DECREASE IN THE DIVERSITY OF PYRROLIZIDINE ALKALOIDS OF *Senecio pterophorus* AFTER INVASION

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Contribución del alumno en este trabajo

Yo, Alejandro Sánchez, comencé el Proyecto Fin de Máster el 3 de febrero de 2014 basándome en un trabajo experimental de muestreo biogeográfico, extracción de alcaloides y análisis cromatográficos de *Senecio pterophorus* obtenidos por el equipo de Eva Castells. En los primeros dos meses identifiqué los alcaloides pirrolizidínicos (PAs) de las muestras foliares de unos 500 individuos, comparando las características cromatográficas de los datos brutos obtenidos con GC-FID mediante tres técnicas: comparación del tiempo de retención (RT) con muestras de PAs conocidas (QC "quality control") y patrones comerciales mediante GC-FID, comparación de los RT y datos de fragmentación obtenidos por GC-MS, y comparación de fragmentación, peso molecular y abundancia relativa de cada compuesto a partir de los análisis de LC-MS/MS. Para el análisis de los patrones de fragmentación mediante GC-MS (una muestra por población) utilicé el programa AMDIS 2.1. Comprobando la masa y los fragmentos de masa de los picos de abundancia y conociendo sus RT relacioné cada pico con un PA distinto, extrapolando la identificación de los distintos PAs al resto de muestras en función del RT de cada pico de abundancia. Finalmente calculé la concentración de cada PA usando como referencia la concentración conocida del patrón estándar (monocrotalina).

En abril comencé los análisis estadísticos de las concentraciones absolutas y relativas de los PAs para las distintas regiones: modelo lineal generalizado mixto (GLMM), análisis de componentes principales (PCA) y análisis de clúster jerárquico. Finalmente, analicé la correlación entre las distancias químicas entre poblaciones con datos de distancias genéticas cedidos por Roser Vilatersana, miembro del equipo de Eva Castells, mediante el test de Mantel utilizando los programas R 2.15.0 y SPSS Statistics 19. Para las tablas y figuras utilicé fundamentalmente con el programa SigmaPlot 10.

En mayo comencé a redactar esta memoria siguiendo el formato e instrucciones de la revista *Biological Invasions*; supervisada y revisada periódicamente por Eva Castells.

Abstract

Pyrrolizidine alkaloids (PAs) are N-based plant secondary metabolites that function as chemical defenses against vertebrate and invertebrate herbivores. PAs can be highly variable at intraspecific level, both in their absolute and relative concentrations. Changes in the chemical composition of exotic plants when they invade a new environment have been poorly explored. Here we studied the biogeographical variation on PAs in *Senecio pterophorus* (Asteraceae) in the native region in Eastern South Africa, an expanded region in Western South Africa, and two introduced regions in Australia and Europe. PAs in *S. pterophorus* were represented by the highly toxic 1,2-unsaturated PAs and the less toxic 1,2-saturated PAs. Our results show a change in the plant chemical composition after invasion. Total PAs concentrations were highest in Australia compared to any other region. Plants from Europe contained the highest relative concentrations of 1,2-saturated PAs. The positive correlation between the chemical and the genetic distances estimated between populations suggests that the chemical profiles in the non-native regions were related to the plant dispersal routes. The decrease in the chemical diversity and the change in the absolute PAs concentrations in *S. pterophorus* after invasion may have consequences in the interactions between plants and herbivores in the novel habitats.

Keywords: Biogeographical survey · Chemical defenses · Chemical diversity · Exotic plants · Pyrrolizidine alkaloids · *Senecio pterophorus*

Introduction

Pyrrolizidine alkaloids (PAs) are a wide class of N-based plant secondary metabolites integrated by more than 360 structures formed by the esterification of a necine base moiety with an alkyl necic acid (Hartmann 1999; Witte et al. 1993). PAs are present in *ca* 3% of all flowering plants (Smith and Culvenor 1981), mostly in some genera of the *Asteraceae* such as *Senecio* and *Eupatorium*, *Boraginaceae* and, to a lesser extent, *Fabaceae* and *Orchidaceae* (Mattocks 1986; Ober and Hartmann 1999).

PAs function as plant chemical defenses, being strong feeding deterrents for generalist insect herbivores (Hartmann 1999; Müller-Schärer et al. 2004). PAs also cause liver toxicity in vertebrates, including livestock and humans (Cheeke 1988; Mattocks 1986), and mutagenesis in insects (Frei et al. 1992). Individual PAs differ in their toxicity depending on their chemical structure. Thus, PAs with a double-bond at the C1-C2 positions in their necine base (1,2-unsaturated PAs) (Fig. 1) can produce carcinogenicity, mutagenicity, genotoxicity, teratogenicity and fetotoxicity, and some of them can be pneumotoxic or neurotoxic (IPCS 1988; Schmeller et al. 1997; Wiedenfeld et al. 2008). Meanwhile, 1,2-saturated PAs (Fig. 1) have a much lower toxicity (Wiedenfeld et al. 2008).

The relative composition of secondary metabolites within a species can be highly variable (Kleine and Müller 2011; Macel and Klinkhamer 2010). Due to the different toxicity of individual PAs, changes in the plant chemical composition during biological invasions could have evolutionary consequences on the invaded ecosystems by modifying interactions between plants and herbivores and offering a considerable greater potential to invasion success (Keane and Crawley 2002; Pimentel et al. 2005; Wolf et al. 2012).

According to Evolution of Increased Competitive Ability (EICA) hypothesis, exotic species colonizing a new environment are released from their natural enemies causing a

reallocation of resources from defences to growth (Blossey and Nötzold 1995). Thus, plants from the introduced habitats are expected to evolve lower concentrations of chemical defences compared with the native habitats. Therefore, plants which contains two classes of PAs with distinct biological activity would a stronger decrease of the more toxic 1,2-unsaturated PAs compared with the less toxic 1,2-saturated PAs after enemy release. In addition, other factors such as resource availability could change the levels of defense investment (Orians and Ward 2011).

In addition to the effects of biotic and abiotic factors in the novel habitats, changes in the levels of chemical defences could also be caused by non-adaptative events such as demographic bottlenecks, genetic drift or the plant invasive pathways. Indeed, genetic diversity tends to decrease after an invasion because the new populations are established by a smaller number of individuals compared with the source populations, resulting in lower genetic variability in the introduced populations (Nei et al. 1975; Slatkin and Excoffier 2012). Some examples that confirm this phenomenon are the high genetic variability of the native populations of *Rubus alceifolius* in contrast to its areas of introduction (Amsellem et al. 2000) or a reduction in genetic variation of *Eicchornia paniculata* in its colonised area (Husband and Barrett 1991). Because chemical diversity is related to genetic diversity (Böszörményi et al. 2009; Nan et al. 2003), we would expect a reduction of the chemical diversity in the introduced regions compared to the native region.

Senecio pterophorus DC (*Asteraceae*) is a perennial shrub native from Eastern Cape and KwaZulu-Natal Provinces in South Africa, expanded to Western South Africa, and introduced into Australia and Europe (Castells et al. 2013; Hilliard 1977). In Australia, *S. pterophorus* is considered as noxious species causing heavy productivity losses in agricultural areas reducing diversity, displacing native species and hybridizing with other *Senecio* species (Parson and Cuthbertson 1992). Despite the negative effects

that *S. pterophorus* may cause on invaded ecosystems, its chemical composition has been poorly explored (Castells et al. 2014; Liddell and Logie 1993).

Here we compared the concentrations and profile of PAs from plants collected in the native region (Eastern South Africa), an expanded region (Western South Africa) and two cross-continental introductions (Australia and Europe) which covered the entire known distributional area of *S. pterophorus* in its native and invasive range. We determined the absolute and relative PA concentrations of 421 individual plants from 44 populations across these four regions to study the change in chemical defences after invasion. We hypothesized that introduced plants will have 1) lower absolute concentrations of PAs, and 2) a lower chemical diversity compared with plants from the native region.

Materials and methods

Species description and distributional area

Senecio pterophorus DC (*Asteraceae*) is a perennial shrub native to Eastern Cape and southern parts of KwaZulu-Natal Provinces in South Africa, where it forms scattered populations in forest margins and grasslands (Castells et al. 2013; Hilliard 1977). This species was expanded into the Western Cape Province near Cape Town during the early 20th century, where is considered an invasive species (Levyns 1950). The first record in Australia dates from 1908 in Melbourne and from 1930 *S. pterophorus* spread along the southeastern coast in South Australia and Victoria forming persistent populations (Scott and Delfosse 1992). In continental Europe the introduction of *S. pterophorus* is comparatively recent (Castells et al. 2013), first recorded in 1982 near Barcelona, at the northeastern Iberian Peninsula (Casasayas 1989; Chamorro et al. 2006). In 1990 it was also found on the Ligurian coast in northwestern Italy (Barberis et al. 1998, Castells et al. 2013).

