# Pyrrolizidine alkaloid variation in *Senecio vulgaris* populations from native and invasive ranges

**Dandan Cheng** [Corresp., 1] , **Viet-Thang Nguyen** [2, 3] , **Noel Ndihokubwayo** [2, 4] , **Jiwen Ge** [5] , **Patrick PJ Mulder** [6]

[1] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences (Wuhan), Wuhan, China

[2] School of Environmental Studies, China University of Geosciences (Wuhan), Wuhan, China

[3] Faculty of Biology and Agriculture Engineering, Thai Nguyen Uiniversity of Education, Thai Nguyen, Vietnam

[4] Département des Sciences Naturelles, Ecole Normale Supérieure, Bujumbura, Burundi

[5] Hubei Key Laboratory of Wetland Evolution & Ecological Restoration, China University of Geosciences (Wuhan), Wuhan, China

[6] RIKILT, Wageningen University & Research, Wageningen, The Netherlands

Corresponding Author: Dandan Cheng
Email address: dandan.cheng@cug.edu.cn

Biological invasion is regarded as one of the greatest environmental problems facilitated by globalization. Some hypotheses about the invasive mechanisms of alien invasive plants consider the plant–herbivore interaction and the role of plant defense in this interaction. For example, the "Shift Defense Hypothesis" (SDH) argues that introduced plants evolve higher levels of qualitative defense chemicals and decreased levels of quantitative defense, as they are released of the selective pressures from specialist herbivores but still face attack from generalists. Common groundsel (*Senecio vulgaris*), originating from Europe, is a cosmopolitan invasive plant in temperate regions. As in other *Senecio* species, *S. vulgaris* contains pyrrolizidine alkaloids (PAs) as characteristic qualitative defense compounds. In this study, *S. vulgaris* plants originating from native and invasive ranges (Europe and China, respectively) were grown under identical conditions and harvested upon flowering. PA composition and concentration in shoot and root samples were determined using Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). We investigated the differences between native and invasive *S. vulgaris* populations with regard to quantitative and qualitative variation of PAs. We identified 20 PAs, among which senecionine, senecionine N-oxide, integerrimine N-oxide and seneciphylline N-oxide were dominant in the roots. In the shoots, in addition to the 4 PAs dominant in roots, retrorsine N-oxide, spartioidine N-oxide and 2 non-identified PAs were also prevalent. The roots possessed a lower PA diversity but a higher total PA concentration than the shoots. Most individual PAs as well as the total PA concentration were strongly positively correlated between the roots and shoots. Both native and invasive *S. vulgaris* populations shared the pattern described above. However, there was a slight trend indicating lower PA diversity and lower total PA concentration in invasive *S. vulgaris* populations than native

populations, which is not consistent with the prediction of SDH.

1    **Pyrrolizidine alkaloid variation in *Senecio vulgaris* populations from native and**
2                        **invasive ranges**

3    Dandan Cheng[1, *]

4    Viet Thang Nguyen [2,3]

5    Noel Ndihokubwayo [2,4]

6    Jiwen Ge[5]

7    Patrick P. J. Mulder [6]

9    [1] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences,
10        Wuhan, Hubei, 430074, China

11   [2] School of Environmental Studies, China University of Geosciences (Wuhan), Wuhan, Hubei, 430074,
12        China

13   [3] Faculty of Biology, Thai Nguyen University of Education, No. 20, Luong Ngoc Quyen Street, Thai
14        Nguyen City, Vietnam

15   [4] Ecole Normale Supérieure, Département des Sciences Naturelles, Boulevard du 28 Novembre, B.P 6983
16        Bujumbura, Burundi

17   [5] Hubei Key Laboratory of Wetland Evolution & Ecological Restoration, China University of
18        Geosciences (Wuhan), Wuhan, 430074, China

19   [6] RIKILT Wageningen University & Research, Akkermaalsbos 2, 6708 WB Wageningen, The
20        Netherlands

22   * Corresponding author, E-mail: dandan.cheng@cug.edu.cn

23   **Abstract**

24   Biological invasion is regarded as one of the greatest environmental problems facilitated by

25   globalization. Some hypotheses about the invasive mechanisms of alien invasive plants consider

26   the plant–herbivore interaction and the role of plant defense in this interaction. For example, the

27   "Shift Defense Hypothesis" (SDH) argues that introduced plants evolve higher levels of

28   qualitative defense chemicals and decreased levels of quantitative defense, as they are released of

29   the selective pressures from specialist herbivores but still face attack from generalists.

30   Common groundsel (*Senecio vulgaris*), originating from Europe, is a cosmopolitan invasive

31   plant in temperate regions. As in other *Senecio* species, *S. vulgaris* contains pyrrolizidine

32   alkaloids (PAs) as characteristic qualitative defense compounds. In this study, *S. vulgaris* plants

33   originating from native and invasive ranges (Europe and China, respectively) were grown under

34   identical conditions and harvested upon flowering. PA composition and concentration in shoot

35   and root samples were determined using Liquid Chromatography–Tandem Mass Spectrometry

36   (LC-MS/MS). We investigated the differences between native and invasive *S. vulgaris*

37   populations with regard to quantitative and qualitative variation of PAs.

38   We detected 22 PAs in *S. vulgaris*, among which senecionine, senecionine N-oxide,

39   integerrimine N-oxide and seneciphylline N-oxide were dominant in the roots. In the shoots, in

40   addition to the 4 PAs dominant in roots, retrorsine N-oxide, spartioidine N-oxide and 2 non-

41   identified PAs were also prevalent. The roots possessed a lower PA diversity but a higher total

42   PA concentration than the shoots. Most individual PAs as well as the total PA concentration

43   were strongly positively correlated between the roots and shoots. Both native and invasive *S.*

44   *vulgaris* populations shared the pattern described above. However, there was a slight trend

45   indicating lower PA diversity and lower total PA concentration in invasive *S. vulgaris*

46   populations than native populations, which is not consistent with the prediction of SDH.

47   **Key words:** Biological invasion, Shift Defense Hypothesis (SDH), qualitative defense, Liquid

48   Chromatography–Tandem Mass Spectrometry (LC-MS/MS), secondary metabolite, diversity

## Introduction

An alien invasive plant species is a species that expands its natural range with facilitation from intentional or non-intentional human activities, tending to hazard biodiversity, ecosystem services and human well-being in its new range (Vilà & Hulme, 2017). Many hypotheses have been proposed to explain the invasive mechanisms of alien invasive plants (Catford et al., 2009). Some explanations focus on plant-herbivore interactions and the role of plant defense. For instance, the "Enemy Release Hypothesis" (ERH) states that in a new range, introduced plants may leave behind their specialist herbivores and gain a rapid increase in distribution and abundance (Keane & Crawley, 2002). Loss of enemies leads to lower defense levels as plants allocate fewer resources to defense and more to growth, as according to the "Evolution of Increased Competitive Ability" (EICA) hypothesis (Blossey & Notzold, 1995). The "Shift Defense Hypothesis" (SDH) argues that invasive plants decrease the level of quantitative defense but increase their qualitative defense, as invasive plants still face pressure from generalist herbivores even though they escaped attack from specialists (Doorduin & Vrieling, 2011; Joshi & Vrieling, 2005; Müller-Scharer et al., 2004).

Chemical defense in plants can be divided into qualitative defense and quantitative defense in relation to their effect on herbivores. Plant secondary metabolites (SMs) involved in qualitative defense are toxic to many herbivores and not very costly to produce. Those involved in quantitative defense are based on digestibility-reducing chemicals and more expensive to produce and to maintain due to the typically higher complexity of the molecules (Feeny, 1976; Rhoades & Cates, 1976). Specialist and generalist herbivores react in different ways to toxic SMs: generalist herbivores are deterred by high concentrations of toxic chemicals, while specialists are often adapted to these chemicals and use them as a cue to find their host plant. Thus, plants containing high concentrations of toxic chemicals suffer more from specialist herbivores (Cates, 1980). Hence, specialist and generalist herbivores inflict different selective pressures on plants, and the concentration of SMs is balanced by the opposing selective forces of specialists and generalists ("Specialist-Generalist Dilemma", van der Meijden, 1996).

Moreover, different plant metabolites, even from the same groups of chemicals, may have different effects on herbivores (Kleine & Müllller, 2010; Macel et al., 2005; van Dam et al., 1995). It is assumed that plants with a more diverse and/or with higher concentrations of SMs can better protect themselves when the specialist herbivores adapted to the qualitative defense

80   chemicals are absent. Therefore, for introduced plants variation in both concentration and

81   composition of defense chemicals is important to defend themselves against the guild of

82   herbivores in a new range.

83   *Senecio* and *Jacobaea*, possessing pyrrolizidine alkaloids (PAs) as their characteristic defense

84   compounds, have been chosen in several studies as model species to assess the quantitative and

85   qualitative variation in SMs in native and introduced populations. PAs act as deterrents or toxins

86   to non–adapted herbivores and pathogens. However, specialist herbivores that are adapted to PAs

87   can utilize them from host plants for their own benefit, such as for a food cue and oviposition

88   (Joosten & van Veen, 2011; Macel, 2011; Trigo, 2011). Higher concentrations of PAs have been

89   found in invasive rather than native populations of *Jacobaea vulgaris* (syn. *Senecio jacobaea;*

90   Joshi & Vrieling, 2005; Lin et al., 2015), and invasive *Senecio pterophorus* was found to have a

91   higher concentration of PAs than its conspecific relatives (Caño et al., 2009; Castells et al.,

92   2014). Beside PAs from *Senecio* and *Jacobaea*, more than 350 PAs have been identified in an

93   estimated 6,000 plants in the Boraginaceae, Asteraceae, and Leguminosae families (Stegelmeier

94   et al., 1999).  In this study, we selected *Senecio vulgaris* (common groundsel, Senecioneae:

95   Asteraceae) as a model organism for the comparison of quantitative and qualitative PA variation

96   between native and invasive populations. *S. vulgaris*, a cosmopolitan weed in temperate regions,

97   probably originated from southern Europe (Kadereit, 1984), and has spread to America, North

98   Africa, Asia, Australia and New Zealand in the 18th century (Robinson et al., 2003). The

99   occurrence of *S. vulgaris* was first recorded in China in the 19th century, and it is nowadays

100   mainly distributed in northeastern and southwestern China (Li & Xie 2002; Xu et al., 2012). *S.*

101   *vulgaris* plants of some European and Canadian populations contain high amounts (>0.6 mg/g

102   fresh weight) of PAs (von Borstel et al., 1989). Handley et al. (2008) investigated the invasive

103   mechanisms of this species with respect to the interaction between plants and pathogens and the

104   outcomes did not support the EICA hypothesis. Zhu et al (2017) found that although *S. vulgari*s

105   might have been introduced into China on multiple occasions, the Chinese populations contained

106   smaller genetic diversity compared to European populations.

