can be expected in the Far Eastern Clade and ca. 40–45 in the *Vincetoxicum s. str.* Clade.

We have added a total of 46 accessions (26 of which belong to the *Vincetoxicum s. str.* Clade or the Far Eastern Clade) to the dataset of Liede-Schumann et al. (2012), and have conducted a new phylogenetic analysis. We have reconstructed the biogeographic history focusing on the *Vincetoxicum s. str.* Clade and the Far Eastern Clade. In addition, we have performed climate niche modelling on selected subclades of the *Vincetoxicum s. str.* Clade to ask whether the expansion of *Vincetoxicum s. str.* has followed along similar climatic conditions.

The purpose of our paper is therefore (1) to elucidate the phylogeny and biogeography of the *Vincetoxicum s. str.* Clade; (2) to identify the climatic conditions under which species of the *Vincetoxicum s. str.* Clade have been able to expand the range of this clade; (3) to ask which characters might have enabled the species of the *Vincetoxicum s. str.* Clade to expand their range, to the degree that some species have become invasive.

2. Materials and methods

2.1. Phylogeny

2.1.1. Taxon sampling

As a basis, the dataset of Liede-Schumann et al. (2012) was used. In addition, 46 new accessions of Tylophorinae were obtained with a focus on European and Asian material. In total, our dataset comprises 47 species attributed to the Far Eastern Clade and the *Vincetoxicum s. str.* Clade, as well as two unnamed accessions attributed to these two clades. In addition, two species of *Vincetoxicum*-like habit, but uncertain affiliation (*V. brachystelmoide*, *V. linifolium*) were newly included. Our sampling was aimed at covering the area of the genus *Vincetoxicum s.l.* as completely as possible, with particular emphasis on the Far Eastern Clade and the *Vincetoxicum s. str.* Clade, for which there are gaps only in the now independent Central Asian states of the former Soviet Union and the Korean Peninsula. Thus, our dataset represents the most comprehensive Tylophorinae sampling hitherto analyzed.

2.1.2. DNA isolation, PCR amplification and sequencing

The same extraction methods and amplification protocols were used as described in Liede-Schumann et al. (2012). Plastid *trnT-L*, *trnL-F* and *psbA-trnH* spacers, the *trnL* intron and the *trnG* intron were obtained as well as the internal transcribed spacer region (ITS) and the external transcribed spacer (ETS). In total, 268 sequences were obtained newly for this study (ENA accession numbers LN880556–LN88082; see Table S1 [Supplementary Information]; see there also for authors of species and voucher information).

2.1.3. Phylogenetic analysis

A first analysis included 140 accessions (130 Tylophorinae, 10 outgroup). The complete dataset is available under <http://purl.org/phylo/treebase/phyloids/study/TB2:S15858>. Bayesian inference (BI) under the settings and with the models of DNA evolution detailed in Liede-Schumann et al. (2012) was conducted using MrBayes v.3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). In addition, Maximum Likelihood (ML) analysis was carried out using GARLI v.2.0 (Zwickl, 2006) with the models of DNA evolution specified for each partition in the configuration file. A GARLI bootstrap with 100 replicates and two search replicates each was conducted using the same models of DNA evolution. From the results of these analyses (Fig. 1), a new dataset was constructed, summarizing multiple accessions of the same taxon into a consensus taxon, provided these accessions were retrieved in the same clade and originated from the same area (see below). Thus, the dataset was reduced to 121 terminal taxa (111 Tylophorinae, 10 outgroup). All subsequent analyses were carried out using this reduced dataset.

Congruency between the nuclear and the plastid partition of the dataset was assessed conducting separate Maximum Likelihood (ML) bootstrap analyses of both partitions, retaining only clades with BS support $\geq 70\%$. Because no incongruence at this level could be detected (Fig. S1), all subsequent analyses were performed using the combined dataset. The best-fit nucleotide substitution model for both the chloroplast and the nuclear dataset was identified as GTR+G+I using jModelTest 2.1.1 (Posada, 2008) under the Akaike information criterion (AIC).

The occurrence of the two uncommon characters, possession of phenanthroindolizidine alkaloids and autogamy, was assembled from literature records and their known distribution was mapped onto the phylogeny using Mesquite vs 3.03 (Maddison and Maddison, 2015).