Sampling

S. pterophorus was sampled across the entire known distribution of *S. pterophorus* in the native, expanded and introduced ranges as detailed in Castells et al. (2013). We sampled 14 populations in the native range (Eastern South Africa), 5 in the expanded range in Western South Africa, 13 in Australia and 12 in Europe. Plant foliage from *ca* 10 individuals per population (a total of 421 plants) was collected and dried with silica gel. The sampling was conducted during the plant flowering period, in December 2009 and January 2010 in the southern hemisphere (South Africa and Australia) and in July 2010 in the northern hemisphere (Europe).

Analysis of pyrrolizidine alkaloids (PAs)

Dried leaf material for individual was grinded and extracted with 70:30 MeOH: 1N HCl. Monocrotaline (1 mg/mL) was added as internal standard. PAs were reduced to tertiary amines by adding excess Zn dust and extracted with CH_2Cl_2 with hexadecane (0.01% v/v) as an injection standard after increasing the pH with NH_3OH.

PAs were analyzed by gas chromatograph coupled with a flame ionization detector (GC-FID) (Agilent® 6890, Santa Clara, CA, USA) on a capillary column (HP-1, 30 m, 0.25 μm). The oven temperature was increased from 165ºC to 253ºC at 4ºC/min. One representative sample per population was also analyzed by GC-MS (Agilent® 6890, Santa Clara, CA, USA) using the same column and temperature program detailed above. PAs were identified by their retention times in comparison with commercial standards (senecionine, retrorsine, seneciphylline; Sigma-Aldrich, St. Louis, MO, USA), and with mass spectra obtained by GC-MS and analyzed using AMDIS 2.1 (DTRA/NIST, 2000) (Table S1). Additionally, GC data from one sample per population was compared with chromatographic and mass spectrometric data analyzed by LC-

MS/MS (Castells et al. 2014). PAs concentrations were calculated using monocrotaline as a reference standard.

Genetic analysis

The genetic distance between populations was estimated on 365 individuals by AFLPs (Amplified Fragment Length Polymorphism) (Vilatersana et al. unpublished). The methodology used for DNA extraction and AFLP analyses is detailed in Vilatersana et al. (2007). In short, total genomic DNA was extracted using the CTAB method (Doyle and Dickson 1987, Tel-Zur et al. 1999). Three selective primer pairs showing a high reproducibility and variation across plant regions were chosen for the analysis (EcoRI-ACT/MseI-CAA, EcoRI-AGG/MseI-CTC, and EcoRI-AAG/MseI-CAA). Ambiguous, non-reproducible or marginal AFLP loci were excluded from the dataset. The neighbour-joining analysis was performed using the midpoint rooting mode and the programs NEIGHBOR and CONSENSE and the pairwise genetic distance between populations were estimated using AFLP-SURV v.1.0 software.

Statistical analysis

Differences in PA concentration and profile of the expanded (Western South Africa) and introduced regions (Australia and Europe) compared with native region (Eastern South Africa) were tested on a generalized linear mixed model (GLMM) with individuals within populations as random effects, region as fixed effects, and absolute and relative PA concentrations as the response variables. The native region was set as the intercept. Data corresponding to absolute concentration that not showed a normal distribution were log transformed or square root transformed.

To group individuals and populations based on their chemotypes, i.e. a similar profile of the PA relative abundance, we performed a principal component analysis

(PCA) and a hierarchical cluster analysis (average linkage method UPGMA). The correlation between the chemical distances between populations calculated from the cluster analysis and the genetic distances obtained from Vilatersana et al. (unpublished) was tested with a Mantel test based on Pearson's product-moment correlation and carrying out 999 permutations.

All statistical analyses were performed using R 2.15.0 (R Developed Core Team) except for the UPGMA which was performed using SPSS Statistics 19.0 (IBM, SPSS Inc Armonk, NY, USA). Specifically, for the GLMM was used the "nlme" package, whereas for the PCA was used the "stats (prcomp)" package and for the Mantel test was used the "vegan" package; all in R 2.15.0. The UPGMA was analyzed by SPSS Statistics 19.0.

Results

A total of 24 compounds were present in *S. pterophorus* across all samples: 16 1,2-unsaturated PAs (all with a retronecine base) and 8 1,2-saturated PAs (3 platynecine and 5 rosmarinecine base PAs) (Table 1; Table S1; Fig. S1). Plants from Western South Africa did not show significant different concentrations compared with the native region (Table 2; Fig. 2). Plants from Australia had higher absolute concentrations of total PAs, as well as 1,2-unsaturated and 1,2-saturated PAs compared with plants from the native region at Eastern South Africa (Table 2; Fig. 2). All individual PAs were found at highest concentrations in Australia except for senecivernine, retrorsine, eruciflorine, usaramine, hydroxy-rosmarinine and one unknown PA that were present at lower concentrations compared with the native plants (Table 2; Fig. 3). Plants from Europe had significantly lower total concentrations compared with the native region, caused by the extremely low concentrations of 1,2-saturated PAs, in particular rosmarinine and isomers. However, no differences were found between European and native plants for

the 1,2-unsaturated PAs because the higher concentrations of acetylseneciphylline in European plants was accompanied by a decrease in senecivernine and retrorsine, among other minor PAs (Table 2; Fig. 2, 3). Retrorsine was found in higher concentration in the native plants (Table 2; Fig. 3).

In terms of relative concentrations, both the Western South Africa and Australian plants showed lower levels of 1,2-unsaturated PAs and higher levels of 1,2-saturated PAs compared with the native region at Eastern South Africa (Table 2; Fig. 2). In these two regions, virtually all relative concentrations of 1,2-unsaturated PAs were lower than the native region, except for hydroxy-seneciphylline in the Western South Africa and seneciphylline in Australia. High relative concentrations of 1,2-saturated PAs in these two regions were mainly determinate by rosmarinine (I) and (II) in Western South Africa and rosmarinine (I) in Australia (Table 2; Fig. 3). In contrast, the relative concentration of 1,2-unsaturated PAs in Europe was higher and the 1,2-saturated PAs lower compared with the native region (Table 2; Fig 2). Both seneciphylline and acetylseneciphylline contributed to the high concentration of 1,2-unsaturated PAs; while rosmarinine and isomers were lower than the native region, especially rosmarinine (I) (Table 2; Fig. 3).

In order to classify the plants according to their chemical profile, we performed a multivariate analysis using the relative abundance of individual PAs. A PCA including all individual plants revealed that vectors were oriented according to the structural similarities of individual PAs. Thus, compounds sharing the necine base (retronecine, rosmarinecine) and structural isomers were positioned together. When plants were averaged by region, the PCA and cluster analysis showed the existence of four distinct chemotypes, that were named after the most abundant or distinct compound (Fig. 4, 5): 1) Rosmarinine chemotype, with high levels of rosmarinecine-base PAs, which included all populations from Australia and Western South Africa and five populations of

Eastern South Africa; 2) Seneciphylline chemotype, with high levels of seneciphylline, senecionine and acetylseneciphylline, and present in all European plants and two populations from Eastern South Africa; 3) Senecionine chemotype, with high levels of senecionine and retrorsine, and present in four populations from Eastern South Africa, and 4) Retrorsine chemotype, with high levels of retrorsine and rosmarinine, and present in three populations from Eastern South Africa.

Thus, the chemical diversity was reduced after invasion. Plants from the native region were represented in the four chemotypes, while plants from each non-native region belonged to a single chemotype (Fig. 4). This decrease in chemical diversity was also shown by the overall closest distance within each non-native region compared to the distance within the native region (Fig. 5, Fig. S2).

A positive correlation was found between the chemical distance, calculated by the cluster analysis (Fig. 5), and the genetic distance, estimated by AFLPs (Vilatersana et al. unpublished), for population pairs across all regions (Mantel test, $p<0.05$) (Fig. 6).