107   In this study, *S. vulgaris* plants from seeds collected from 6 native (Europe) and 6 invasive

108   (China) populations were grown under identical conditions in a greenhouse. PAs were extracted

109   from the roots and shoots of harvested *S. vulgaris* plants and measured using Liquid

110   Chromatography–Tandem Mass Spectrometry (LC-MS/MS). According to the SDH, invasive

111    plants tend to evolve higher levels of qualitative defense chemicals. Hence, we hypothesized that

112    plants from invasive *S. vulgaris* populations would produce higher concentrations of PAs than

113    those from native ranges. We also compared PA profiles in the native and invasive populations.

**Materials and Methods**

**Studies species**

116    *Senecio vulgaris* can complete its life cycle in as little as 8 weeks, producing an average of

117    38,300 seeds per generation and can be found in gardens, lawns, roadsides, field margins, arable

118    lands, waste places and coastal habitats. Variation in capitula morphology, seed dormancy and

119    growth form have been observed in different *S. vulgaris* populations (Robinson et al, 2003). No

120    surveys have yet been undertaken on the amount of herbivory naturally occurring in *S. vulgaris*

121    populations. However, it is known that *S. vulgaris* can be the host plant of generalist herbivores

122    such as the leafminer *Liriomyza trifolii* and the Western tarnished plant bug (*Lygus hesperus*)

123    (Minkenberg & Lenteren, 1986; Barlow et al., 1999). The cinnabar moth (*Tyria jacobaeae*), flea

124    beetle (*Longitarsus jacobaeae*) and ragwort seed fly (*Botanophila seneciella*) are specialists that

125    have be used as biological control for *Jacobaea vulgaris* in North America and Australia. The

126    first two insects have been observed also to feed on *S. vulgaris*, but it is unknown whether the

127    ragwort seed fly can feed on *S. vulgaris*. Furthermore, a rust fungi *Puccinia lagenophorae* can

128    infect *S. vulgaris* plants and is used as biological control of *S. vulgaris* (Frantzen & Hatcher,

129    1997). In China, we observed that leafminers and seed flies caused damage to natural

130    populations of *S. vulgaris* and we also observed heavy herbivory by aphids on *S. vulgaris* plants

131    grown in the greenhouse for this study. The insects have not yet been identified, and it remains to

132    be determined whether these are specialists or not.

133    Some *S. vulgaris* biotypes showed increased resistance to various herbicides such as simazine,

134    atrazine, bromacil, pyrazon, buthidazole and linuron. Therefore, *S. vulgaris* is considered as a

135    troublesome weed, especially in horticulture where frequent cultivation occurs (Robinson et al.,

136    2003). The morphology of *S. vulgaris* plants resembles that of some other *Senecio* species used

137    as Chinese traditional medicinal plants, implicating a risk to human health if they are used as

138    medicine or otherwise consumed by mistake (Yang et al., 2011).

139 **Plant resources, growth and harvesting**

140 We used seeds collected from 6 native and 6 invasive *S. vulgaris* populations in Europe and

141 China (Table 1). Achenes from 6-20 individual plants per population were kept in paper bags,

142 air-dried and stored in the laboratory. Seeds from 4-7 individuals in each population were

143 selected for germination. Substrate made from coconut soil and sand (1:1 by volume) was placed

144 into 12–cell boxes (size of one cell: 3.7 × 3.7 × 5 cm) for seed germination. One seed was sown

145 in each cell. After sowing, the boxes were covered with a transparent top and placed in a climate

146 room (20ºC). The sowed seeds were watered by means of a small sprayer.

147 For plant rearing, we prepared substrate as described above and added slow release fertilizer

148 (N:P:K= 14:13:13, Osmocote, The Scotts Company, USA) along with a potting medium

149 comprising 20 g of fertilizer and 3 kg of substrate. Once 2–4 true leaves had appeared, the plants

150 were transplanted into bigger pots (size: 8 × 8 × 9 cm) containing the substrate and fertilizer and

151 left to grow in a greenhouse.

152 When some of the plants began to flower, their first capitula were pruned. A week later, when

153 the majority of plants had developed 5-10 capitula, they were then harvested. The shoots and

154 roots were separated at their root crowns using secateurs. The shoots were rinsed using tap water.

155 The fresh weight of the roots and shoots was separately measured. The samples were kept

156 separately in plastic bags and then placed in liquid nitrogen prior to storage in a freezer at -80ºC.

157 Following this, the samples were freeze-dried in an ALPHA 1-2 LD laboratory freeze-dryer

158 (Martin Christ, Lower Saxony, Germany). The dry weight of the roots and shoots was measured

159 before they were ground into a fine powder and homogenized using a vortex machine.

160 Approximately 10 mg of the powder was placed into 2 mL Eppendorf tubes and stored at -20ºC

161 until PA extraction.

162 **PA extraction and analysis**

163 The extraction and analysis of PAs was performed as described in detail in our previous work

164 (Joosten et al., 2010; Cheng et al., 2011). In brief, approximately 10 mg of the fine powdered

165 plant material was used to extract PAs with 1 mL 2% formic acid solution in water. At a

166 concentration of 1 μg mL$^{-1}$, heliotrine was added as internal standard to the extraction solvent.

167 The plant extract solution was shaken for 0.5 h. Solid plant material was removed by

168 centrifugation at 2,600 rpm for 10 min and filtered through a 0.2 μm nylon membrane (Acrodisc

169    13 mm syringe filter, Pall Corporation, NY, USA). An aliquot of the filtered solution (25 μL)

170    was diluted with water (975 μL) and 5 μL was injected into the LC-MS/MS system (Acquity

171    UPLC coupled to a Quattro Premier XE tandem mass spectrometer (Waters, Milford, MA,

172    USA)), using an Acquity BEH C18, 150 × 2.1 mm, 1.7 μm (Waters, USA) UHLPC column,

173    maintained at 50°C, for separation of the PAs. As mobile phase A 6.5 mM ammonia in water was

174    used and as mobile phase B acetonitrile. An analytical run was applied, starting at 100% A which

175    was linearly changed to 50% B in 12 min, where after the mobile phase was returned to 100% A

176    in 0.2 min. Total run time was set at 15 min and the flow was kept at 0.4 mL min$^{-1}$.

177    Quantification of the extracts was performed against a calibration range of PA standards (0-500

178    ng mL$^{-1}$) in a blank plant extract. Ten analytical standards were available for quantification

179    (Table 2). The concentrations of the remaining PAs were determined semi-quantitatively by

180    comparison of their peak area with that of a related standard as indicated in Table 2. The limit of

181    detection (LOD) for individual PAs in leaf tissue was approximately 0.5 μg g$^{-1}$ dry weight. LC-

182    MS/MS analytical settings used for detection and quantification of PAs are listed in Table 2.

183    **Data analysis**

184    The Shannon index of PA diversity (H') in each sample was calculated according to the formula:

185    $H' = -\Sigma\, p_i * \ln p_i$, where $p_i$ is the relative abundance of each of the 20 individual PAs in a sample.

186    The homogeneity of PA distribution in each sample (evenness, J') was calculated as: J' =

187    H'/ln(s), where s is the total number of occurring PAs in a sample. The calculation was

188    conducted using the R package "vegan" (Simpson et al., 2009).

189    Variation in PA composition was evaluated using the concentrations of all of the 20 individual

190    PAs detected in the shoots and roots (except usaramine N-oxide and riddelliine which were only

191    rarely detected, see Table 3). Differences in PA composition among the populations and between

192    the shoots and roots were evaluated using an Adonis test, a nonparametric MANOVA, in which

193    populations and plant parts (shoots or roots) were defined as factor variables.

194    We visualized the variation in PA composition using a nonmetric multidimensional scaling

195    (NMDS) method, which is analogous to a principal component analysis (PCA) or

196    multidimensional scaling (MDS), but without distribution assumptions (Goslee & Urban, 2007).

197    Heatmaps were constructed to show difference between populations by using the R package

198    "pheatmap" (Kolde, 2015).

199 We calculated the Sn/Sp ratio from the concentration of 4 PAs using the formula: (senecionine +

200 senecionine N-oxide)/(seneciphylline + seneciphylline N-oxide). The ratios were square root

201 transformed and used in a Kruskal-Wallis test to assess whether the ratios differed between

202 populations. Between-population homoscedasticity was checked using Breusch-Pagan tests.

203 Total PA concentration and the individual concentrations of 20 PAs was log10 transformed and

204 then used in analysis of PA concentration. Paired Wilcoxon rank tests were used to confirm

205 whether the concentration of total PA and the individual PAs differed between the roots and

206 shoots, while Spearman's rank correlation tests were conducted to investigate the correlation

207 between roots and shoots. Breusch-Pagan tests were used to assess equality of variance between

208 the groups. *P*-values of the results were adjusted using sequential Bonferroni method when

209 multiple tests were performed.

210 To confirm whether for roots and shoots the concentration, relative abundance of individual PAs,

211 and total PA concentration differed among populations and between ranges, nested ANOVA

212 tests were conducted in SPSS (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp.,

213 Armonk, NY, USA). Equality of variance between the groups was assessed using Levene's tests.

214 To conduct nested ANOVA tests, we selected the 13 PAs that had an average relative abundance

215 of more than 1%. Concentration of PAs was log transformed. Relative abundance of PAs was

216 calculated as individual PA percentage of the total PA concentration and root square transformed.

217 Except nested ANOVA tests, all analyses were performed with R version 3.1.2 (R Core Team

218 2015).

219 **Results**

220 **PA diversity**

221 Of the 21 PAs reported from *S. vulgaris* in the literature, 16 PAs were included in the mass

222 spectrometric method and detected in our samples (Figure 1). An additional eight putative PA N-

223 oxides, with unknown identity were detected, of which it could be ascertained, based on their

224 protonated molecular mass, fragmentation spectra and retention times, that they were different

225 from the 21 PAs reported previously (Table S1). In many cases, both forms of PAs (tertiary

226 amine and N-oxide) were detected in at least part of the samples. Exceptions were senecivernine

227 and usaramine N-oxide that were detected in a number of samples, but their counterparts

228 senecivernine N-oxide and usaramine were below the limit of detection in all samples. Similarly,

229 no tertiary amine counterparts of the 8 unknown PA N-oxides could be identified. Thus, in total

230 22 PAs were detected (Table 3).