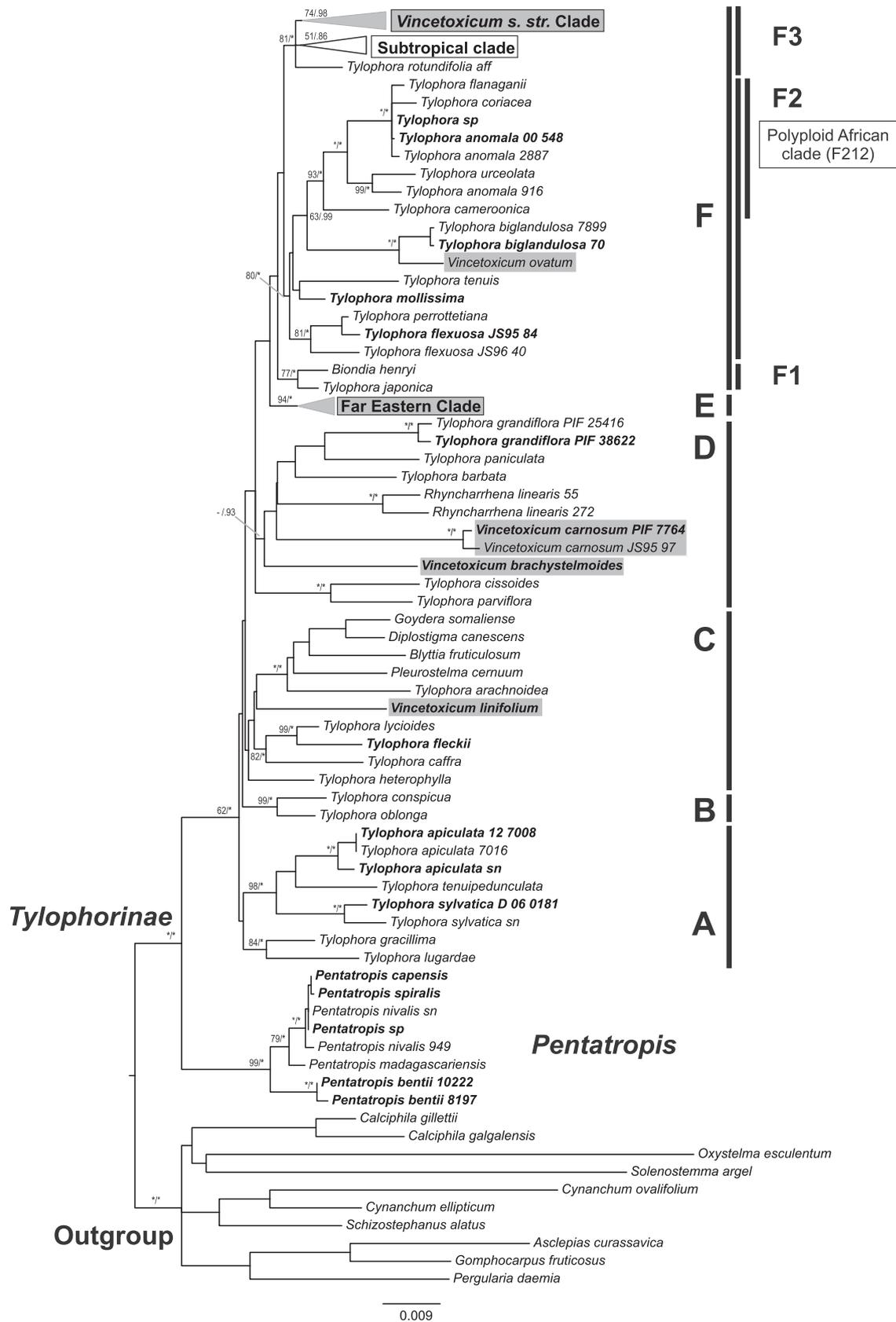


Fig. 1. General phylogenetic structure of Tylophorinae, based on *trnT-L*, *trnL-F* and *psbA-trnH* spacers, the *trnL* intron and the *trnG* intron, the internal transcribed spacer region (ITS) and the external transcribed spacer (ETS). Topology is identical for Maximum Likelihood and Bayesian Analysis using MrBayes. Far Eastern Clade, Subtropical Clade and *Vincetoxicum s. str.* Clade, which are shown in more detail in Fig. 3, are summarized by triangles. Numbers indicate bootstrap support (100 replicates) for Maximum likelihood analysis/Posterior Probability for Bayesian analysis. * Indicates 100% bootstrap support/1.00 Posterior Probability. Support values are only shown for major clades. Taxa in bold indicate accessions newly sequenced for the present study and grey shading indicates plants of the erect “*Vincetoxicum*” habit. Clade designations correspond to Liede-Schumann et al. (2012).