Discussion

Levels of chemical defenses after plant introduction

The EICA hypothesis predicts a decrease in plant chemical defenses after invasion as a result of a decrease in herbivore consumption in the novel environment. In the case of *S. pterophorus*, which contains two classes of PAs with distinct biological activity, we would expect a stronger decrease of the more toxic 1,2-unsaturated PAs compared with the less toxic 1,2-saturated PAs after enemy release. In Australia, one of the introduction areas, we observed an increase in the absolute PA concentrations compared with the native region for both 1,2-unsaturated and 1,2-saturated compounds, which is not in accordance with the EICA hypothesis. However, the relative concentrations of 1,2-unsaturated PAs were decreased, and 1,2-saturated PAs were increased, in

comparison with the native region. This observed shift from 1,2-unsaturated to 1,2-saturated PAs, that is a decrease in resource allocation to the more toxic compounds, is consistent with a lower herbivore selective pressure in Australia compared with the native region. Plants from Europe had lower concentrations of total PAs and 1,2-saturated PAs compared with the native region, but higher relative abundance of 1,2-unsaturated PAs, mostly due to the extremely low concentrations of the 1,2-saturated PAs. Finally, plants from the extended regions had similar total concentrations than the native populations, but a shift was found from 1,2-unsaturated PAs to 1,2-saturated PAs similar to the Australian plants. Overall, we found no consistent trends in the changes in absolute PAs in the two cross-continental introductions compared with the native region, and the changes in the PA relative abundance was similar in Australia and Western South Africa.

The fact that we analysed the PAs from plants collected *in-situ* should be considered. The EICA hypothesis is an evolutionary hypothesis that predicts a decrease of the genetically determined levels of chemical defences. In our study we ignore what part of the changes in PA concentrations were genetically or environmentally determined, and we acknowledge that differences in the abiotic environment across regions may have contributed to the PA concentrations independently to the selective role of herbivores. Whether the changes in PAs are solely caused by herbivory or also by the local environmental conditions should be further evaluated in a common garden experiment. Nevertheless, our results are relevant to predict the potential effects of the phenotypic changes in PA on herbivores after invasion. Herbivore consumption is regulated by the total levels of plant chemical defences in natural conditions, regardless whether these defences are genetically or environmentally determined. Thus, an increase in total PA concentrations and 1,2-unsaturated PAs in Australia, and the

relative increase of 1,2-unsaturated PAs in Europe could increase the plant resistance to herbivores, and change their selective pressure in the invasive range.

Diversity of PAs after plant introduction

According to our predictions, the diversity of PAs in *S. pterophorus* was lower in the non-native regions compared with the native region in Eastern South Africa. Thus, plant populations from the native area belonged to four distinct chemotypes (rosmarinine, seneciphylline, senecionine and retrorsine chemotypes) while the introduced populations belonged only to a single chemotype (Australia and Western South Africa to rosmarinine chemotype; Europe to seneciphylline chemotype). These results show that chemical diversity changed with plant invasions, revealing a decline in the diversity of PAs in plants that colonized new environments compared with plants from the native populations. Despite there are a large number of articles related to chemical diversity for many chemical groups, such as terpenoids (Bravo-Monzón et al. 2014; Kleine and Müller 2011) or PAs (Cheng et al. 2011; Hartmann and Dierich 1998; Macel et al. 2002), most studies only address the impact of this diversity on herbivores regardless of the genetic component.

Chemical and genetic distance

The positive correlation between the chemical and the genetic distances for population pairs suggests that the overall chemical profiles in the non-native regions were related to the plant dispersal routes. The absence of correlation between the phenotypic differences and the genetic distances may be indicative of an ongoing evolutionary selection for a particular plant trait. In contrast, here we found that the populations that were genetically closest they were also more similar in their chemical profile. This result suggests that the divergence in the chemical profiles in Australia and Europe

could have been caused by different introduction events with the source populations belonging to the same chemotype in the introduced and native populations. A more detail analyses of the correlation between the genetic and chemical distances are needed to determine whether the origin of each non-native population explains the chemical profile in the introduced population.

Several studies have assessed the relationship between genetic diversity and chemical diversity of plants (Wolf et al. 2012), with results range from a highly positive correlation (Böszörményi et al. 2009; Nan et al. 2003) to the absence of correlation (Trindade et al. 2008; Wolf et al. 2012) depending on the plant species and sampling methodology.

Conclusion

We found no consistent change in the absolute concentrations of PAs in the introduced plant populations. In two introduced areas, Western South Africa and Australia, a shift from 1,2-unsaturated PAs to 1,2-saturated PAs was found. This change together with the decrease in the chemical diversity in *S. pterophorus* after invasion may have consequences in the interactions between plants and herbivores in the novel habitats and eventually plant invasion success.

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Table 1 List of pyrrolizidine alkaloids (PAs) of *Senecio pterophorus* identified by GC-FID and classified by their necine base (Type)

Type	PAs	Code	RT (min)	Mass (MW)	Fragment mass
1,2-unsaturated PAs	Senecivernine	SV	17.063	335	93, 120, 136
	Senecionine	SC	17.285	335	93, 120, 136
	Seneciphylline	SP	17.575	333	94, 120, 136
	Spartioidine	ST	18.261	333	93, 120, 136
	Integerrimine	IG	18.415	335	93, 120, 136
	Jacobine	JB	19.947	351	95, 120, 136
	Hydroxy-seneciphylline	HSP	20.211	349	94, 120, 136
	Acetylseneciphylline	ASP	20.590	375	94, 120, 136
	Acetylspartioidine	AST	21.403	375	93, 119, 136
	Retrorsine	RT	21.472	351	93, 120, 136
	Jaconine	JN	21.525	387	95, 120, 136
	Riddelliine	RD	21.570	349	94, 120, 136
	Unknown (I)	UKI	21.869	363	93, 120, 136
	Eruciflorine	ER	22.526	351	93, 120, 136
	Usaramine	US	22.544	351	93, 120, 136
	Unknown (II)	UKII	22.926	393	93, 120, 136
1,2-saturated PAs	Platyphylline (I)	PLI	18.752	337	82, 122, 140
	Platyphylline (II)	PLII	18.864	337	82, 122, 140
	Platyphylline (III)	PLIII	20.123	337	82, 122, 140
	Rosmarinine (I)	ROI	21.048	353	82, 138, 154
	Rosmarinine (IV)	ROIV	22.037	353	82, 138, 156
	Rosmarinine (II)	ROII	22.484	353	82, 138, 156
	Rosmarinine (III)	ROIII	23.732	353	82, 138, 156
	Hydroxy-rosmarinine	HRO	25.045	369	82, 138, 156

Code, PA acronym; RT, retention time; Mass, molecular weight; Fragment mass, major mass fragment detected by flame ionization

Table 2 Generalized linear mixed model (GLMM) results corresponding to differences in PAs concentration and relative concentration found in plants collected at the expanded region in Western South Africa and two cross continental introductions (Australia and Europe) compared with native region (Eastern South Africa). The intercept corresponds to the native region. Values represent the mean ± s.e.