231 Senecionine, integerrimine, seneciphylline, and their respective N-oxides were present in the

232 roots and shoots of all plants and all populations. Spartioidine, retrorsine and their respective N-

233 oxides were found in all populations and in more than 90% of the individual root and shoot

234 samples. Riddelliine N-oxide was detected in ten populations (83%), while senecivernine was

235 detected in five populations (42%). Two PAs, riddelliine and usaramine N-oxide, were rarely

236 found; usaramine N-oxide was only detected in the root and shoot extracts of one plant from a

237 native population located in Potsdam, Germany, while riddelliine was found in two plants

238 originating from Potsdam and in one plant from an invasive population in Shennongjia, China.

239 Six of the eight unidentified PA N-oxides were found in all populations, and three of them (unk

240 3-5) were found in more than 90% of the shoot and root samples (Table 3, Table S2-3).

**Variation in PA composition**

242 Overall PA diversity (H') as well as evenness (J') was higher in shoots than in roots,   and lower

243 in the invasive populations than in native ones (Figure 2). Differences in PA composition were

244 significant between organs (shoots and roots) and among populations (two factor Adonis test;

245 organ: df = 1, $r^2$ = 0.41, $p$ = 0.005; populations: df = 11, $r^2$ = 0.19, p = 0.005; Figure 3).

246 Senecionine N-oxide was the dominant component of the PA profile in the roots, followed by

247 integerrimine N-oxide, senecionine and seneciphylline N-oxide (Figure 4a). In the shoots, the

248 four above mentioned PAs were also prevalent, in combination with retrorsine N-oxide and two

249 unidentified PA N-oxides (unk 4 and 5, Figure 4b). The ratio between the concentration of

250 senecionine and that of seneciphylline (Sn/Sp ratio, including the free base and N-oxide forms of

251 these PAs) for individual plants ranged from 0.56 to 6.87; the ratio at population level was

252 greater than 1 and differed significantly between populations (ANOVA test: df = 11 and 43, F =

253 9.7, $P$ < 0.001, Figure 5).

254 Generally, the relative abundance of individual PAs was significantly different among

255 populations (Table 4). However, the clustering of the populations did not show any

256 geographically related pattern (Figure 6).

257 **Variation in PA concentration**

258 Within the plants, significantly higher concentrations of senecionine, integerrimine (and their N-

259 oxides), senecivernine and 2 unidentified PA N-oxides (unk 3 and 6) were present in the roots

260 than in the shoots, but the concentrations of spartioidine, riddelliine N-oxide and 5 unidentified

261 PA N-oxides (unk 2, 4, 5, 7 and 8) were significantly lower (Table 3). The concentrations of

262 seneciphylline, seneciphylline N-oxide, spartioidine N-oxide, retrorsine N-oxide and an

263 unidentified PA N-oxide (unk 1) tended to be higher in the shoots, but statistically the

264 differences were not significant. A significant correlation between roots and shoots was found

265 regarding the total PA concentration, as well as between most of the individual PAs, except for

266 some unidentified PA N-oxides (Unk 1, 2, 4. 5, Table 3).

267 The concentration of the individual PAs and that of total PA was generally higher in plants from

268 the native populations than in those from the invasive populations (Table S2-3). The difference

269 between populations was often significant. However, significant differences between the ranges

270 were only found for retrorsine and retrorsine N-oxide (Table 4). These two PAs were minor

271 compounds in the PA profile of plants from both ranges (Table 3).

272 **Discussion**

273 The great asset of LC-MS/MS is that it can analyse PA tertiary amine and N-oxide forms

274 simultaneously in a single run with high sensitivity and specificity in combination with minimal

275 sample clean-up. However, like other mass spectrometric techniques that have evolved in recent

276 years, such as LC-QToF-MS (Skoneczny et al., 2015) and LC-Orbitrap-MS (These et al., 2013),

277 it requires a comprehensive set of authentic analytical standards for a full quantitative result.

278 Furthermore, although most LC-MS techniques are capable of annotating tentative PAs –based

279 on their fragmentation spectra and or elementary composition– to establish the chemical

280 structure of the unknowns, additional techniques, such as NMR are required.

281 It has been reported that PA profiles of the aboveground parts of *S. vulgaris* plants comprise

282 seneciphylline, senecionine, retrorsine and the corresponding E-geometrical isomers, spartioidine,

283 integerrimine and usaramine (Hartmann & Zimmer, 1986; Pieters & Vlietinck, 1988). In *S.*

284 *vulgaris* PAs are primarily produced as N-oxides in the roots, which is also the dominant form of

285 PAs in the other parts of the plant (Hartmann & Dierich, 1998). Apart from the PAs mentioned

286 above, riddelliine, senecivernine, platyphylline and neoplatyphylline have been reported in the

287   aerial parts of *S. vulgaris* plants (von Borstel et al., 1989; Yang et al., 2011), as well as

288   neosenkirkine (von Borstel et al., 1989) and othonnine (Xiong et al., 2012). The 21 PAs with

289   identified structures detected from *S. vulgaris* plants in previous studies have been summarized

290   in Table S1 and structures of most of them were shown in Figure 1.

291   Of these 21 PAs reported previously, 16 PAs were included in the mass spectrometric method,

292   most of which were detected in this study. However, due to a lack of suitable reference standards,

293   we were unable to search for platyphylline, neoplatyphylline, neosenkirkine, or othonnine in the

294   root and shoot extracts of *S. vulgaris*. However, three unidentified PA N-oxides (Unk 3-5), with

295   the same molecular mass as retrorsine N-oxide and that could be structural isomers of the latter

296   were present in more than 90% of the samples. In particular, one PA N-oxide (Unk 5) comprised

297   about 10% of the total PA concentration in shoot samples, although a reliable quantification of

298   this compound due to lack of a standard could not be made. It would be worthwhile to elucidate

299   the structure of these three PAs and explore whether they are dominant in the PA profiles of

300   certain *S. vulgaris* plants.

301   Some studies have found either senecionine (Hartmann & Zimmer, 1986) or seneciphylline to be

302   dominant (Lüthy et al., 1983), while others have found both PAs to be dominant in *S. vulgaris*

303   (von Borstel et al., 1989; Brown & Molyneux, 1996). In this study, senecionine was generally

304   present in higher concentrations than seneciphylline.

305   The shoots and roots of *S. vulgaris* plants differed in that shoots showed more divergent PA

306   profiles and that the shoots had a lower total PA content than the roots. Although there were

307   significant differences in PA variation between the shoots and roots, these parts were positively

308   correlated regarding the concentrations of total PAs and most of individual PAs (Table 3). This

309   pattern could be explained by the processes of PA synthesis and accumulation in *S. vulgaris*

310   plants, as PAs are primarily produced as senecionine N-oxide in the roots, while structural

311   transformation mainly occurs in the shoots. Usually there is little turnover of PAs once being

312   produced and they translocate to plant tissues mainly via the phloem (Hartmann & Dierich,

313   1998). Similar patterns regarding differences and correlations of PAs between the roots and

314   shoots have been found in *J. vulgaris* (Cheng et al., 2011; Joosten et al., 2011).

315   Higher PA concentrations in the belowground parts compared with the aboveground parts of *S.*

316   *vulgaris* plants have been found in the vegetative stage. It has been reported that when the plants

317    have produced buds, the highest PA concentrations are found in the capitula, while the stems and

318    leaves generally contain lower total PA concentrations compared to the roots (Hartmann &

319    Zimmer, 1986). This consistent with our finding that the total PA concentration was lower in the

320    shoots than in the roots when the *S. vulgaris* plants were not yet flowering.

321    The indexes of PA diversity and evenness were somewhat lower for plants from invasive ranges

322    than those from the native range (Figure 2). This indicated that invasive *S. vulgaris* populations

323    tended to produce less diverse PA profiles than the native ones. However, this trend is much

324    weaker than observed in some other invasive species. For instance, native *J. vulgaris* populations

325    expressed four chemotypes (Macel et al., 2004), while in invasive *J. vulgaris* populations one

326    chemotype dominated (Joshi & Vrieling, 2005). PA diversity in *S. pterophorus* (native to South

327    Africa) was reduced after introduction in Europe and Australia (Castells et al., 2014).

328    Furthermore, invasive *Tanacetum vulgare* plants contained a smaller number of qualitative

329    defense compounds than the native ones (Wolf et al., 2011).

330    We also found that invasive *S. vulgaris* populations did not produce higher concentrations of

331    individual and of total PAs than native populations. These results did not agree with our

332    prediction deduced from the SDH. Some studies have found that PA levels of related species

333    significantly increased in the invaded range. For example, invasive populations of *S.*

334    *inaequidens*, *S. pterophorus* and *J. vulgaris* all showed a significantly higher total PA

335    concentration than their native conspecifics (Joshi & Vrieling, 2005; Caño et al., 2009; Castells

336    et al., 2014; Lin et al., 2015). Some other invasive species appear to have evolved towards

337    decreased chemical defense levels but they may have developed other compensatory

338    mechanisms that contribute to their invasion success. For instance, invasive genotypes of *Sapium*

339    *sebiferum* evolved a reduced defense and resistance ability, but were more tolerant and

340    outperformed the native genotypes under higher levels of herbivore attack (Zou et al., 2008).

341    The prerequisite of the EICA hypothesis and SDH is that invasive plants face a lower specialist

342    herbivore pressure in invasive ranges. We could confirm that *S. vulgaris* populations in China

343    were attacked by insect herbivores but we did not determine whether the insects were specialists

344    or not. Although it is likely that most insects will be generalists, it is not impossible that there

345    may be one or more specialists among them that have adapted to *S. vulgaris*, since *S. vulgaris*

346    has a long invasive history and more than 60 congeneric species have been identified in China

347    (Chen, 1999). Since there are significant variations between populations, a good revisiting study

348 on the EICA hypothesis and SDH needs enough populations for a robust statistical analysis, and
349 it is also important to describe and cluster invasive populations by analysis of their genetic
350 structure in the different ranges; otherwise it remains difficult to determine whether the
351 differences between native and invasive populations are the result of evolution or of pre-adaption
352 (Pan et al., 2013; Turner et al., 2014; Siemann et al., 2016; Schrieber et al., 2016).