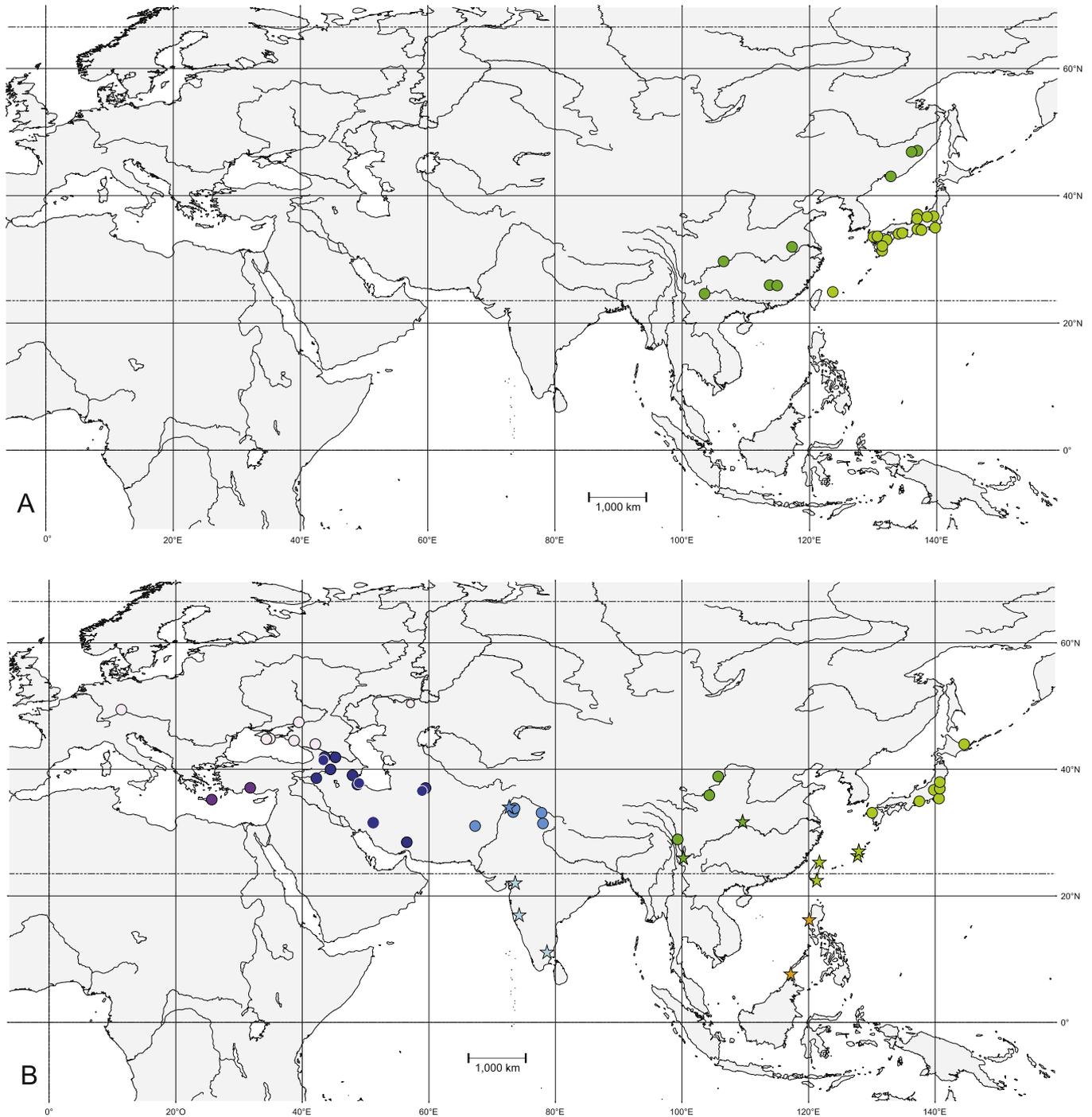


Fig. 2. Distribution of the sequenced Eurasian *Vincetoxicum* samples. A. Far Eastern Clade. B. Subtropical Clade (stars) and *Vincetoxicum* s. str. Clade (dots). Colours correspond to Fig. 3.

2.2. Biogeographical analyses

The distribution range of Tylophorinae was divided into nine biogeographical areas (Fig. 3), as follows: A (Africa and Arabia), B (Australasia, Philippines, Indochina), C (Japan and Taiwan), D (China, Russian Far East), E (Indian subcontinent), F (Pakistan), G (Irano-Turanian region), H (Eastern Mediterranean), and I (Eurasia north of the Caucasus). Because our analysis suggests that species boundaries are not yet correctly resolved in several cases, we have coded the origin of the actual specimen and not the presumed species range, which, in any case, is not known to span more than two (adjacent) biogeographical areas. The Package BioGeoBEARS

(Matzke, 2012, 2013a) was used in R Studio (version 0.98.1091) and R (version 3.0.2; R Core Team, 2013) adapting the sample script provided by Matzke (<http://phylo.wikidot.com/bio-geobears#toc17>) to the *Vincetoxicum* dataset. Separate analyses were conducted for the assumption that one species can occupy two, three, or four areas (numareas = 2, 3, or 4).

As input tree for the BioGeoBEARS analysis, an ultrametric tree was obtained by applying non-parametric rate smoothing (NPRS) to the best tree derived from ML analysis using TreeEdit (Rambaut and Charleston, 2002) as described by Moore (2012). Root age was set at 18.35 ma, following the results of Liede-Schumann et al. (2012). Polytomies were resolved in Mesquite vs

3.03 (Maddison and Maddison, 2015) using the option “resolve polytomies (to 0-length branches)”. Zero-length branches were considered as having a length of $1.0E-6$, the minimal length accepted by BioGeoBEARS.

2.3. Species distribution modelling

2.3.1. Occurrence data

We have estimated the potential distribution of nine *Vincetoxicum* species (*V. arnottianum*, *V. canescens*, *V. creticum*, *V. funebre*, *V. glaucum*, *V. pumilum*, *V. scandens*, *V. stocksii*, and *V. tmoleum*), known from Balkan Peninsula, Anatolia, Middle East, to western part of Himalayas. These species represent four subclades of the *Vincetoxicum* s. str. Clade and have been selected because they span a wide geographical range (Fig. 4), have been reliably identified, and are known from a sufficient number of populations for a modelling approach. These species were selected because they occupy an area outside, but adjacent to, the shared range of the *Vincetoxicum* s. str. Clade and the Far Eastern Clade in order to gain insight in the climatic conditions members of the *Vincetoxicum* s. str. Clade had to tolerate when they were expanding their range. We considered it more instructive to look at the conditions under which the species just outside the common range of the two clades are living than to look at the invasive species themselves, because the latter are considered widely tolerant of climatic conditions (e.g., DiTommaso et al., 2005; Sanderson and Antunes, 2013).

The occurrence data for all species were collected from herbaria (KUH, RAW, IRAN, ISL, PMNH, and TARI; abbreviations follow Thiers, 2012), personal collections in the field by RK and AP, and complemented by data from TROPICOS (Botanical information system at the Missouri Botanical Garden – www.tropicos.org), and, in the case of *V. canescens*, *V. tmoleum* and *V. creticum*, literature data (Browicz, 1975). Data derived from GBIF (The Global Biodiversity Information Facility, Backbone Taxonomy, accessed via <http://www.gbif.org/species/> on 12 December 2013) were used only if the identification could be verified. If available, longitude/latitude coordinates given on the specimens were used; otherwise, the sampling site given on the specimen was located on Google Earth (2011). Specimens with untraceable, ambiguous or too general locality data (only at province or country level) were omitted from the dataset. The coordinates were then introduced in a Geographical Information System (GIS) database using ArcGIS 10 (ESRI, 2011). Prior to using the occurrences to predict the potential

distribution of the selected *Vincetoxicum* s. str. species, we used the “trim duplicates” option from ENM Tools 1.3 (Warren et al., 2010) to ensure the independency of the occurrence data from the environmental data. The final occurrence dataset comprised 220 spatially unique data points (Placemark file available from the authors upon request).