Type	PAs	PA concentration (mg/g dry wt.)				Relative concentration (%)			
		Intercept	Expanded	Australia	Europe	Intercept	Expanded	Australia	Europe
Total PAs		0.93 ± 0.2	-0.26 ± 0.4	1.13 ± 0.3***	-0.73 ± 0.3*	ND	ND	ND	ND
1,2-unsaturated PAs		1.54 ± 0.2	-0.52 ± 0.3	0.77 ± 0.2**	-0.30 ± 0.3	69.83 ± 4.5	-28.60 ± 8.7**	-15.93 ± 6.4*	26.08 ± 6.6***
	Senecivernine	0.29 ± 0.0	-0.21 ± 0.1*	-0.13 ± 0.1*	-0.23 ± 0.1***	3.93 ± 0.6	-3.48 ± 1.2*	-3.58 ± 0.9***	-3.58 ± 0.9***
	Senecionine	-1.67 ± 0.3	-0.45 ± 0.6	1.33 ± 0.4**	0.19 ± 0.5	20.22 ± 2.9	-12.35 ± 5.6*	-9.31 ± 4.2*	1.63 ± 4.3
	Seneciphylline	0.56 ± 0.1	0.15 ± 0.3	1.19 ± 0.2***	0.23 ± 0.2	15.53 ± 2.4	4.40 ± 4.7	14.30 ± 3.6***	23.28 ± 3.6***
	Spartioidine	0.22 ± 0.0	0.06 ± 0.1	0.30 ± 0.1***	0.07 ± 0.1	2.87 ± 0.4	0.57 ± 0.8	0.01 ± 0.6	3.52 ± 0.6***
	Integerrimine	0.22 ± 0.0	-0.10 ± 0.1	0.04 ± 0.0	-0.04 ± 0.0	2.47 ± 0.3	-1.65 ± 0.7*	-1.58 ± 0.5**	-0.25 ± 0.5
	Hydroxy-seneciphylline	0.06 ± 0.0	0.08 ± 0.0	0.12 ± 0.0**	0.01 ± 0.0	0.47 ± 0.2	0.91 ± 0.4*	0.10 ± 0.3	0.33 ± 0.3
	Acetylseneciphylline	0.24 ± 0.0	-0.03 ± 0.1	0.33 ± 0.1***	0.26 ± 0.1***	3.55 ± 1.3	-1.03 ± 2.5	1.79 ± 1.8	16.04 ± 1.9***
	Acetylspartioidine	0.04 ± 0.0	0.02 ± 0.0	0.19 ± 0.0***	0.08 ± 0.0*	0.64 ± 0.3	-0.23 ± 0.6	0.52 ± 0.4	1.23 ± 0.4*
	Retrorsine	0.61 ± 0.1	-0.40 ± 0.2*	-0.37 ± 0.1**	-0.53 ± 0.1***	15.56 ± 2.3	-12.00 ± 4.6*	-14.81 ± 3.4***	-13.64 ± 3.5***
	Riddelliine	0.09 ± 0.0	-0.01 ± 0.0	0.12 ± 0.0**	-0.04 ± 0.0	0.70 ± 0.3	-0.03 ± 0.5	0.00 ± 0.4	0.13 ± 0.4
	Unknown (I)	0.07 ± 0.0	-0.03 ± 0.0	0.01 ± 0.0	-0.04 ± 0.0*	0.26 ± 0.0	-0.16 ± 0.1*	-0.15 ± 0.0**	-0.16 ± 0.1**
	Eruciflorine	0.10 ± 0.0	-0.10 ± 0.0**	-0.10 ± 0.0***	-0.09 ± 0.0***	1.87 ± 0.7	-1.87 ± 1.3	-1.87 ± 1.0	-1.85 ± 1.0
	Usaramine	0.09 ± 0.0	-0.09 ± 0.1	-0.09 ± 0.0*	-0.09 ± 0.0*	1.21 ± 0.4	-1.21 ± 0.8	-1.21 ± 0.6*	-1.21 ± 0.6*
	Unknown (II)	0.07 ± 0.0	-0.07 ± 0.0*	-0.05 ± 0.0**	-0.07 ± 0.0***	0.37 ± 0.1	-0.34 ± 0.1*	-0.34 ± 0.1**	-0.35 ± 0.1***
1,2-saturated PAs		0.80 ± 0.1	0.40 ± 0.3	1.26 ± 0.2***	-0.62 ± 0.2**	30.16 ± 4.5	28.60 ± 8.7**	15.93 ± 6.4*	-26.08 ± 6.6***
	Platyphylline (I)	0.11 ± 0.0	0.09 ± 0.0***	0.10 ± 0.0***	-0.01 ± 0.0	1.16 ± 0.3	1.99 ± 0.6**	-0.18 ± 0.4	0.42 ± 0.4
	Platyphylline (II)	0.08 ± 0.0	-0.03 ± 0.0	0.03 ± 0.0	-0.06 ± 0.0*	0.47 ± 0.1	-0.31 ± 0.2	-0.32 ± 0.1*	-0.39 ± 0.1*
	Platyphylline (III)	0.01 ± 0.0	0.01 ± 0.0	0.06 ± 0.0***	-0.01 ± 0.0	0.08 ± 0.0	0.16 ± 0.1	0.12 ± 0.1*	-0.01 ± 0.1
	Rosmarinine (I)	0.66 ± 0.1	0.27 ± 0.24	1.25 ± 0.2***	-0.55 ± 0.2**	18.30 ± 2.8	11.60 ± 5.4*	19.25 ± 4.0***	-16.54 ± 4.1***
	Rosmarinine (IV)	0.16 ± 0.0	0.04 ± 0.1	0.22 ± 0.0***	-0.15 ± 0.0**	1.40 ± 0.2	0.06 ± 0.5	0.21 ± 0.4	-1.33 ± 0.4**
	Rosmarinine (II)	0.19 ± 0.0	0.35 ± 0.1***	0.31 ± 0.1***	-0.18 ± 0.1**	7.22 ± 2.4	15.18 ± 4.6**	-2.21 ± 3.4	-7.03 ± 3.5
	Rosmarinine (III)	0.01 ± 0.0	0.01 ± 0.0	0.01 ± 0.0***	-0.01 ± 0.0**	0.69 ± 0.2	0.46 ± 0.3	-0.13 ± 0.2	-0.67 ± 0.2*
	Hydroxy-rosmarinine	0.11 ± 0.0	-0.07 ± 0.0	-0.02 ± 0.0	-0.07 ± 0.0*	0.81 ± 0.2	-0.56 ± 0.3	-0.67 ± 0.2*	-0.53 ± 0.3*

Significant p-values are indicated by *p < 0.05, **p < 0.005 and ***p < 0.0005 for expanded and introduced regions

Notice that a transformation of some data corresponding to absolute concentrations was necessary to get a normal distribution

Figure captions

Fig. 1 Structures of the two classes of pyrrolizidine alkaloids (PAs) found in *S. pterophorus*: **a)** 1,2-unsaturated PAs, represented by a retronecine-base PAs (pictured senecionine) and **b)** 1,2-saturated PAs, including platynecine-base or rosmarinecine-base PAs (pictured platyphylline and rosmarinine)

Fig. 2 a) Absolute concentration and **b)** relative concentration of PAs according to their saturation at C1-C2 position at the necine base from plants collected at the native, the expanded and two introduced regions. The insert graph shows the total PA concentrations averaged by region. Bars represent the mean (± s.e.) by regions. Significant differences of the non-native regions compared with native region are indicated by *$p < 0.05$, **$p < 0.005$ and ***$p < 0.0005$

Fig. 3 a) Concentration and **b)** relative concentration of PAs averaged by region (mean ± s.e.), excluding PAs with a relative concentration less than 1% in all regions. Codes corresponding to PAs listed in Table 1. Significant differences of non-native regions compared with native region were tested on a generalized linear mixed model (GLMM) considering the last one as intercept (Table 2)

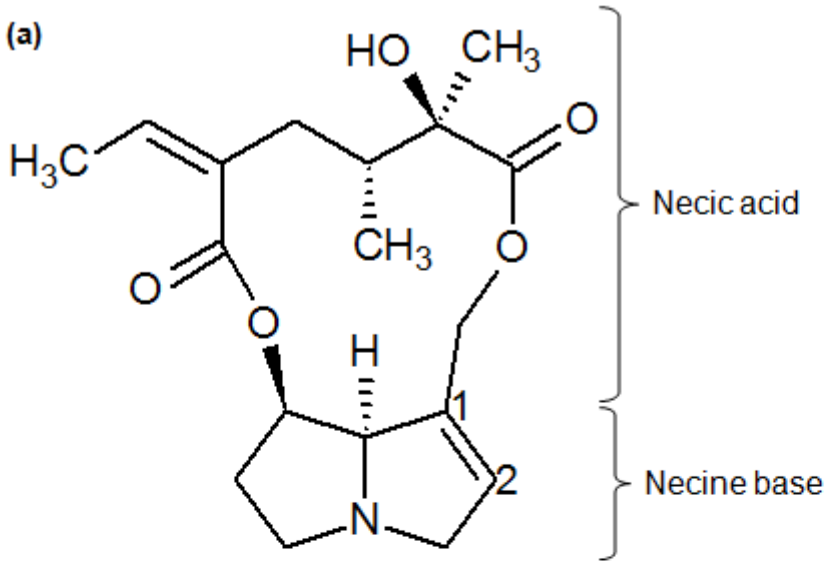
Fig. 4 Principal component analysis **a)** individually by plants and **b)** averaged by populations, and grouped by chemotypes according to the hierarchical cluster analysis. Codes of individual PAs are found in Table 1. Each colour represents the following regions: red = Australia (A01–A13), blue = Europe (E01–E12), green = Western Cape Province in South Africa (S01–S05) and yellow = Eastern Cape Province in South Africa (S06–S19)