353 Taking into account the high PA levels present in *S. vulgaris* and the toxic effect that PAs exert
354 on most herbivores, it reasonable to assume that PAs play an important role in the chemical
355 defense of *S. vulgaris*. However, there are also other metabolites that can function as chemical
356 defense in *S. vulgaris*. For instance, an oplopane sesquiterpene and jacaranone were identified
357 from *S. vulgaris* (Liu et al., 2010). Both compounds (or similar compounds) have a negative
358 effect on insect feeding (Lajide et al., 1996; Reina et al., 2001; Xu et al., 2003). It will be
359 interesting to investigate whether the levels of other qualitative defense compounds such as
360 oplopane sesquiterpenes and jacaranone are higher in invasive *S. vulgaris* populations than in
361 native ones, as the SDH would predict. It may be advantageous to use a non-targeted analysis
362 approach to explore for metabolites of potential significance, as was recently shown in the study
363 of Skoneczny et al. (2017).

364 **Conclusions**

365 As the *Senecio vulgaris* plants from native and invasive ranges were grown under identical
366 conditions, the differences in PA concentration and PA composition between ranges and between
367 populations might thus be explained by their genetic variation. In our study the invasive *S.*
368 *vulgaris* populations had slightly less diverse PA profiles and tended to have lower
369 concentrations of individual PAs compared to the native populations. This finding is in contrast
370 to the predictions of the SDH. However, the current findings should also be treated with caution
371 given the limited number of populations sampled, the lack of background information about
372 herbivore guilds feeding on *S. vulgaris* and the limited knowledge on the genetic structure of *S.*
373 *vulgaris* populations in the different ranges. Future studies should focus on sampling a larger
374 number of populations and screening for a wider array of plant metabolites in order to address
375 these questions.

376

377 **Acknowledgements**

378 We are very thankful to K Vrieling, T de Jong, E.Castells, H Müller‐Schärer, J Joshi, R Abbott,
379 G Korbecka, X Wei and X Yang for seed collecting. J Li, L Feng, Y Shi and X Deng are thanked
380 for their help in plant rearing and harvesting.

381

382 **References**

383 **Barlow VM, Godfrey LD, Norris RF**. 1999. Population dynamics of *Lygus hesperus*
384 (Heteroptera: Miridae) on selected weeds in comparison with alfalfa. *Journal of Economic*
385 *Entomology* 92:846-852.

386 **Blossey B, and Notzold R. 1995**. Evolution of increased competitive ability in invasive
387 nonindigenous plants: a hypothesis. *Journal of Ecology* 83:887-889.

388 **Brown MS, and Molyneux RJ. 1996**. Effects of water and mineral nutrient deficiencies on
389 pyrrolizidine alkaloid content of *Senecio vulgaris* flowers. *Journal of the Science of Food*
390 *and Agriculture* 70:209-211.

391 **Caño L, Escarre J, Vrieling K, and Sans FX. 2009**. Palatability to a generalist herbivore,
392 defence and growth of invasive and native Senecio species: testing the evolution of
393 increased competitive ability hypothesis. *Oecologia* 159:95-106.

394 **Castells E, Mulder PP, and Perez-Trujillo M. 2014**. Diversity of pyrrolizidine alkaloids in
395 native and invasive Senecio pterophorus (Asteraceae): implications for toxicity.
396 *Phytochemistry* 108:137-146.

397 **Cates RG. 1980**. Feeding patterns of monophagous, oligophagous, and polyphagous insect
398 herbivores: the effect of resource abundance and plant chemistry. *Oecologia* 46:22-31.

399 **Catford JA, Jansson R, and Nilsson C. 2009**. Reducing redundancy in invasion ecology by
400 integrating hypotheses into a single theoretical framework. *Diversity and Distributions*
401 15:22-40.

402 **Cheng D, Kirk H, Mulder PPJ, Vrieling K, and Klinkhamer PGL. 2011**. Pyrrolizidine

403     alkaloid variation in shoots and roots of segregating hybrids between *Jacobaea vulgaris* and

404     *Jacobaea aquatica*. *New Phytologist* 192:1010-1023.

405 **Chen Y**. 1999. *Asteraceae: Flora Reipublicae Popularis Sinicae (in Chinese)*. 77(1):301.

406     Beijing: Science Press.

407 **Doorduin LJ, and Vrieling K. 2011**. A review of the phytochemical support for the shifting

408     defence hypothesis. *Phytochemistry Reviews* 10:99-106.

409 **Feeny P. 1976**. Plant Apparency and Chemical Defense. *Recent Advances in Phytochemistry*

410     10:1-40.

411 **Frantzen J, and Hatcher P**. 1997. A fresh view on the control of the annual plant *Senecio*

412     *vulgaris*. *Integrated Pest Management Reviews* 2:77-85.

413 **Goslee SC, and Urban DL. 2007**. The ecodist package for dissimilarity-based analysis of

414     ecological data. *Journal of Statistical Software* 22:1-19.

415 **Handley RJ, Steinger T, Treier UA, and Mueller-Schaerer H. 2008**. Testing the evolution of

416     increased competitive ability (EICA) hypothesis in a novel framework. *Ecology* 89:407-

417     417.

418 **Hartmann T, and Dierich B. 1998**. Chemical diversity and variation of pyrrolizidine alkaloids

419     of the senecionine type: biological need or coincidence? *Planta* 206:443-451.

420 **Hartmann T, and Zimmer M. 1986**. Organ-specific distribution and accumulation of

421     pyrrolizidine alkaloids during the life history of two annual *Senecio* species. *Journal of*

422     *Plant Physiology* 122:67-80.

423 **Joosten L, Mulder PPJ, Vrieling K, van Veen J, and Klinkhamer PGL. 2010.** The analysis

424     of pyrrolizidine alkaloids in *Jacobaea vulgaris*; a comparison of extraction and detection

425     methods. *Phytochemical Analysis*, 21:197-204.

426 **Joosten L, Cheng D, Mulder PPJ, Vrieling K, van Veen J, and Klinkhamer PGL. 2011**. The

427     genotype dependent presence of pyrrolizidine alkaloids as tertiary amine in *Jacobaea*

428     *vulgaris*. *Phytochemistry* 72:214–222.

429  **Joosten L, and van Veen JA. 2011**. Defensive properties of pyrrolizidine alkaloids against

430     microorganisms. *Phytochemistry Reviews* 10:127-136.

431  **Joshi J, and Vrieling K. 2005**. The enemy release and EICA hypothesis revisited: incorporating

432     the fundamental difference between specialist and generalist herbivores. *Ecollogy Letters*

433     8:704-714.

434  **Kadereit JW. 1984**. The origin of *Senecio vulgaris* (Asteraceae). *Plant Systematics and*

435     *Evolution* 145:135-153.

436  **Keane RM, and Crawley MJ. 2002**. Exotic plant invasions and the enemy release hypothesis.

437     *Trends in Ecology & Evolution* 17:164-170.

438  **Kleine S, and Mülller C. 2010**. Intraspecific plant chemical diversity and its relation to

439     herbivory. *Oecologia* 166:175-186.

440  **Kolde R. 2015.** Pheatmap: Pretty Heatmaps. R package version 3.3.3. https://cran.r-

441     project.org/web/packages/pheatmap/

442  **Lajide L, Escoubas P, and Mizutani J**. 1996. Cyclohexadienones-insect growth inhibitors from

443     the foliar surface and tissue extracts of *Senecio cannabifolius*. *Cellular & Molecular Life*

444     *Sciences CMLS* 52:259-263.

445  **Li Z, and Xie Y. 2002**. *Invasive Alien Species in China (in Chinese)*. Beijing: China Forestry

446     Publishing House.

447  **Lin T, Klinkhamer PGL, and Vrieling K. 2015**. Parallel evolution in an invasive plant: effect

448     of herbivores on competitive ability and regrowth of *Jacobaea vulgaris*. *Ecology Letters*

449     18:668-676.

450  **Liu Y, Zhang Z, and Wang Y. 2010.** Chemical constituents in *Senecio vulgaris* (in Chinese

451     with English abstract). *Chinese Traditional and Herbal Drugs*, 41: 1608-1612

452  **Lüthy J, Heim T, and Schlatter C. 1983**. Transfer of [3H] pyrrolizidine alkaloids from *Senecio*

453     *vulgaris* L. and metabolites into rat milk and tissues. *Toxicology Letters* 17:283-288.

454 **Macel M, Bruinsma M, Dijkstra SM, Ooijendijk T, Niemeyer HM, and Klinkhamer PGL.**
455     **2005**. Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore
456     species. *Journal of Chemical Ecology* 31:1493-1508.

457 **Macel M, Vrieling K, and Klinkhamer PGL. 2004**. Variation in pyrrolizidine alkaloid patterns
458     of *Senecio jacobaea*. *Phytochemistry* 65:865-873.

459 **Minkenberg O, Lenteren JC**. 1986. The leafminers, *Liriomyza bryoniae* and *L. trifolii* (Diptera:
460     Agromyzidae), their parasites and host plants: a review. *Agricultural University*
461     *Wageningen Papers* 86:1-50.

462 **Müller-Scharer H, Schaffner U, and Steinger T. 2004**. Evolution in invasive plants:
463     implications for biological control. *Trends in Ecology & Evolution* 19:417-422.

464 **Pan XY, Jia X, Fu DJ, and Li B**. **2013.** Geographical diversification of growth-defense
465     strategies in an invasive plant. *Journal of Systematics and Evolution* 51:308-317.

466 **Pieters LA, and Vlietinck AJ. 1988**. Spartioidine and Usaramine, Two Pyrrolizidine Alkaloids
467     from *Senecio vulgaris*. *Planta Medica* 54:178-179.

468 **R Core Team**. 2015. R: A language and environment for statistical computing. R Foundation for
469     Statistical Computing. Vienna, Austria.

470 **Reina M, González-coloma A, Gutiérrez C, Cabrera R, Rodríguez ML, Fajardo V, and**
471     **Luis V**. 2001. Defensive Chemistry of *Senecio miser*. *Journal of Natural Products* 64:6-11.

472 **Rhoades DF, and Cates RG. 1976**. Toward a General Theory of Plant Antiherbivore
473     Chemistry. *Recent Advances in Phytochemistry* 10:168-213.

474 **Robinson DE, O'Donovan JT, Sharma MP, Doohan DJ, and Figueroa R. 2003**. The biology
475     of Canadian weeds. 123. *Senecio vulgaris* L. *Canadian Journal of Plant Science* 83:629-
476     644.

477 **Schrieber K, Wolf S, Wypior C, Höhlig D, Hensen I, and Lachmuth S**. **2016.** Adaptive and
478     non-adaptive evolution of trait means and genetic trait correlations for herbivory resistance
479     and performance in an invasive plant. *Oikos* doi: 10.1111/oik.03781.