2.3.2. Environmental data

To estimate the potential distribution of the studied *Vincetoxicum* s. str. species we have used the current climatic data from WorldClim database (Hijmans et al., 2005a,b). WorldClim database comprises a set of 19 so-called bioclimatic variables (Hijmans et al., 2005a,b), that have been shown to define the eco-physiological tolerances of a species and thus shaping its distribution area (Nix, 1986; Hijmans and Graham, 2006). Because selecting a subset of these variables has been shown to produce better models than using the whole set of 19 variables (Beaumont et al., 2005), we have followed the methodology described by Sahlean et al. (2014) for selecting the most meaningful variables for each species. This process was done by running an initial model in Maxent (Phillips et al., 2006; Phillips and Dudik, 2008) using all variables and then selecting only the climatic variables that had a contribution greater or equal to 5% in generating the initial model (Table 1). This process was conducted for each species (Table 1). In order to match the accuracy of the available occurrences with the environmental data, we have trained the models at 5 km spatial resolution. In addition, the training region used to build the models was set for each species individually based on their known distribution derived from the occurrence dataset and it represents the accessible area (Barve et al., 2011).

Because frost tolerance is uncommon in Asclepiadoideae, but necessary for survival in the distribution range of the *Vincetoxicum* s. str. Clade, we took the number of frost days as a proxy for exposure to subzero temperatures and estimated the average number of frost days to which the nine species studied are exposed. The numbers of frost days have been calculated based on the CRU TS3.10 dataset (available at www.cru.uea.ac.uk) (Harris et al., 2014). The CRU TS3.10 dataset comprises six climatic variables (including FRS, frost days) for the years between 1901 and 2000 (Harris et al., 2014), on a monthly basis. In order to calculate the average number of days of frost we have used the FRS (frost days) variable that was averaged for the whole period in order to obtain the average number of frost days that each *Vincetoxicum* s. str. species

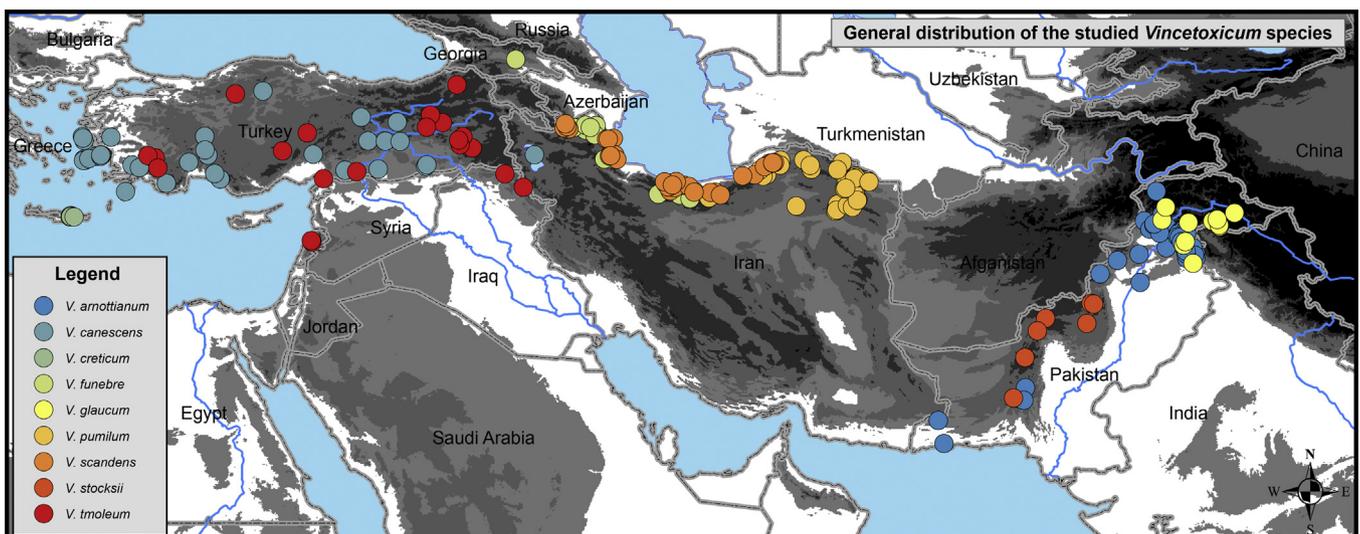


Fig. 4. General distribution of all *Vincetoxicum* s. str. species used in the modelling study.

Table 1

Variable contributions for the initial (I) and final (F) models. Factors with a contribution >10 in the final models have been marked bold.