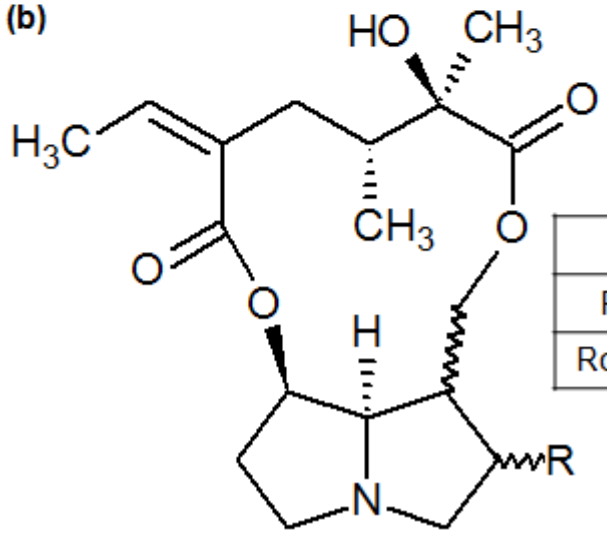
Fig. 5 Hierarchical cluster analysis of chemical variation for populations. PA profile for each chemotype is represented by vertical bar charts, excluding PAs with a relative

concentration less than 1% in all regions. Codes corresponding with PAs listed in Table 1. Population codes are corresponding with the following regions: A01–A13 = Australia, E01–E12 = Europe, S01–S05 = Western Cape Province in South Africa and S06–S19 = Eastern Cape Province in South Africa

Fig. 6 Pairwise relationship between the genetic and chemical distances for populations pairs from all native and non-native populations. Statistical significance corresponds to a Mantel test

Fig. 1





Type	-R
Platynecine	-H
Rosmarinecine	-OH

Fig. 2

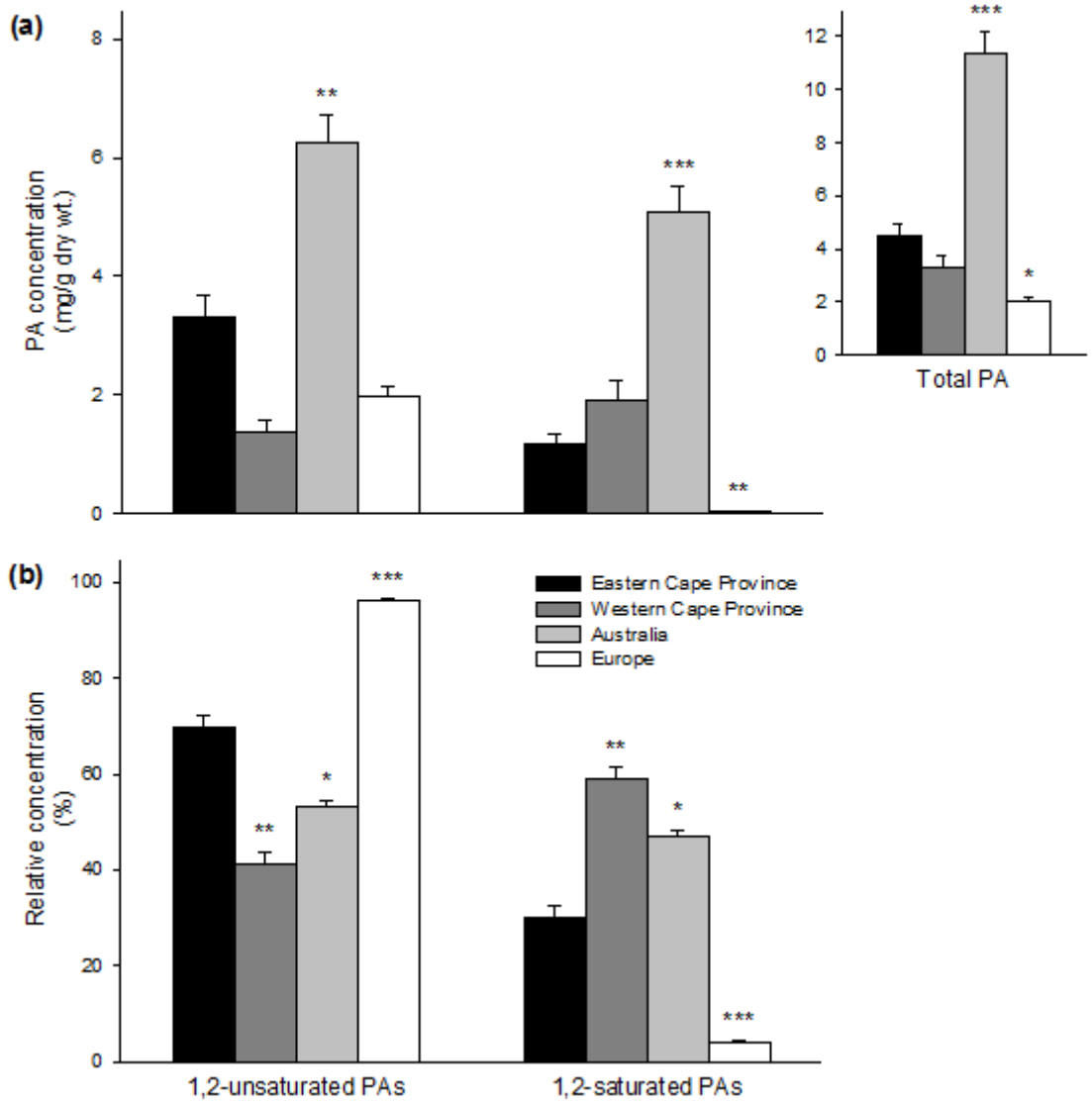

(a)
PA concentration (mg/g dry wt.)
0
2
4
6
8
**
***
**
Total PA
0
2
4
6
8
10
12
***
*
(b)
Relative concentration (%)
0
20
40
60
80
100
**
*
***
**
*
***
Eastern Cape Province
Western Cape Province
Australia
Europe
1,2-unsaturated PAs
1,2-saturated PAs

Fig. 3

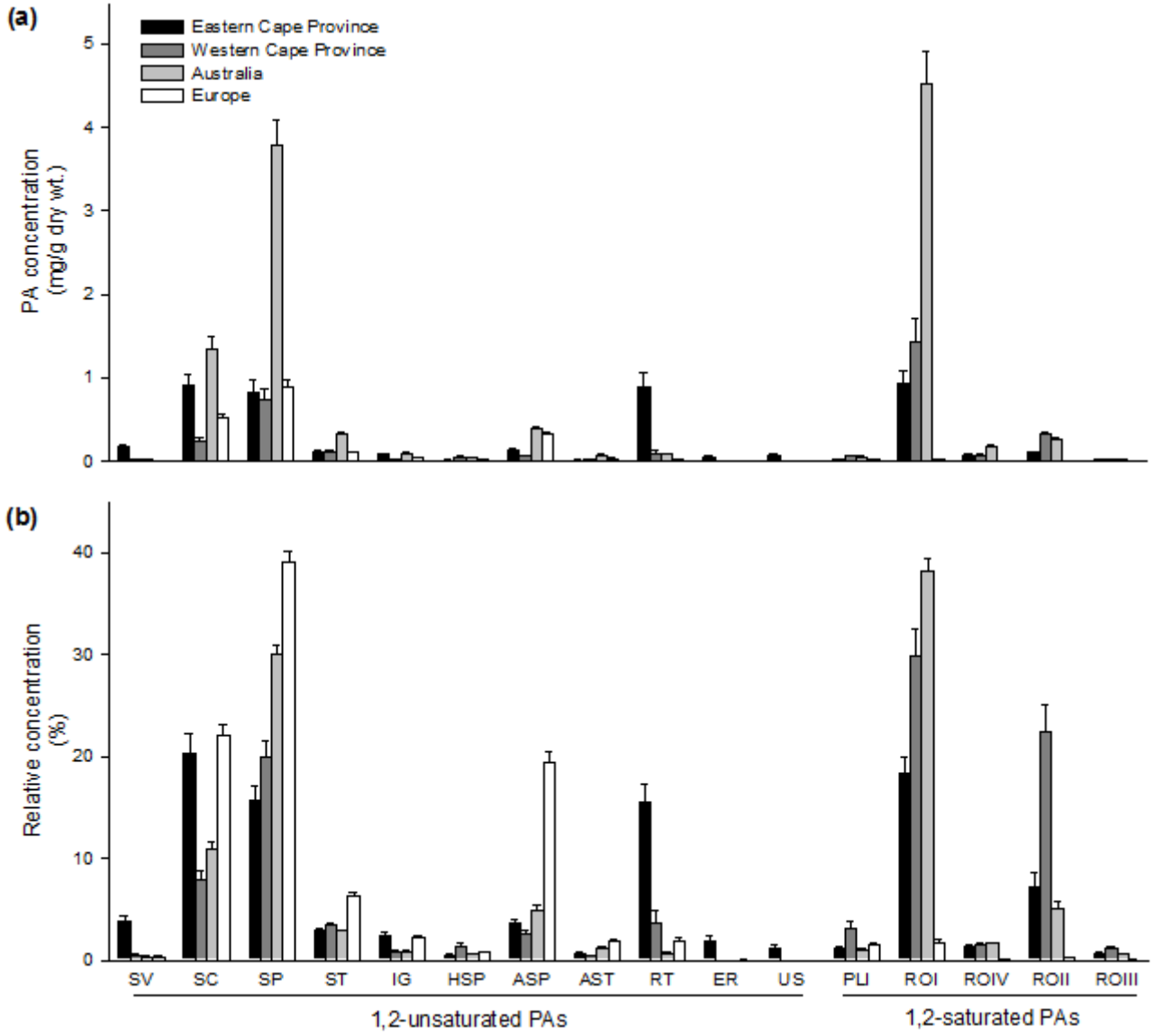

(a)
Eastern Cape Province
Western Cape Province
Australia
Europe
PA concentration (mg/g dry wt.)
0
1
2
3
4
5
(b)
Relative concentration (%)
0
10
20
30
40
SV
SC
SP
ST
IG
HSP
ASP
AST
RT
ER
US
PLI
ROI
ROIV
ROII
ROIII
1,2-unsaturated PAs
1,2-saturated PAs