480   **Siemann E, Dewalt SJ, Zou J, and Rogers WE**. 2016. An experimental test of the EICA
481        Hypothesis in multiple ranges: invasive populations outperform those from the native range
482        independent of insect herbivore suppression. *AoB Plants* 9:plw087.

483   **Simpson GL, Solymos P, Stevens M, and Wagner H. 2009**. Vegan: community ecology
484        package. *Time International* 1997:15–17.

485   **Skoneczny D, Weston P A, Zhu X C, Gurr GM, Callaway RM, and Weston LA. 2015.**
486        Metabolic profiling of pyrrolizidine alkaloids in foliage of two *Echium* spp. invaders in
487        Australia - a case of novel weapons? *International Journal of Molecular Sciences*.
488        16:26721-26737.

489   **Skoneczny D, Weston P A, Zhu X, Gurr GM, Callaway RM, Barrow RA and Weston LA.**
490        **2017.** Metabolic Profiling and Identification of Shikonins in Root Periderm of Two Invasive
491        *Echium* spp. Weeds in Australia. *Molecules*, 22:330-348.

492   **Stegelmeier BL, Edgar JA, Colegate SM, Gardner DR, Schoch TK, Coulombe RA,**
493        **Molyneux RJ.** 1999. Pyrrolizidine Alkaloid Plants, Metabolism and Toxicity. *Journal of*
494        *Natural Toxins*, 8:95.

495   **These A, Bodi D, Ronczka S, Lahrssen-Wiederholt M, Preiss-Weigert A. 2013.** Structural
496        screening by multiple reaction monitoring as a new approach for tandem mass
497        spectrometry: presented for the determination of pyrrolizidine alkaloids in plants. *Analytical*
498        *Bioanalytical Chemistry*. 405:9375–9383

499   **Trigo JR. 2011**. Effects of pyrrolizidine alkaloids through different trophic levels.
500        *Phytochemistry Reviews* 10:83-98.

501   **Turner KG, Hufbauer RA, and Rieseberg LH**. 2014. Rapid evolution of an invasive weed.
502        *New Phytologist* 202:309-321.

503   **van Dam NM, Vuister LWM, Bergshoeff C, de Vos H, and van der Meijden ED. 1995**. The
504        'raison d'etre' of pyrrolizidine alkaloids in *Cynoglossum officinale* Deterrent effects against
505        generalist herbivores. *Journal of Chemical Ecology* 21:507-523.

506 **van der Meijden E. 1996**. Plant defence, an evolutionary dilemma: Contrasting effects of

507 (specialist and generalist) herbivores and natural enemies. *Entomologia Experimentalis et*

508 *Applicata* 80:307-310.

509 **Vilà M, and Hulme PE. 2017.** *Impact of biological invasions on ecosystem services*: Springer

510 International Publishing.

511 **von Borstel K, Witte L, and Hartmann T. 1989**. Pyrrolizidine alkaloid patterns in populations

512 of *Senecio vulgaris*, *Senecio vernalis* and their hybrids. *Phytochemistry* 28:1635-1638.

513 **Wolf VC, Berger U, Gassmann A, and Müller C. 2011**. High chemical diversity of a plant

514 species is accompanied by increased chemical defence in invasive populations. *Biological*

515 *Invasions* 13:2091-2102.

516 **Xiong A, Yang L, Ji L, Wang Z, Yang X, Chen Y, Wang X, Wang C, and Wang Z. 2012**.

517 UPLC-MS based metabolomics study on *Senecio scandens* and *S. vulgaris*: an approach for

518 the differentiation of two *Senecio* herbs with similar morphology but different toxicity.

519 *Metabolomics* 8:614-623.

520 **Xu H, Qiang S, Genovesi P, Ding H, Wu J, Meng L, Han Z, Miao J, Hu B, and Guo J. 2012**.

521 An inventory of invasive alien species in China. *NeoBiota* 15:1-26.

522 **Xu H, Zhang N, and Casida JE**. **2003.** Insecticides in Chinese medicinal plants: survey leading

523 to jacaranone, a neurotoxicant and glutathione-reactive quinol. *Journal of Agricultural and*

524 *Food Chemistry* 51:2544-2547.

525 **Yang X, Yang L, Xiong A, Li D, and Wang Z. 2011**. Authentication of Senecio scandens and

526 S. vulgaris based on the comprehensive secondary metabolic patterns gained by UPLC-

527 DAD/ESI-MS. *Journal of pharmaceutical and Biomedical Analysis* 56:165-172.

528 **Zhu BR, Barrett SCH, Zhang D Y, and Liao WJ. 2017.** Invasion genetics of senecio vulgaris :

529 loss of genetic diversity characterizes the invasion of a selfing annual, despite multiple

530 introductions. *Biological Invasions*, 19:255-267.

531 **Zou J, Siemann E, Rogers WE, and DeWalt SJ. 2008**. Decreased resistance and increased

532   tolerance to native herbivores of the invasive plant *Sapium sebiferum*. *Ecography* 31:663-

533   671.

Sites of origin of native and invasive populations of *Senecio vulgaris.*

| Range | Population code | Location | Coordinates | |
|---|---|---|---|---|
| Native | Barcelona | Barcelona, Spain | Lat 41.67 | Long 2.73 |
| | Pulawy | Puławy, Poland | Lat 51.40 | Long 21.96 |
| | St. Andrews | St. Andrews, UK | Lat 56.33 | Long -2.78 |
| | Fribourg | Fribourg, Switzerland | Lat 46.79 | Long 7.15 |
| | Obidos | Óbidos, Portugal | Lat 39.36 | Long -9.16 |
| | Potsdam | Potsdam, Germany | Lat 52.40 | Long 13.07 |
| Invasive | Slj.djh | Dajiuhu,Shennongjia, China | Lat 31.49 | Long 109.99 |
| | Dl.hsj | Heishijiao, Dalian, China | Lat 38.87 | Long 121.56 |
| | Lj.lsh | Lashihai, Lijiang, China | Lat 26.9 | Long 100.14 |
| | Slj.myz | Muyuzhen, Shennongjia, China | Lat 31.46 | Long 110.40 |
| | Lj.xyl | Xianyulu, Lijiang, China | Lat 26.87 | Long 100.24 |
| | Dali.sts | Santasi, Dali, China | Lat 26.70 | Long 100.15 |

LC-MS/MS analytical settings used for detection and quantification of pyrrolizidine alkaloids (PAs).

\* Y = standard available, N = standard not available.

| No. | Pyrrolizidine alkaloid | Code | Retention time (min) | Precursor mass (m/z) | Fragment Mass | Collision energy | Standard available [*] | PA used for (semi) quantification |
|-----|------------------------|------|----------------------|----------------------|---------------|------------------|------------------------|-----------------------------------|
| 1 | senecionine | Sn | 9.54 | 336.2 | 94.0; 120.0 | 40; 30 | Y | senecionine |
| 2 | senecionine N-oxide | Sn.ox | 6.68 | 352.2 | 94.0; 120.0 | 40; 30 | Y | senecionine N-oxide |
| 3 | integerrimine | Ir | 9.35 | 336.2 | 94.0; 120.0 | 40; 30 | Y | integerrimine |
| 4 | integerrimine N-oxide | Ir.ox | 6.55 | 352.2 | 94.0; 120.0 | 40; 30 | Y | integerrimine N-oxide |
| 5 | senecivernine | Sv | 9.79 | 336.2 | 94.0; 120.0 | 40; 30 | N | integerrimine |
| 6 | senecivernine N-oxide | Sv.ox | 6.75 | 352.2 | 94.0; 120.0 | 40; 30 | N | integerrimine N-oxide |
| 7 | retrorsine | Rt | 8.19 | 352.2 | 94.0; 120.0 | 40; 30 | Y | retrorsine |
| 8 | retrorsine N-oxide | Rt.ox | 5.74 | 368.2 | 94.0; 120.0 | 40; 30 | Y | retrorsine N-oxide |
| 9 | usaramine | Us | 7.98 | 352.2 | 94.0; 120.0 | 40; 30 | N | retrorsine |
| 10 | usaramine N-oxide | Us.ox | 5.62 | 368.2 | 94.0; 120.0 | 40; 30 | N | retrorsine N-oxide |
| 11 | seneciphylline | Sp | 8.76 | 334.2 | 94.0; 120.0 | 40; 30 | Y | seneciphylline |
| 12 | seneciphylline N-oxide | Sp.ox | 6.07 | 350.2 | 94.0; 138.0 | 40; 30 | Y | seneciphylline N-oxide |
| 13 | spartioidine | St | 8.58 | 334.2 | 120.0; 138.0 | 30; 30 | N | seneciphylline |
| 14 | spartioidine N-oxide | St.ox | 6.01 | 350.2 | 94.0; 138.0 | 40; 30 | N | seneciphylline N-oxide |
| 15 | riddelliine | Rd | 7.58 | 350.2 | 94.0; 138.0 | 40; 30 | Y | riddelliine |
| 16 | riddelliine N-oxide | Rd.ox | 5.20 | 366.2 | 94.0; 118.0 | 40; 30 | Y | riddelliine N-oxide |
| 17 | unknown N-oxide 1 | Unk1 | 4.78 | 366.2 | 94.0; 118.0 | 40; 30 | N | riddelliine N-oxide |
| 18 | unknown N-oxide 2 | Unk2 | 4.84 | 366.2 | 94.0; 118.0 | 40; 30 | N | riddelliine N-oxide |
| 19 | unknown N-oxide 3 | Unk3 | 4.88 | 368.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |
| 20 | unknown N-oxide 4 | Unk4 | 5.55 | 368.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |
| 21 | unknown N-oxide 5 | Unk5 | 5.78 | 368.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |
| 22 | unknown N-oxide 6 | Unk6 | 6.22 | 370.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |
| 23 | unknown N-oxide 7 | Unk7 | 6.57 | 402.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |
| 24 | unknown N-oxide 8 | Unk8 | 6.82 | 402.2 | 94.0; 138.0 | 40; 30 | N | retrorsine N-oxide |

Pyrrolizidine alkaloids (PAs) variation in roots and shoots of *Senecio vulgaris* plants from native and invasive populations and grown under greenhouse conditions.

[1] Presence percentage = number of root/ shoot samples from which a certain individual PA was detected /number of total root/shoot sample) × 100 (%).

[2] Unit of concentration: µg/g dry weight. For the PA N-oxides with unknown identity (entries 17-24) the concentrations are estimates, based on comparison of the peak area with that of riddellliine N-oxide (entries 17 and 18) or retrorsine N-oxide (entries 19-24).