| Variable | Western Himalayan subclade | | | | | | Irano-Turanian subclades | | | | | | Eastern Mediterranean subclade | | | | | |
|--|----------------------------|-------------|-------------------|-------------|--------------------|-------------|--------------------------|-------------|-------------------|-------------|--------------------|-------------|--------------------------------|-------------|--------------------|-------------|-------------------|-------------|
| | <i>V. arnottianum</i> | | <i>V. glaucum</i> | | <i>V. stocksii</i> | | <i>V. pumilum</i> | | <i>V. funebre</i> | | <i>V. scandens</i> | | <i>V. canescens</i> | | <i>V. creticum</i> | | <i>V. tmoleum</i> | |
| | I | F | I | F | I | F | I | F | I | F | I | F | I | F | I | F | I | F |
| Annual mean temperature | | | 0 | | | | 0.1 | | 29.7 | 48.9 | | | 4.1 | | | | | 3.9 |
| Mean diurnal range (mean of monthly) | 0.1 | | 8.6 | 9.7 | | | 4.6 | | | | 3.7 | | 28.8 | 36.5 | 16.1 | 18.2 | | |
| Isothermality (BIO2/BIO7) (*100) | 1.2 | | 0.3 | | | | 0.2 | | | | 4.6 | | 2.2 | | | | 9.2 | 11.2 |
| Temperature seasonality (standard deviation *100) | 0.6 | | | | | | 9.2 | 17.9 | 9.8 | 8 | 12.7 | 22.6 | 0.4 | | | | | 3.8 |
| Max temperature of warmest month | 1.4 | | | | 27.1 | 23.3 | 0.6 | | 0.2 | | | | 1.4 | | 54.2 | 60 | 0.3 | |
| Min temperature of coldest month | 1.4 | | 6.4 | 7.5 | | | 7 | 18.7 | | | 3.1 | | 1.4 | | | | | |
| Temperature annual range (BIO5–BIO6) | 3.8 | | | | 3.1 | | 4.9 | | 4.9 | | 10.3 | 9.6 | 0.9 | | 23.5 | 21.8 | 1.8 | |
| Mean temperature of wettest quarter | 4.1 | | | | | | 4 | | | | 5.5 | 7.9 | | | | | 17.9 | 24.1 |
| Mean temperature of driest quarter | | | 0.8 | | 55.7 | 52.2 | | | 0.8 | | 22.1 | 27.4 | | | | | 0.2 | |
| Mean temperature of warmest quarter | | | 0.3 | | | | | | 1.1 | | 0.9 | | 0.5 | | | | 0.6 | |
| Mean temperature of coldest quarter | 0.4 | | 0.7 | | | | 31.1 | 16.2 | 30.8 | 5.6 | 0.9 | | 4.1 | | | | 20.4 | 25.7 |
| Annual precipitation | 5.5 | 5.1 | | | | | | | | | | | 0.1 | | | | | |
| Precipitation of wettest month | | | | | 0.1 | | | | | | 0.5 | | 0.3 | | 3.1 | | 0.6 | |
| Precipitation of driest month | 17.8 | 9.6 | | 49.5 | 0.8 | | 1 | | 1.8 | | 22.8 | 5.4 | 4.4 | | | | 4.8 | |
| Precipitation seasonality (coefficient of variation) | 2.7 | | 8.6 | 33.3 | 13.1 | 24.5 | 5.4 | 8.6 | 4.4 | | 2 | | 23.9 | 32.6 | 1.5 | | 0.5 | |
| Precipitation of wettest quarter | | | 0.3 | | | | | | 0.5 | | 3 | | 0.2 | | 1.6 | | 28.4 | 30.7 |
| Precipitation of driest quarter | 15.3 | 30.1 | | | | | 1.6 | | 0.2 | | 7.9 | 27.1 | 2.6 | | | | 7.2 | 8.3 |
| Precipitation of warmest quarter | 42.4 | 55.1 | | | | | 25.8 | 38.5 | 9.7 | 35.7 | | | 10.7 | 17.3 | | | 0.1 | |
| Precipitation of coldest quarter | 3.2 | | 6.4 | | | | 4.4 | | 6.1 | 1.9 | | | 14.1 | 13.6 | | | 0.3 | |

would experience in nature. All calculations have been carried out in ArcGIS 10, using Map Algebra tool.

2.3.3. Model design and testing

For estimation of the current distribution of the studied *Vincetoxicum s. str.* species, we used Maxent 3.3.3 k (Phillips et al., 2006; Phillips and Dudik, 2008), together with occurrence data for each species as well as climatic data. The potential distribution of studied species was estimated using cross validation (Phillips et al., 2006), with a number of replicates corresponding to 75% of the available occurrence points. Using this method ensures that for each model some occurrences are omitted from training the model and are used to test the model; however, the occurrences are not repeated and hence, the replicates are independent. “Fade by clamping” option was used to limit our models to not extrapolate into regions without similar climates (Phillips et al., 2006; Phillips and Dudik, 2008; Webber et al., 2011). In this way, we have ensured that climatic conditions not experienced by the *Vincetoxicum s. str.* species are not included in our predictions. The other Maxent settings used to generate the models were left as default, including 10,000 background points (Phillips et al., 2006, 2009; Phillips and Dudik, 2008; Barbet-Massin et al., 2012), prevalence of 0.5 and 500 maximum iterations (Phillips et al., 2006; Phillips and Dudik, 2008). The models were reported using a 10% omission error threshold in the training occurrence dataset (Phillips et al., 2006). This threshold allows to produce a binary prediction

(presence/absence) for each species, assuming 10% error in the available occurrence dataset.

In order to test the models obtained, we have used the Area Under the (Receiver Operating Characteristic (ROC)) Curve (AUC) generated by Maxent while it estimated the potential distribution of the studied species. The AUC ranges from 0 to 1, where 1 means perfect match between the predicted and the observed phenomenon (Swets, 1988). Swets (1988) ranked the models based on the AUC values as “very good” if > 0.9, “good” if > 0.8, and “useful” if > 0.7. However, even if AUC is the classic way of testing the accuracy of the Maxent models (Phillips et al., 2006), in recent years it has been shown that AUC is not the most reliable method for testing the presence-only or presence-background based models (such as produced by Maxent) (Lobo et al., 2008, 2010). Hence, we further used the proportion of false absences (omission error) in the occurrence data to test the performance of our models. The main assumption behind this is that a performant model would predict most of the occurrences of the modelled species distribution (Pearson et al., 2006). We also used a third method for model evaluation called partial ROC as proposed by Peterson et al. (2008). In order to calculate the partial ROC, we have used the partial ROC software developed by Barve Narayani from University of Kansas, using 75% of the data for testing, and 100 bootstraps. Then, the range of the obtained values was used to test the observed partial ROC value using z-test. All maps and spatial data manipulations were done in ArcGIS 10 (ESRI, 2011).