Fig. 4

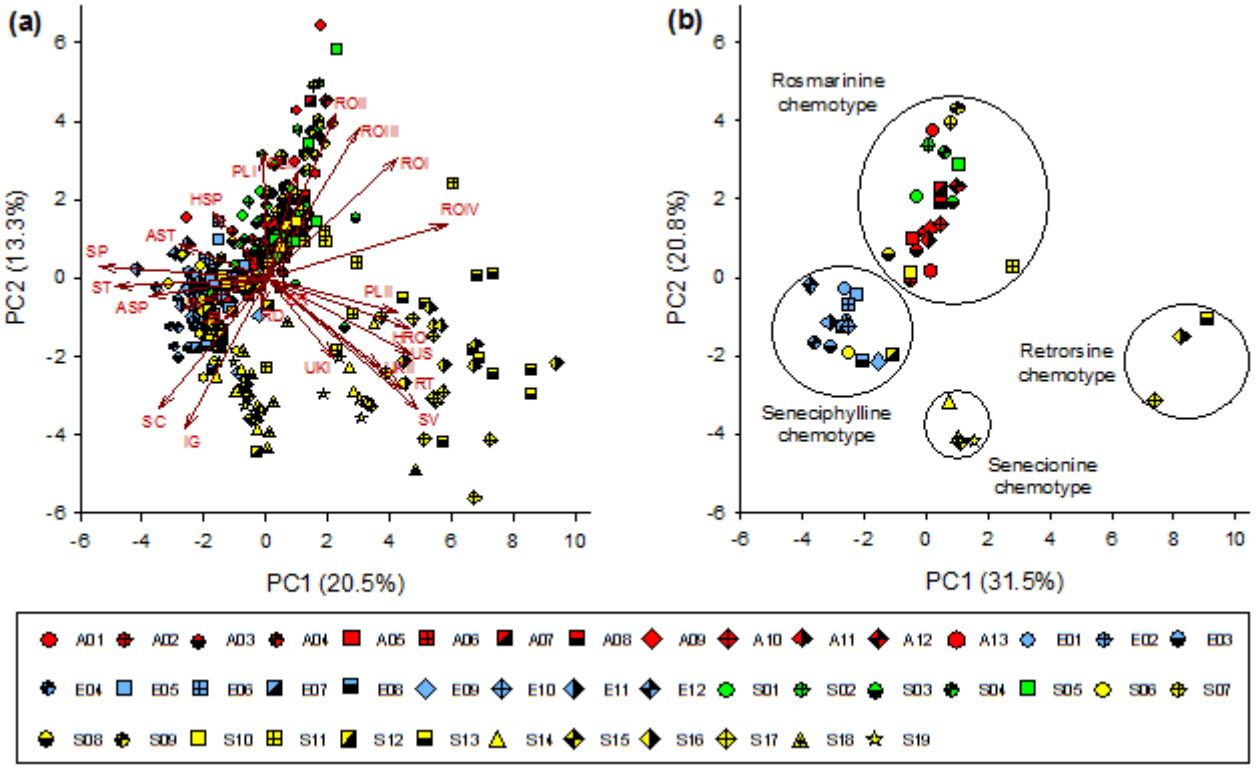
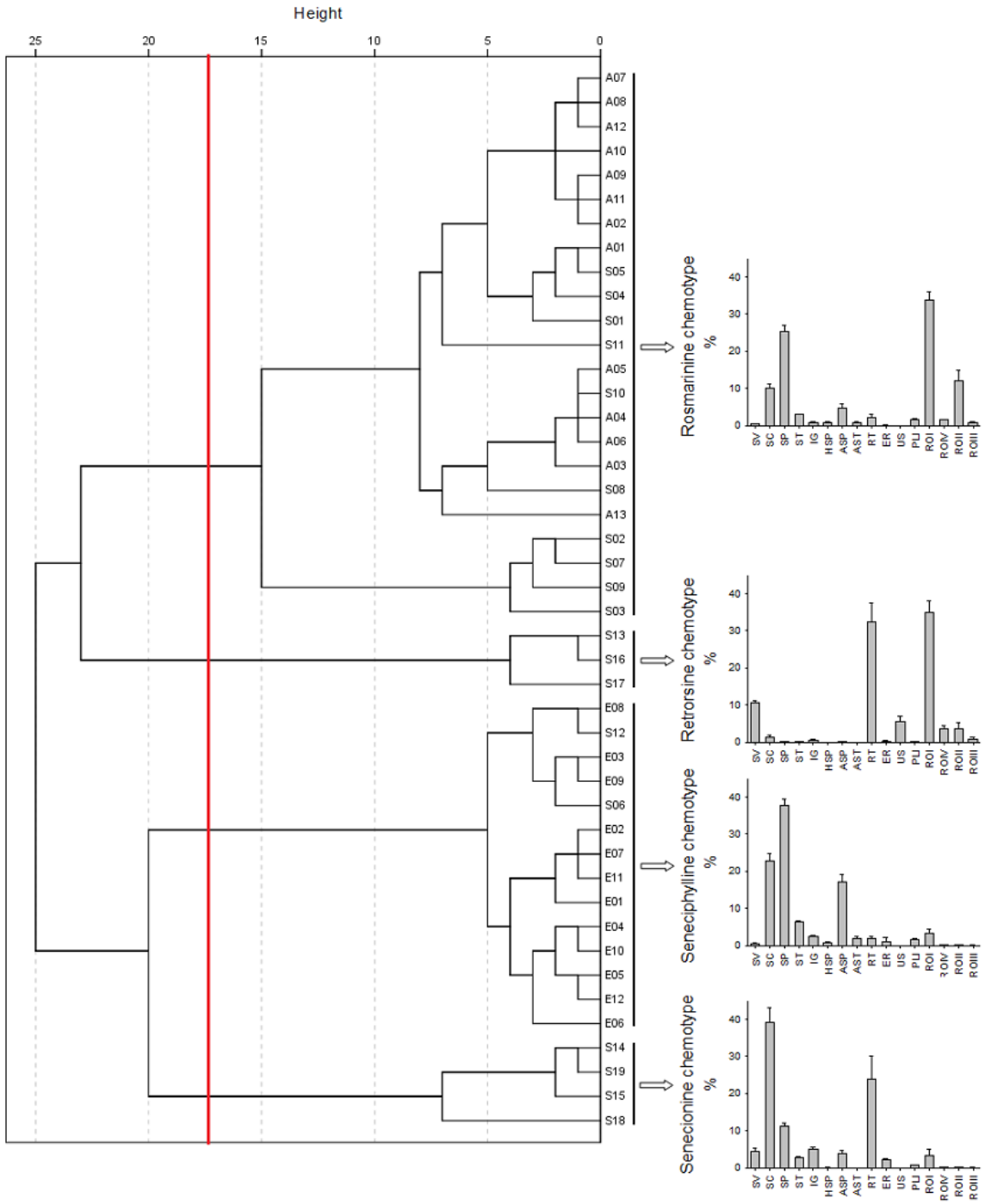

(a)
(b)
PC1 (20.5%)
PC2 (13.3%)
PC1 (31.5%)
PC2 (20.8%)
ROII
ROIII
ROI
ROIV
PLI
HSP
AST
SP
ST
ASP
PLII
UKI
SV
SC
IG
Rosmarinine chemotype
Seneciphylline chemotype
Retrorsine chemotype
Senecionine chemotype
A01 A02 A03 A04 A05 A06 A07 A08 A09 A10 A11 A12 A13 E01 E02 E03
E04 E05 E06 E07 E08 E09 E10 E11 E12 S01 S02 S03 S04 S05 S06 S07
S08 S09 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19