[3] Difference of concentration of total PA and the individual PAs between roots and shoots was investigated by paired Wilcoxon rank tests and P-values of the tests are shown.

[4] Correlation between roots and shoots in relation to concentration of total PA and the individual PA was investigated by Spearman rank correlation tests; R and P-values of the tests are shown.

[5] < LOD: All samples below the limit of detection (0.1 µg/g dry weight).

Level of significance: *p < 0.05, **p < 0.01, ***p < 0.001.

| No | Pyrrolidine alkaloid | Code | PAs in roots | | | | PAs in shoots | | | | Between roots and shoots | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Presence (%) [1] | Mean conc. [2] | Min conc. | Max conc. | Presence (%) | Mean conc. | Min conc. | Max conc. | Difference [3] (df = 1, 58) | Correlation [4] (df = 1, 58) |
| 1 | senecionine | Sn | 100.0 | 129.1 | 2.8 | 397.6 | 100.0 | 30.9 | 1.2 | 84.7 | *** | 0.65*** |
| 2 | senecionine N-oxide | Sn.ox | 100.0 | 1049.0 | 5.7 | 2675.2 | 100.0 | 293.9 | 2.9 | 1231.7 | *** | 0.57*** |
| 3 | integerrimine | Ir | 100.0 | 22.6 | 0.7 | 65.9 | 100.0 | 5.0 | 0.1 | 16.6 | *** | 0.68*** |
| 4 | integerrimine N-oxide | Ir.ox | 100.0 | 248.1 | 1.7 | 998.6 | 98.3 | 59.2 | <LOD | 242.2 | *** | 0.63*** |
| 5 | senecivernine | Sv | 30.5 | 1.7 | <LOD | 18.0 | 18.6 | 0.4 | <LOD | 3.0 | ** | 0.68*** |
| 6 | senecivernine N-oxide | Sv.ox | <LOD[5] | | | | | | | | | |
| 7 | retrorsine | Rt | 94.9 | 2.5 | <LOD | 35.9 | 88.1 | 2.9 | <LOD | 63.2 | ns | 0.72*** |
| 8 | retrorsine N-oxide | Rt.ox | 96.6 | 20.6 | <LOD | 208.8 | 94.9 | 31.6 | <LOD | 582.4 | ns | 0.45** |
| 9 | usaramine | Us | <LOD | | | | | | | | | |
| 10 | usaramine N-oxide | Us.ox | 1.7 | 0.1 | <LOD | 3.4 | 1.7 | 0.2 | <LOD | 12.6 | | |
| 11 | seneciphylline | Sp | 100.0 | 11.5 | 0.4 | 63.6 | 100.0 | 17.1 | 0.3 | 83.5 | ns | 0.62*** |
| 12 | seneciphylline N-oxide | Sp.ox | 100.0 | 92.3 | 0.9 | 376.1 | 100.0 | 161.5 | 1.3 | 1020.1 | ns | 0.50*** |
| 13 | spartioidine | St | 93.2 | 1.8 | <LOD | 6.3 | 89.8 | 2.9 | <LOD | 17.5 | ** | 0.66*** |
| 14 | spartioidine N-oxide | St.ox | 98.3 | 17.3 | <LOD | 57.0 | 100.0 | 29.8 | 0.4 | 212.0 | ns | 0.64*** |
| 15 | riddelliine | Rd | 5.1 | 0.1 | <LOD | 3.4 | 1.7 | 0.1 | <LOD | 5.2 | | |
| 16 | riddelliine N-oxide | Rd.ox | 45.8 | 0.9 | <LOD | 14.4 | 57.6 | 1.8 | <LOD | 46.1 | * | 0.48*** |
| 17 | unknown N-oxide 1 | Unk1 | 32.2 | 0.3 | <LOD | 2.6 | 35.6 | 1.0 | <LOD | 13.5 | ns | 0.29 ns |
| 18 | unknown N-oxide 2 | Unk2 | 61.0 | 1.0 | <LOD | 7.8 | 74.6 | 3.5 | <LOD | 32.1 | ** | 0.28 ns |
| 19 | unknown N-oxide 3 | Unk3 | 96.6 | 9.3 | <LOD | 20.7 | 76.3 | 1.6 | <LOD | 6.7 | *** | 0.48*** |
| 20 | unknown N-oxide 4 | Unk4 | 98.3 | 8.5 | <LOD | 27.5 | 100.0 | 30.6 | 0.7 | 148.2 | *** | 0.27 ns |
| 21 | unknown N-oxide 5 | Unk5 | 94.9 | 18.7 | <LOD | 114.3 | 98.3 | 69.2 | <LOD | 259.1 | *** | 0.14 ns |
| 22 | unknown N-oxide 6 | Unk6 | 88.1 | 4.5 | <LOD | 11.2 | 84.8 | 3.2 | <LOD | 19.8 | ** | 0.36* |
| 23 | unknown N-oxide 7 | Unk7 | 44.1 | 0.6 | <LOD | 5.6 | 81.4 | 4.4 | <LOD | 33.1 | *** | 0.58*** |
| 24 | unknown N-oxide 8 | Unk8 | 74.6 | 1.5 | <LOD | 9.1 | 86.4 | 8.3 | <LOD | 37.1 | *** | 0.53*** |
| | Total PA | | | 1641.8 | 18.4 | 4180.6 | | 758.8 | 16.3 | 2781.3 | *** | 0.58*** |

Results of the nested ANOVA tests of difference among *Senecio vulgaris* populations and ranges (native or invasive) for 13 selected pyrrolizidine alkaloids (PAs).

[a] Nested ANVOA tests were conducted separately for each individual PA (or total PA concentration) from root and shoot samples. Concentration or relative abundance of PAs were used as independent variable, population nested in ranges (df = 10) and range (df = 1) as fixed factors. In total 59 individual plants were used, and they were from 6 native and 6 invasive populations. The relative abundance of the 13 selected PAs was at least 1%, averaged among all samples.

[b] Concentration of PAs was calculated as µg/g dry weight and log transformed for the tests.

[c] Relative abundance of PAs was calculated as individual PA percentage of total PA concentration and root square transformed for the tests.

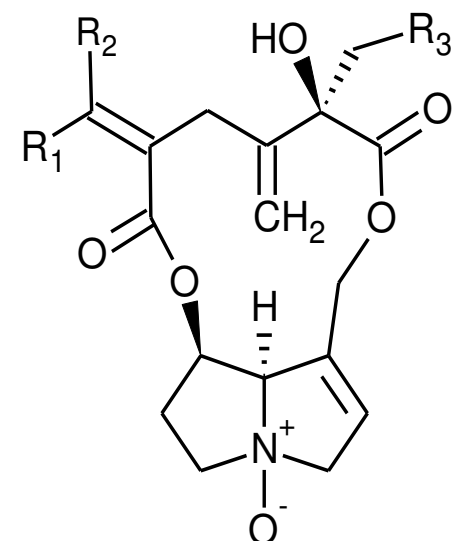
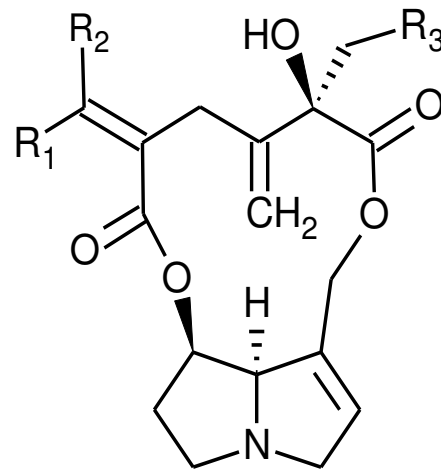
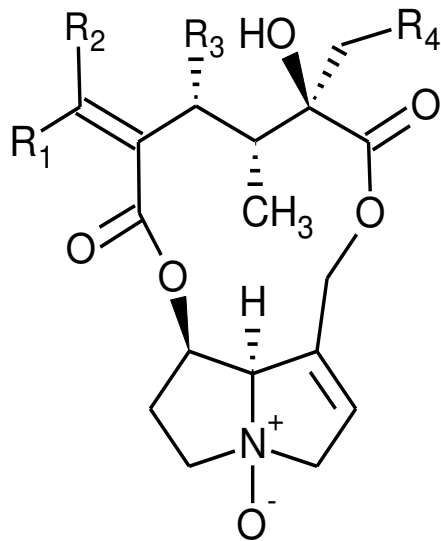
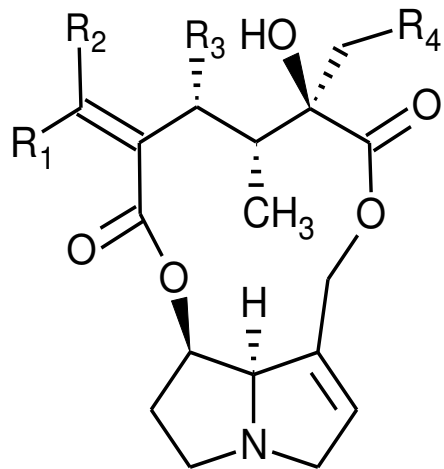
Level of significance: [ns] P > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.