3. Results

3.1. Phylogeny

Clade structure (Fig. 1) of the BI and the ML tree was mostly identical and agrees largely with the tree shown in Liede-Schumann et al. (2012: Fig. 3). *Pentatropis* remains sister to the rest of Tylophorinae, and the two accessions of *Pentatropis bentii* from Somalia and Yemen form a monophyletic sister to the remaining species. The Madagascan *P. madagascariensis* and the Kenyan sample of *P. nivalis* are sister to the four accessions from Pakistan.

Plants of the typical *Vincetoxicum* habit are found in all clades except for the African clades A and B (Fig. 1). The Far Eastern Clade (Fig. 3) is augmented by three samples from the Far Eastern regions of Russia, *V. volubile*, *V. acuminatum* (*Kharkevich s.n.*) and *V. inamoenum* (*s. coll. s.n.*). The *Vincetoxicum s. str.* Clade (Fig. 3), into which almost half of the new samples fall, remains in well supported ($BS_{ML} = 0.81$, $PP = 1$) sister group position to the Subtropical Clade and *Tylophora rotundifolia* aff.

Mean ages of the clades under consideration correspond to the respective nodes in Liede-Schumann et al. (2012; Table S4). Even if these ages can only serve as rough approximations because of the lack of fossils, it needs to be noted that even though the stem age of the Far Eastern Clade is older than the one of the *Vincetoxicum s. str.* Clade, diversification in the Far Eastern Clade started later than in the *Vincetoxicum s. str.* Clade. Almost all records for phenanthroindolizidine alkaloids are derived from species of the Subtropical Clade and the *Vincetoxicum s. str.* Clade (Fig. 3), exceptions are *V. pycnostelma* (Far Eastern Clade) and *T. mollissima* (Table S2). Autogamy has exclusively been reported from members of the Subtropical Clade and the *Vincetoxicum s. str.* Clade (Fig. 3 and Table S3; see also discussion in Liede-Schumann et al., 2012). Four species have both characteristics, *T. hirsuta*, and the three invasive species *V. hirundinaria*, *V. nigrum*, and *V. rossicum*.

3.2. Biogeography

Of the six models tested in BioGeoBEARS, the “+J” models are all significantly favored with the null model rejected at the $p > 0.0001$ cutoff level in all cases (Table S5). There is very little difference between the DEC+J, the DIVALIKE+J and the BAYAREA+J models. This is reflected by all three models resulting in identical ancestral area reconstructions. In all three “+J” models, the j parameter is positive, and the d and e parameters are very small (Table S5), indicating that founder events have played a role in the biogeography of *Vincetoxicum s.l.* (Matzke, 2014). For the DEC+J model, the assumption that one species can maximally occupy two areas results in the same likelihood ($\text{LnL} = -143.23$) as the assumption that it can occupy three ($\text{LnL} = -143.23$) or four ($\text{LnL} = -143.22$) areas.

Reconstruction of ancestral ranges in *Vincetoxicum s.l.* corresponds to the reconstruction in Liede-Schumann et al. (2012: Fig. 5), albeit with far less widespread ranges for most ancestral nodes (Fig. 3). Our enlarged sampling, however, results in a better resolved biogeography for the Far Eastern Clade and the *Vincetoxicum s. str.* Clade.

In the Far Eastern Clade numerous exchanges between Japan and the mainland have taken place, and two species of our dataset (*V. atratum* and *V. pycnostelma*) occur in both areas. For *V. acuminatum*, the Japanese and the mainland sample are not monophyletic, and for *V. inamoenum*, the mainland sample is retrieved in the Far Eastern Clade, while the Japanese sample is retrieved in the *Vincetoxicum s. str.* Clade. In the *Vincetoxicum s. str.* Clade, the Japanese *Tylophora aristolochioides* remains sister to all other species, but with little support ($BS_{ML} = 51$, $PP = 0.98$). The following subclade is also entirely Japanese, consisting of a well supported *V. macrophyllum*–*V. magnificum* group, to which *V. floribundum* is added

without support. The clades branching off next show a tendency for westward expansion. The well-supported subclade ($BS_{ML} = 95$, $PP = 1.0$) of Central Chinese and northern Japanese accessions is followed by a weakly supported ($BS_{ML} = 62$, $PP = 0.94$) clade consisting of the Western Himalayan Subclade ($BS_{ML} = 99$, $PP = 1.0$) and a clade of Eastern Irano-Turanian samples, the latter comprising *V. pumilum* and the recently described endemics *V. assadii* and *V. mozaffarianii* ($BS_{ML} = 73$, $PP = 1.0$). The following clades show an expansion to Kazakhstan and southern Russia (Southern Russian Subclade, $BS_{ML} = 96$, $PP = 1.0$), to the Eastern Mediterranean (Eastern Mediterranean Subclade, $BS_{ML} = 84$, $PP = 1.0$) as well to the Caucasus (Western Irano-Turanian Subclade, $BS_{ML} = 78$, $PP = 0.95$) and into Europe (European Subclade, $BS_{ML} = 98$, $PP = 1.0$). The Western European, invasive *V. nigrum* is unresolved with the Western Irano-Turanian and the European subclades.