Fig. 5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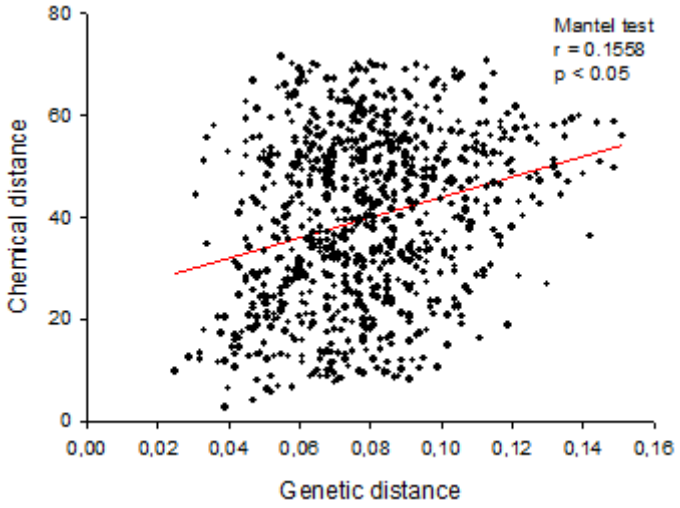
E07
E11
E01
E04
E10
E05
E12
E06
S14
S19
S15
S18
Rosmarinine chemotype
%
Retrorsine chemotype
%
Seneciphylline chemotype
%
Senecionine chemotype
%
SV
SC
SP
ST
IG
HSP
ASP
AST
RT
ER
US
PU
ROI
ROIV
ROII
ROIII

Fig. 6


Mantel test
r = 0.1558
p < 0.05
Chemical distance
Genetic distance
0
20
40
60
80
0,00
0,02
0,04
0,06
0,08
0,10
0,12
0,14
0,16

ELECTRONIC SUPPLEMENTARY MATERIAL

Table S1 Pyrrolizidine alkaloids (PAs) found in *Senecio pterophorus* classified by their necine base (Type) and numbered by average retention time (RT) in GC-FID, and including the mass (MW), the minimum and maximum RT in GC-FID and the average RT in LC-MS/MS

Type	PAs	#	Mass (MW)	GC-FID			LC-MS/MS RT (min)
				Maximum RT (min)	Minimum RT (min)	Average RT (min)	
Internal standard	Monocrotaline	0	325	16.898	17.010	16.927	ND
1,2-unsaturated PAs	Senecivernine	1	335	17.038	17.102	17.063	9.62
	Senecionine	2	335	17.243	17.399	17.285	9.45
	Seneciphylline	3	333	17.512	17.782	17.575	8.71
	Spartioidine	4	333	18.230	18.333	18.261	8.51
	Integerrimine	5	335	18.387	18.449	18.415	9.26
	Jacobine	6	351	19.926	19.964	19.947	7.46
	Hydroxy-seneciphylline	7	349	20.186	20.235	20.211	7.25
	Acetylseneciphylline	8	375	20.559	20.657	20.590	11.31
	Acetylspartioidine	9	375	21.361	21.484	21.403	11.14
	Retrorsine	10	351	21.407	21.732	21.472	8.09
	Jaconine	11	387	21.494	21.571	21.525	8.31
	Riddelliine	12	349	21.501	21.784	21.570	7.50
	Unknown (I)	13	363	21.818	21.957	21.869	ND
	Eruciflorine	14	351	22.446	22.636	22.526	7.33
	Usaramine	15	351	22.505	22.605	22.544	7.90
	Unknown (II)	16	393	22.875	22.991	22.926	ND
1,2-saturated PAs	Platyphylline (I)	17	337	18.722	18.780	18.752	10.23
	Platyphylline (II)	18	337	18.823	18.922	18.864	9.94
	Platyphylline (III)	19	337	20.098	20.137	20.123	9.38
	Rosmarinine (I)	20	353	20.943	21.383	21.048	7.96
	Rosmarinine (IV)	21	353	21.983	22.174	22.037	7.87
	Rosmarinine (II)	22	353	22.441	22.586	22.484	8.43
	Rosmarinine (III)	23	353	23.707	23.807	23.732	8.16
	Hydroxy-rosmarinine	24	369	25.016	25.102	25.045	ND

ESM Figure captions

Fig. S1 Extracted spectrums and chemical structures of pyrrolizidine alkaloids found and identified: **a)** monocrotaline (IS), **b)** senecivernine, **c)** senecionine, **d)** seneciphylline, **e)** spartioidine, **f)** integerrimine, **g)** jacobine, **h)** hydroxy-seneciphylline, **i)** acetylseneciphylline, **j)** acetylspartioidine, **k)** retrorsine, **l)** jaconine, **m)** riddelliine, **n)** unknown (I), **o)** eruciflorine, **p)** usaramine, **q)** unknown (II), **r)** platyphylline (I), **s)** platyphylline (II), **t)** platyphylline (III), **u)** rosmarinine (I), **v)** rosmarinine (IV), **w)** rosmarinine (II), **x)** rosmarinine (III) and **y)** hydroxy-rosmarinine

Fig. S2 Heat map of the chemical distances between population pairs (Pop.) based on Euclidean distances on a cluster analysis. Population codes correspond to the following regions: A01–A13 = Australia, E01–E12 = Europe, S01–S05 = Western Cape Province in South Africa and S06–S19 = Eastern Cape Province in South Africa

Fig. S1

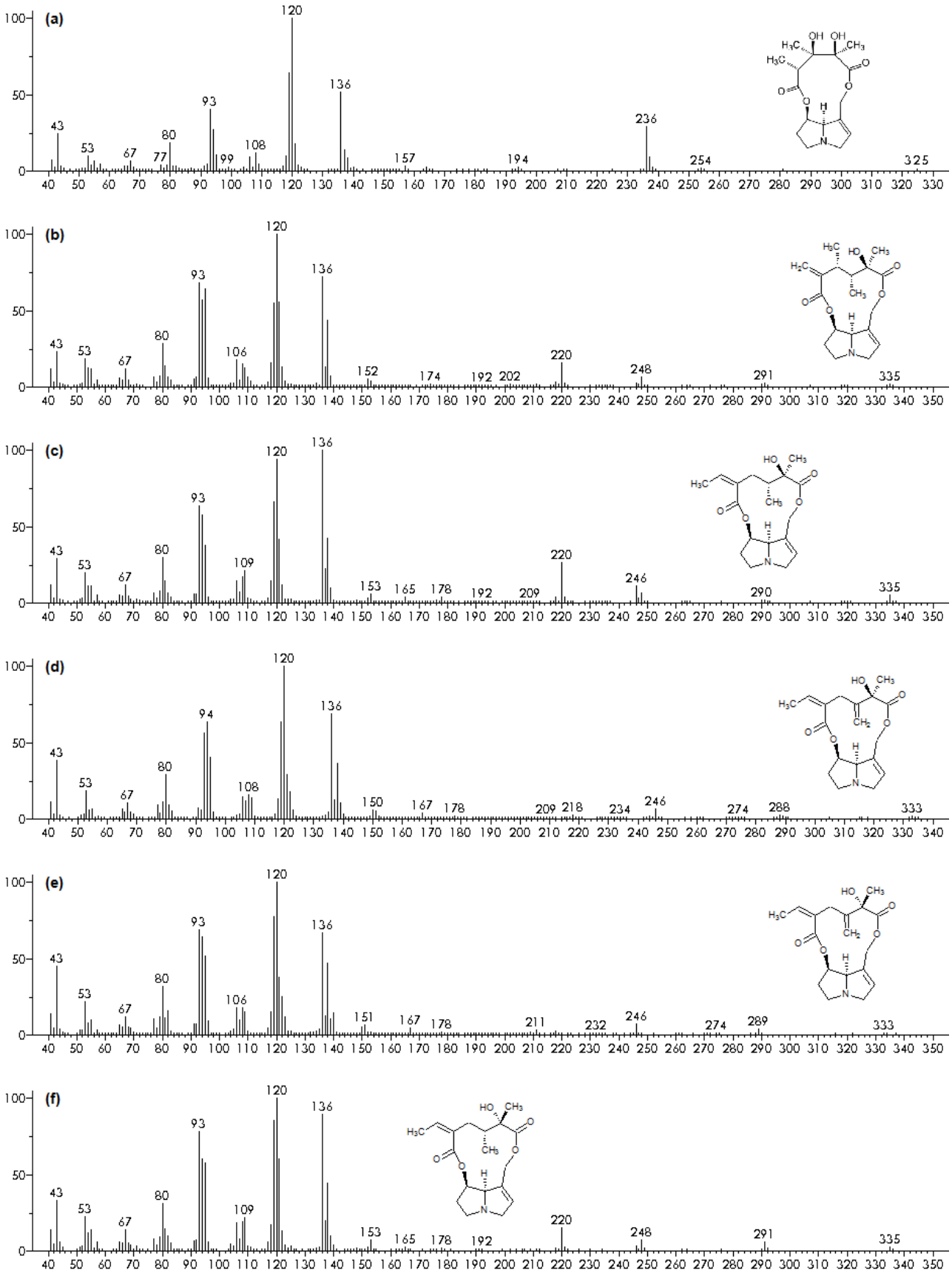


Fig. S1 (cont.)