Concentration of PAs [a, b]

| PA code | Root | | Shoot | |
|---|---|---|---|---|
| | Population (Range) | Range | Population (Range) | Range |
| Sn | 1.24 ns | 0.17 ns | 1.12 ns | 0.37 ns |
| Sn.ox | 2.23* | <0.00 ns | 1.82 ns | 0.33 ns |
| Ir | 0.12 ns | 1.48 ns | 1.34 ns | 0.29 ns |
| Ir.ox | 2.23* | 0.13 ns | 2.59* | 0.69 ns |
| Rt | 2.98** | 13.05** | 3.26** | 12.98*** |
| Rt.ox | 3.22** | 14.15*** | 2.30** | 14.2*** |
| Sp | 1.18 ns | 0.59 ns | 0.81 ns | 1.49 ns |
| Sp.ox | 2.23 ns | 1.26* | 1.62 ns | 1.37 ns |
| St | 2.21* | 2.49 ns | 2.30* | 3.03 ns |
| St.ox | 3.14** | 2.87 ns | 3.13** | 3.32 ns |
| Unk3 | 1.82 ns | 0.42 ns | 2.01 ns | 2.40 ns |
| Unk4 | 3.12** | 2.39 ns | 4.02** | 0.49 ns |
| Unk5 | 3.02** | 1.20 ns | 2.00 ns | 0.10 ns |
| Total PA concentration | 2.05* | 0.11 ns | 1.81 ns | 1.48 ns |

Relative abundance of PAs [a, c]

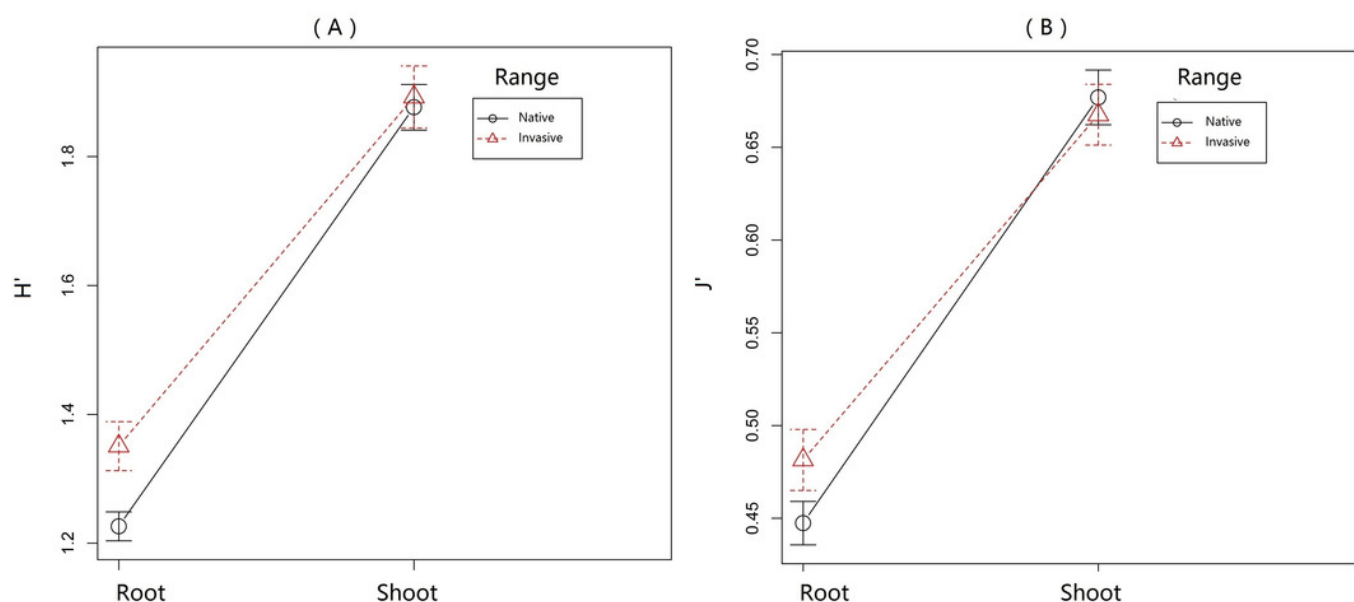
| PA code | Root | | Shoot | |
|---|---|---|---|---|
| | Population (Range) | Range | Population (Range) | Range |
| Sn | 0.35 ns | 1.97 ns | 2.12* | 0.004 ns |
| Sn.ox | 2.68* | 6.67* | 2.10* | 1.64 ns |
| Ir | 2.56* | 0.16 ns | 1.46 ns | 0.51 ns |
| Ir.ox | 6.25** | 0.09 ns | 2.95** | 0.004 ns |
| Rt | 2.21** | 7.96** | 3.66** | 2.39 ns |
| Rt.ox | 3.33 ns | 9.99** | 5.44*** | 3.27 ns |
| Sp | 1.01* | 0.76 ns | 1.60 ns | 0.02 ns |
| Sp.ox | 6.67 ns | 2.83** | 2.27* | 1.06 ns |
| St | 1.74*** | 1.25 ns | 2.83** | 0.57 ns |
| St.ox | 6.34 ns | 15.51** | 10.93*** | 5.09 ns |
| Unk3 | 0.73*** | 0.49 ns | 0.56 ns | 0.68 ns |
| Unk4 | 4.13*** | 1.78 ns | 2.45* | 2.67 ns |
| Unk5 | 3.22** | 1.39 ns | 2.05* | 5.48 ns |

Chemical structures of pyrrolizidine alkaloids and their corresponding N-oxides identified in *Senecio vulgaris* plants.

|  | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Senecionine | $CH_3$ | H | H | H |
| Integerrimine | H | $CH_3$ | H | H |
| Senecivernine | H | H | $CH_3$ | H |
| Retrorsine | $CH_3$ | H | H | OH |
| Usaramine | H | CH | H | OH |

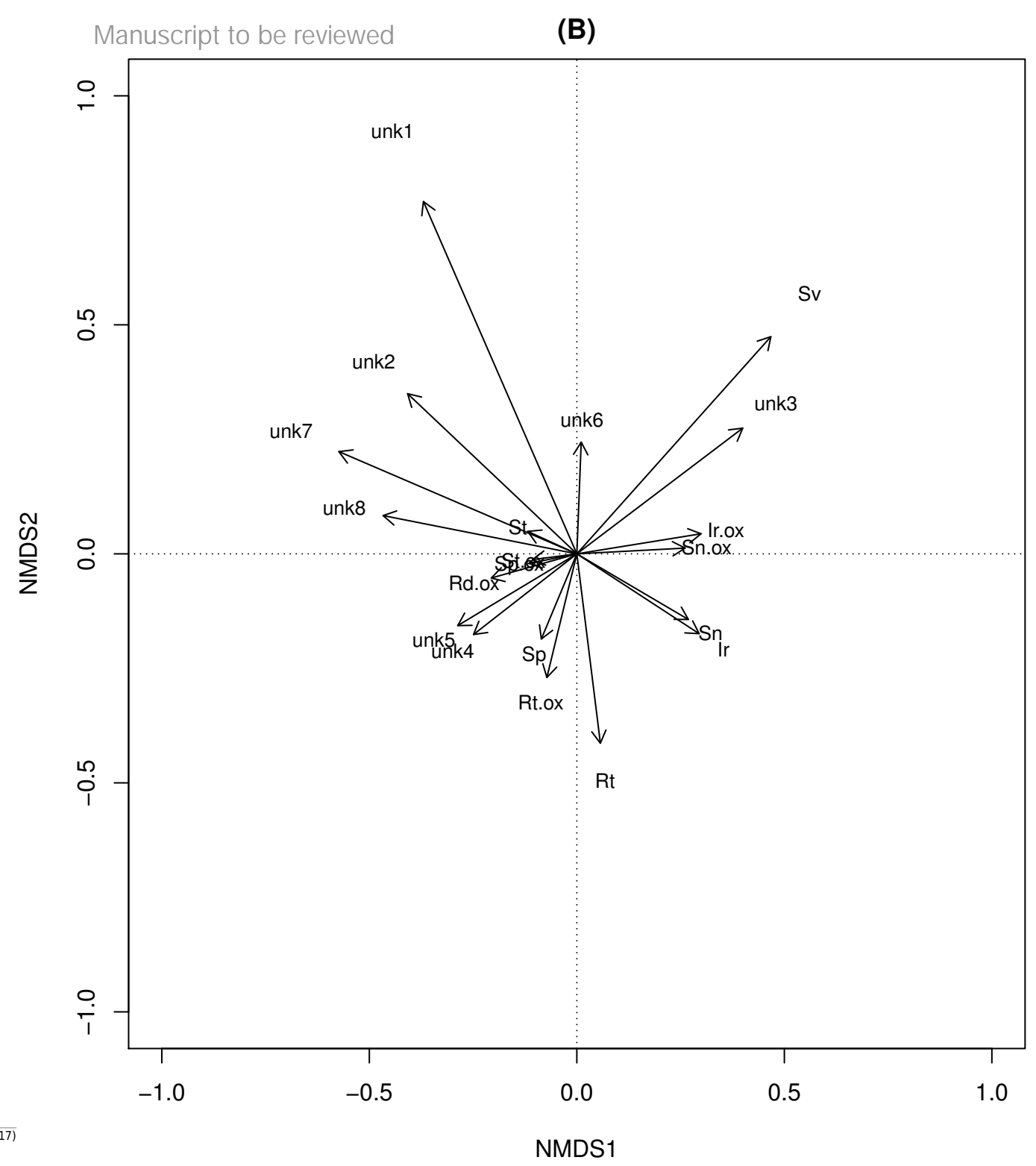
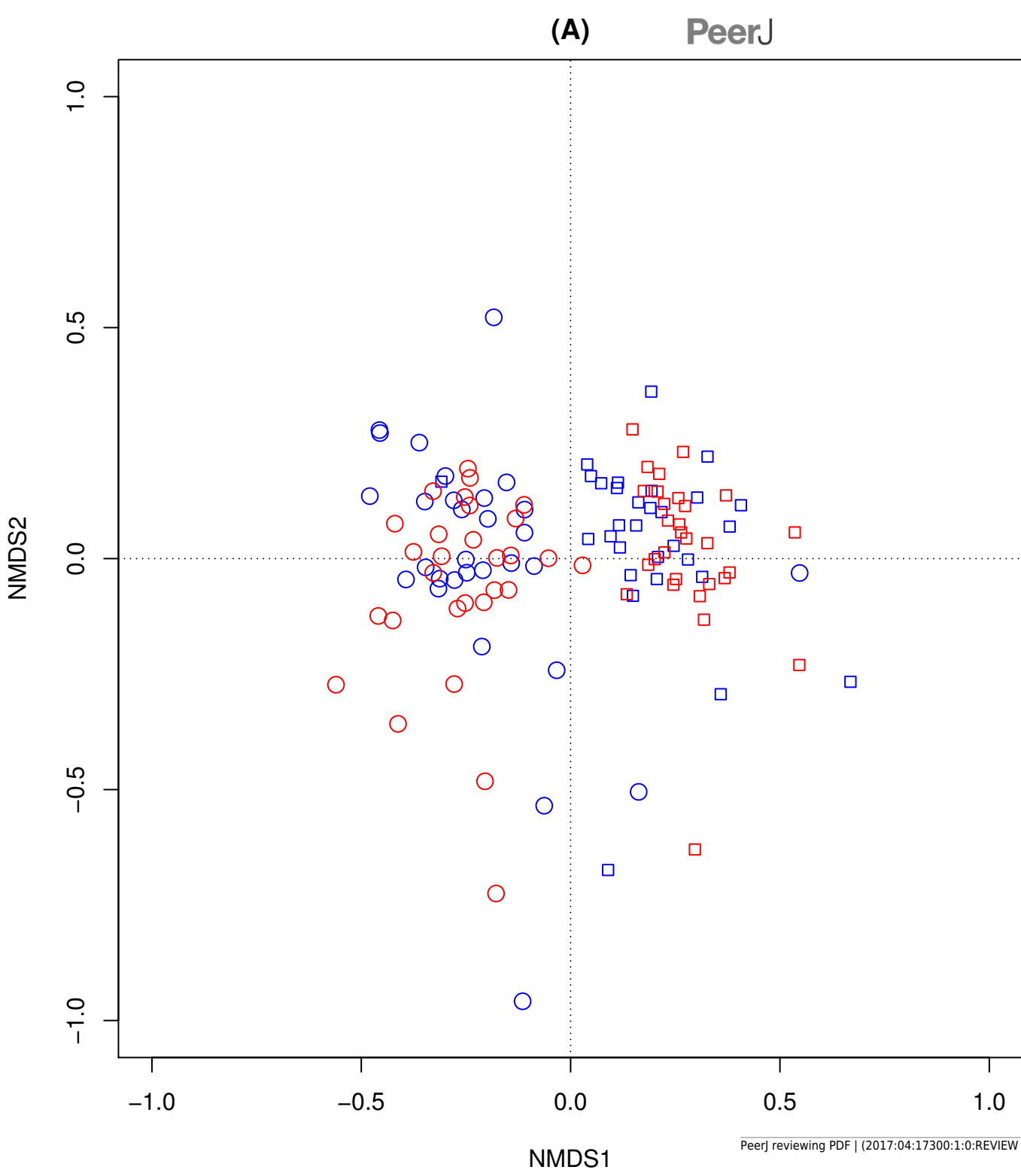
|  | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Seneciphylline | $CH_3$ | H | H |
| Spartioidine | H | $CH_3$ | H |
| Riddelliine | H | H | OH |