3.3. Species distribution modelling

All distribution models performed very well from the point of view of all model accuracy metrics that we have used. The AUC training values ranged from 0.84 to 0.99 and AUC test values from 0.77 to 1.00 (Table S6), which means that our models are very good in their power to estimate the potential distribution of the studied *Vincetoxicum* species. Our models omitted only a very small number of occurrence points, which suggest a great ability of predicting the known distribution of the studied species. The results from the partial ROC analysis are statistically significant for all species, which suggests a significant ability of our models to predict the distribution of Western Himalayan Subclade, the two Irano-Turanian subclades, and the Eastern Mediterranean Subclade (Table 1).

Table 1 indicates the main contributing factors according to initial and final models. The contributing variables vary both between and within the clades. In the Western Himalayan Subclade, precipitation, be it of the driest month, the driest or the warmest quarter, or the seasonality of precipitation, have the most prominent effect on the occurrence of *Vincetoxicum s. str.* species. Only in *V. stocksii* the temperature of the driest quarter is the most relevant variable. In contrast, in the two Irano-Turanian Subclades, temperature seasonality plays a role for all three species, along with precipitation of the warmest and the driest quarter. Mean temperature of the coldest quarter is important for *V. pumilum* and *V. funebre*, mean temperature of the driest quarter for *V. scandens*. Again, each species has its own dependent variable range (Table 1). This trend is even more pronounced in the Eastern Mediterranean Subclade (Table 1). The highest contributing variables for *V. canescens* are mean diurnal temperature range, precipitation seasonality, precipitation of the warmest and coldest quarters. *Vincetoxicum creticum* is only dependent on temperature variables (maximum temperature of warmest month, temperature annual range and mean diurnal range; Table 1). For *V. tmoleum*, in contrast, precipitation of wettest and driest quarters, mean temperature of coldest and wettest quarters, and isothermality are contributing factors (Table 1).

All nine species studied experience a number of days per year with freezing temperatures. For *V. creticum*, the fewest (c. 32 on average), for *V. glaucum* the most (c. 149 on average) number of frost days per year have been recorded. Except for *V. creticum* and *V. canescens*, all species experience on average more than 100 frost days per year (Table 2).

4. Discussion

4.1. Phylogeny

Our phylogenetic analysis in the present study confirms the general clade structure presented in Liede-Schumann et al.

Table 2

Average number of days with temperatures below 0 °C (frost days) at the localities of *Vincetoxicum* populations.

| Species | Number of frost days | |
|-----------------------|----------------------|----------|
| | Average | SD |
| <i>V. arnottianum</i> | 104.2438 | 32.25418 |
| <i>V. canescens</i> | 69.99048 | 37.2615 |
| <i>V. glaucum</i> | 149.1273 | 41.07377 |
| <i>V. creticum</i> | 32.11429 | 3.848562 |
| <i>V. funebre</i> | 146.3938 | 9.758106 |
| <i>V. pumilum</i> | 146.1647 | 19.90552 |
| <i>V. scandens</i> | 138.1629 | 15.826 |
| <i>V. stocksii</i> | 109.7286 | 35.02046 |
| <i>V. tmoleum</i> | 123.225 | 38.02116 |

(2012). Most *Vincetoxicum*-like plants (erect, sometimes apically weakly twining herbaceous plants with clear latex and fascicled roots) are restricted to either the Far Eastern Clade or the *Vincetoxicum s. str.* Clade. However, *V. brachystelmoides*, *V. carnosum*, *V. linifolium*, and *V. ovatum* show that this particular habit has evolved in African and, in particular, Australasian clades as well (Fig. 1).

Our study indicates that species diversity of *Vincetoxicum*-type plants might be higher than hitherto assumed, because especially members of the *Vincetoxicum s. str.* Clade are fairly uniform in terms of floral morphology (see Yamashiro et al., 2004), so that species delimitation based on morphology alone has been fraught with uncertainties. This is illustrated by several accessions which were received as duplicates of the same species, but were not retrieved as monophyletic (Fig. 3). This uncertainty cautions against the uncritical use of the alkaloid data, and a systematic study of the distribution of phenanthroindolizidine alkaloids in the genus would be desirable to confirm its occurrence outside the *Vincetoxicum s. str.* Clade and to trace the evolutionary history of this rare trait in Asclepiadoideae.

4.2. Biogeography

Vincetoxicum s.l. is unique in Asclepiadoideae because it extends to a latitude of 60°N. Similar high latitudes are only reached by two other genera in the subfamily, *Asclepias* L. in North America (*A. ovalifolia* Decne.; to 55°N, Woodson, 1954), and *Diplolepis* R.Br. in South America (*D. australis* (Malme) Hechem and Ezcurra; to 48°S, Hechem et al., 2011). In the Far Eastern Clade, numerous exchanges between the mainland and Japan have occurred, but the low amount of genetic differentiation makes it difficult to trace the patterns of northward migration inside this clade.

In contrast, the genetically more diverse *Vincetoxicum s. str.* Clade shows a clear pattern of north-western expansion that can be traced in its phylogeny (Figs. 2 and 3). The southern slopes of the mountain chains of Himalaya, Hindu Kush, Kopet Dag, Alborz, and Caucasus link the distribution areas of *Vincetoxicum s. str.* between the Far East and Europe (Fig. 4), spanning more than 4000 km.

Our initial assumption that this westward expansion would follow along rather similar climatic niches, is disproved by our data. The nine species studied have thus undergone not only morphological differentiation, but are also adapted to a wide range of climatic niches. In the Western Himalayan Subclade, the area of *V. arnottianum* and *V. glaucum* is determined by precipitation variables. For *V. arnottianum*, our results correspond to Khanum et al. (2013). *Vincetoxicum stocksii*, on the other hand, is endemic to Baluchistan (Ali, 1983), a hot and dry area, and accordingly, temperature of the driest quarter as well as the maximal temperature of the warmest month are the most important factors shaping its distribution area.

In the Eastern Irano-Turanian Subclade, the most widespread species, *V. pumilum*, is a species of the Kopet Dag Range, a rather humid area with moderate temperatures. The two rare endemics from southern Iran (Zagros Mts.), *V. assadii* (central Zagros) and *V. mozzaffarianii* (southern Zagros), for which not enough data for climatic niche modelling were available, are members of this subclade as well, contradicting Zaeifi (1999), who considered *V. assadii* as a relative of *V. tmoleum* and *V. mozzaffarianii* a relative of *V. stocksii*. *Vincetoxicum funebre* and *V. scandens* in the Western Irano-Turanian subclade prefer the rather equitable climates around the Caspian Sea and precipitation in the warmest or driest quarter seem to play a major role for their climatic preferences (Table 1). For *V. scandens*, a species of the hyrcanian forest, temperature and precipitation of the driest period are most important, while for *V. funebre*, a species inhabiting rocky slopes, the temperature of the coldest quarter is the single most decisive factor. For these three species, temperature seasonality is an important factor defining suitable habitats. Only one of the three species of the Eastern Mediterranean Subclade, *V. creticum*, can be considered exclusively Eastern Mediterranean in its distribution. *Vincetoxicum canescens* occupies both coastal habitats in the West and mountainous habitats in the East, ranging into the Irano-Turanian-Floristic Province, where *V. tmoleum* has its main area, which extends into the Mediterranean. All three species, however, avoid very hot and dry areas. Summarizing, our results for these three clades suggest that in *Vincetoxicum s. str.* environmentally mediated divergence plays a role in speciation in closely related allopatric to parapatric species groups. Thus, *Vincetoxicum s. str.* is not conservative (Wiens and Graham, 2005) with regards to climatic niches. Our results agree with those of Anacker and Strauss (2014) who showed on 71 pairs of sister species in the California Floristic Province that the ecological (and reproductive) similarity of sisters was significantly greater than that of sister–non-sister congeners. This was true for each of the six traits they assessed, but 93% of the sister species pairs showed shifts in at least one of these traits, particularly frequently in habitat and soil type.

Nevertheless, all *Vincetoxicum s. str.* species studied share the tolerance to exposure to sub-zero temperatures, which is arguably one of the most important preadaptations for species expanding into higher latitudes. Most likely, the difference between frost sensitivity and frost tolerance is linked to habit, with frost tolerant species capable of dying back in the cold season, resprouting from a subterranean rootstock. This habit is well developed in species of all subclades of the *Vincetoxicum s. str.* Clade, except for *T. aristolochioides*, and *T. floribundum*. We therefore hypothesize that frost tolerance represents a conserved feature in the *Vincetoxicum s. str.* Clade, enabling the plants to radiate in temperate areas. However, species of the Far Eastern Clade are also able to withstand a cold season, and therefore have the potential for radiation. Possibly the fact that the Far Eastern Clade started its radiation later than the *Vincetoxicum s. str.* Clade explains its restriction to a comparatively smaller area, not having had enough time for a wider radiation.

4.3. Invasive species

The three invasive species (*V. hirundinaria*, *V. nigrum*, and *V. rosicum*) belong to the top subclade of the *Vincetoxicum s. str.* Clade, that has its centre of diversity in the Caucasus and the northern shore of the Black Sea. Because species delimitation in this clade is particularly difficult (compare, e.g., Markgraf, 1972; Czerepanov, 1995) and sufficient material for detailed study could not be obtained, this clade was not included in our present modelling analysis. First estimates of climatic preferences (Douglass et al., 2009), which indicate that climate regimes are similar, but not identical in the natural and the invasive ranges of *Vincetoxicum*

s. str. spp., indicate that these species thrive under a rather wide range of climatic conditions. Climatic niche similarity for invasive plants between Eurasia and North America is also suggested by Petitpierre et al. (2012). These three species all possess phenanthroindolizidine alkaloids (Table S2), which probably add to the unpalatability of the plants for unspecialized herbivores in their new environment (DiTommaso et al., 2005; Ernst and Cappuccino, 2005; Maguire et al., 2011). Furthermore, they are all capable of autogamy (Table S3). This combination of cold tolerance, alkaloids, and autogamy is most likely the key to the success of the three species as invasive aliens in North America. However, these results need to be viewed with caution, because the three invasive species have been studied in much more detail than the non-invasive ones, in which alkaloid content or capacity for autogamy might have been overlooked so far.

5. Conclusions

The present study documents the north-westward expansion of the *Vincetoxicum s. str.* Clade and shows that its members can successfully occupy a wide variety of climatic niches, whose only common feature is exposure to subzero temperatures for at least one month per year. Based on these results, the present study suggests several topics for future research. In the areas of overlap between the Far Eastern Clade and the *Vincetoxicum s. str.* Clade (Japan, China), basic taxonomic research is needed to reliably identify the species belonging to the different lineages. Because our study has revealed polyphyly for several presumably widespread species, species delimitation needs to be studied for these complexes in much more detail. In addition, niche studies could show whether the two lineages also occupy similar niches in the overlapping areas. Because Japan is harbouring species of both the Far Eastern Clade and the first branching subclade of the *Vincetoxicum s. str.* Clade, comparative climate niche modelling of Japanese species combined with morphological and genetic studies are needed to throw light onto the acquisition of cold tolerance in *Vincetoxicum*. In the Subtropical and the *Vincetoxicum s. str.* Clades, it would be desirable to test all species for their alkaloid content, and for their potential for self-fertilization. Getting a better picture of the biology of the relatives of the invasive species might lead to the discovery of the desired biocontrol mechanism.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2015.09.021>.

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