# Figure 2

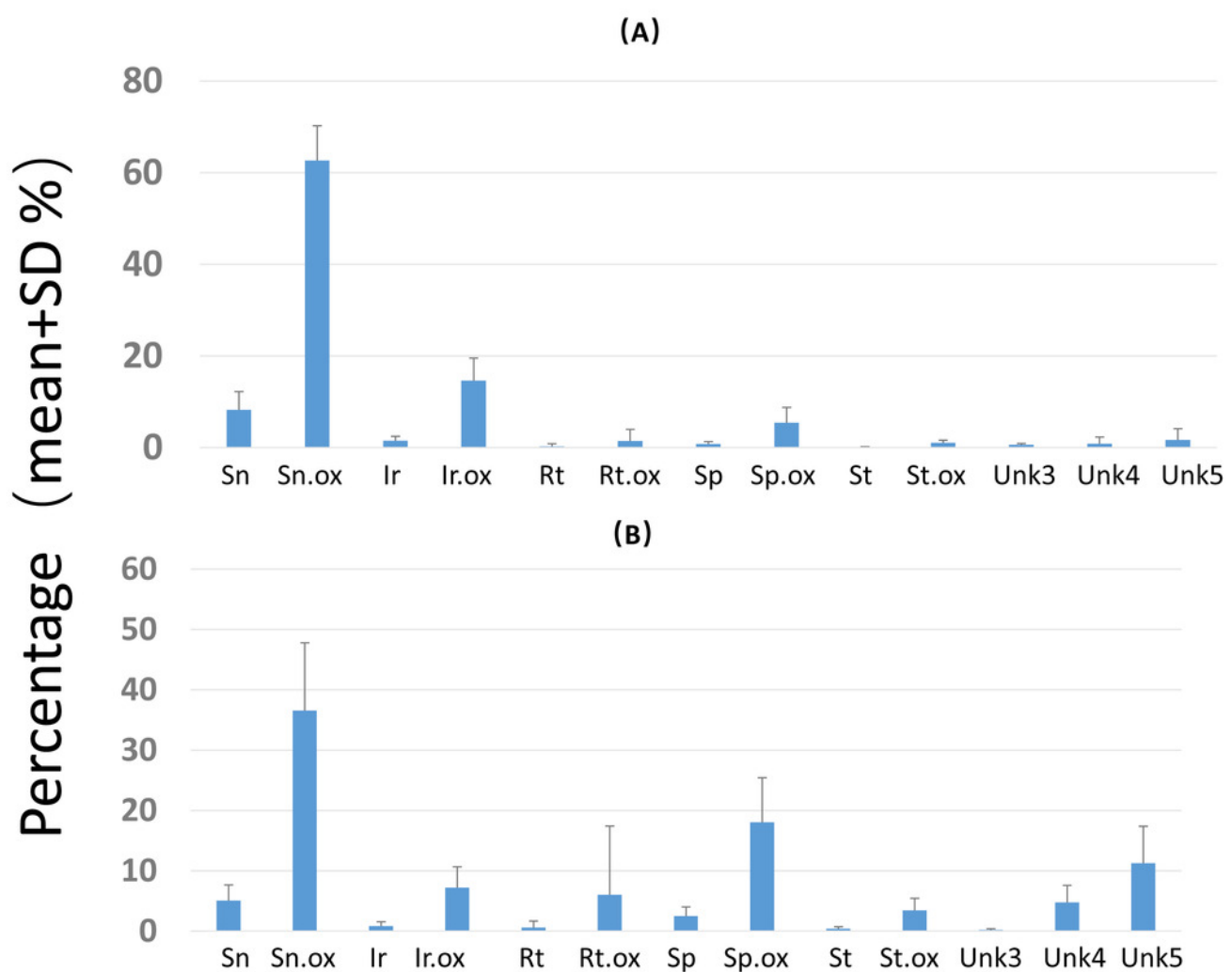
Variation of pyrrolizidine alkaloids (PAs) in roots and shoots of *Senecio vulgaris* from native and invasive populations.

PA diversity was calculated as Shannon index [H′ = −Σ pi * ln pi], where p was the relative abundance of each of the 22 individual PAs in a sample. Homogeneity of PA distribution in each sample was calculated as evenness [J′ = H′/ ln(s)], where s was the total number of occurring PAs in a sample.

Variation of pyrrolizidine alkaloids (PAs) in roots and shoots of *Senecio vulgaris* from native and invasive populations.

(A) Scoring plotting by two-dimension nonparametric multidimensional scaling (NMDS) based on concentration of 20 individual PAs. Square = roots; Dots = shoots. Red symbols were plants from invasive and the blue symbols were from native populations.

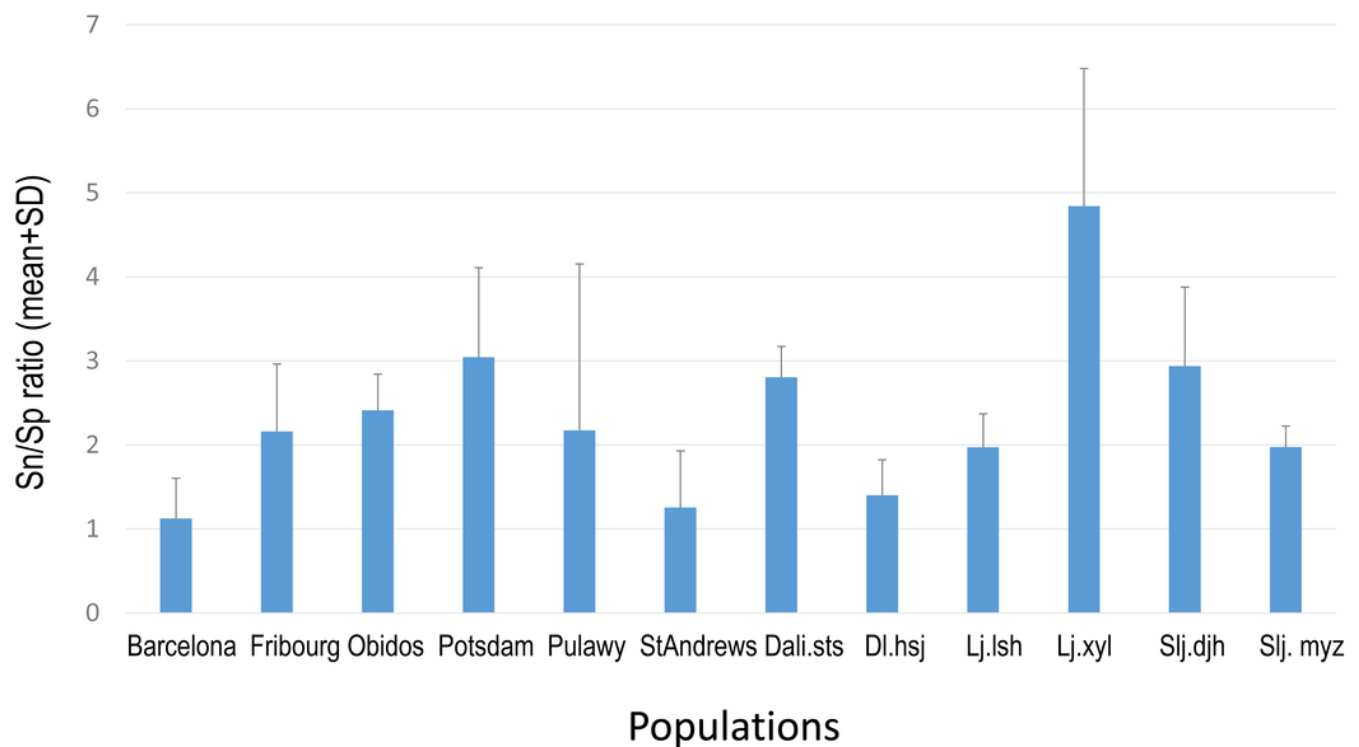
(B) Loading plots of the NMDS. See details of the abbreviation of PAs in Table 2-3.

# Figure 4

Composition of pyrrolizidine alkaloids (PAs) in roots and shoots of *Senecio vulgaris* plants.

Percentage = concentration of an individual PA / total PA concentration × 100. See details of the PAs in Table 2-3.

# Figure 5

Sn/Sp ratio in shoots of *Senecio vulgaris* plants from native and invasive populations.

Sn/Sp ratio = (Senecionine + Senicionine N-oxide)/(Seneciphylline + Seneciphylline N-oxide).

See details of the populations in Table 1.

# Figure 6

Comparison of abundance of selected pyrrolizidine alkaloids (PAs) in roots and shoots of *Senecio vulgare* plants grown under uniform conditions in the greenhouse.

Plants were grew from seeds collected from 6 native and 6 invasive populations. Clustering algorithm and Euclidean distance metric were used on relative abundance values. See details of key to populations (at leaf of heatmap) and to PAs (on the top of heatmap) in Table 1 and Table 2-3. The relative abundance of the 13 selected PAs was at least 1%, averaged among all samples.

**(A)**

**(B)**