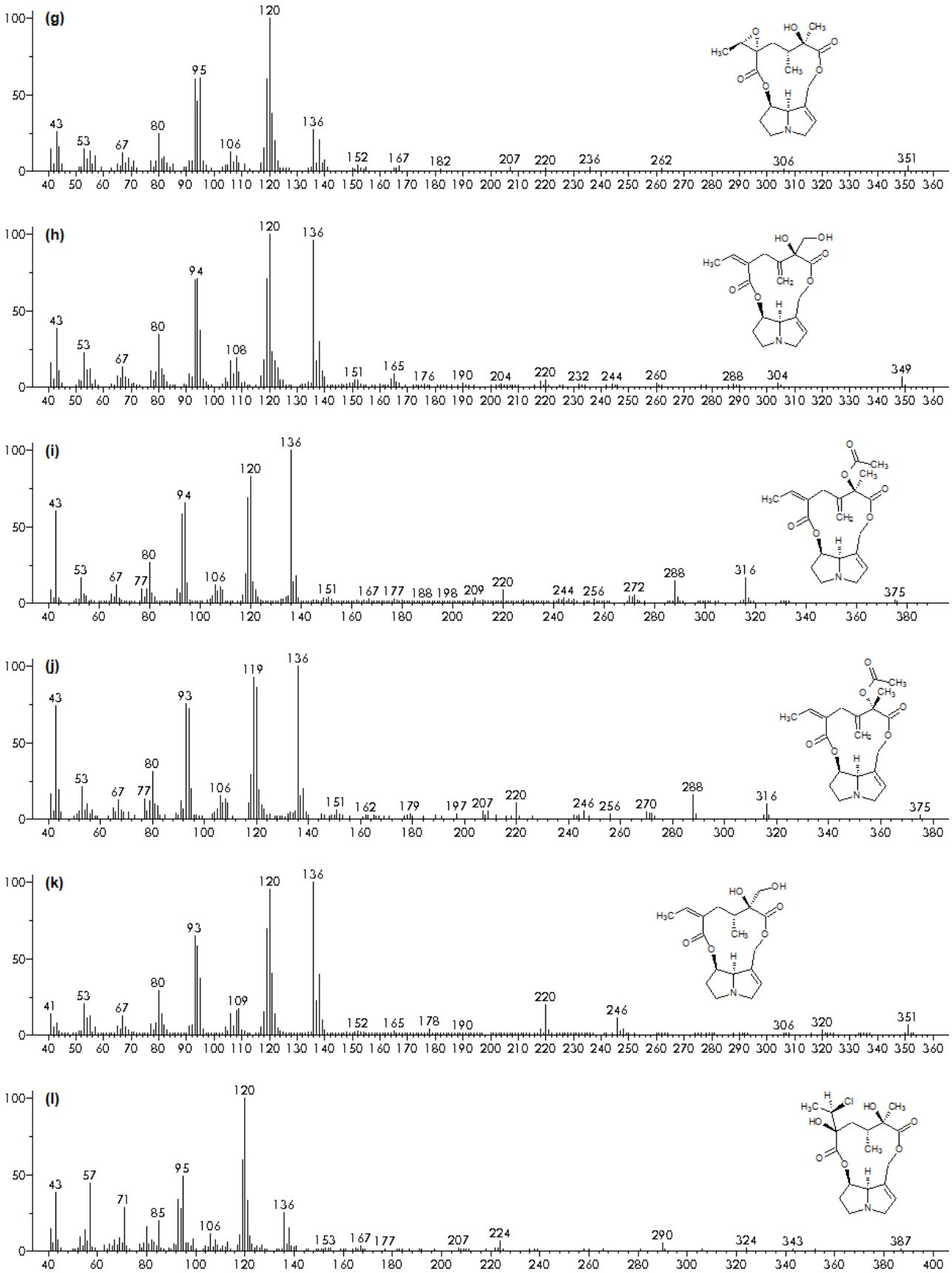


Fig. S1 (cont.)

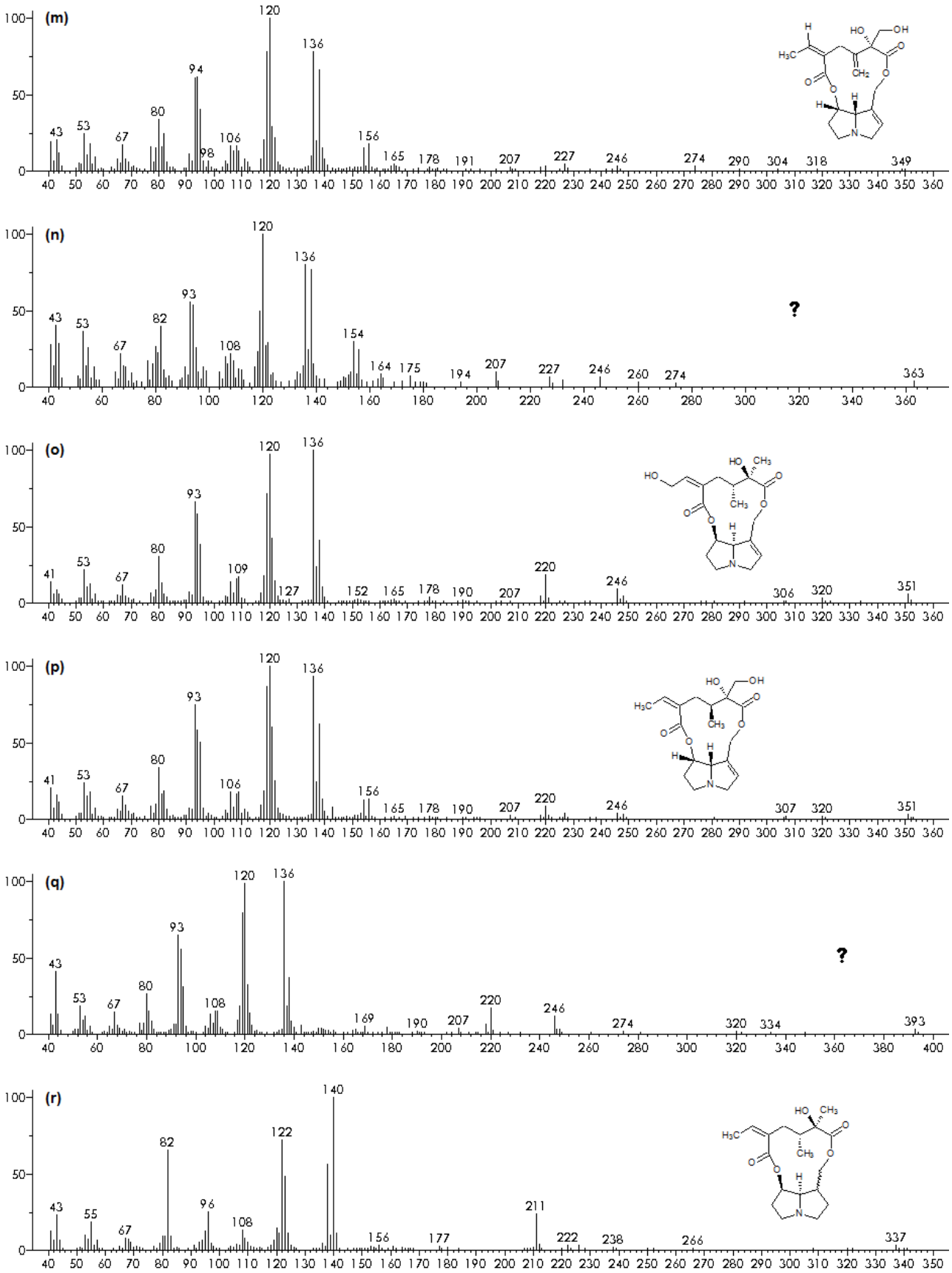
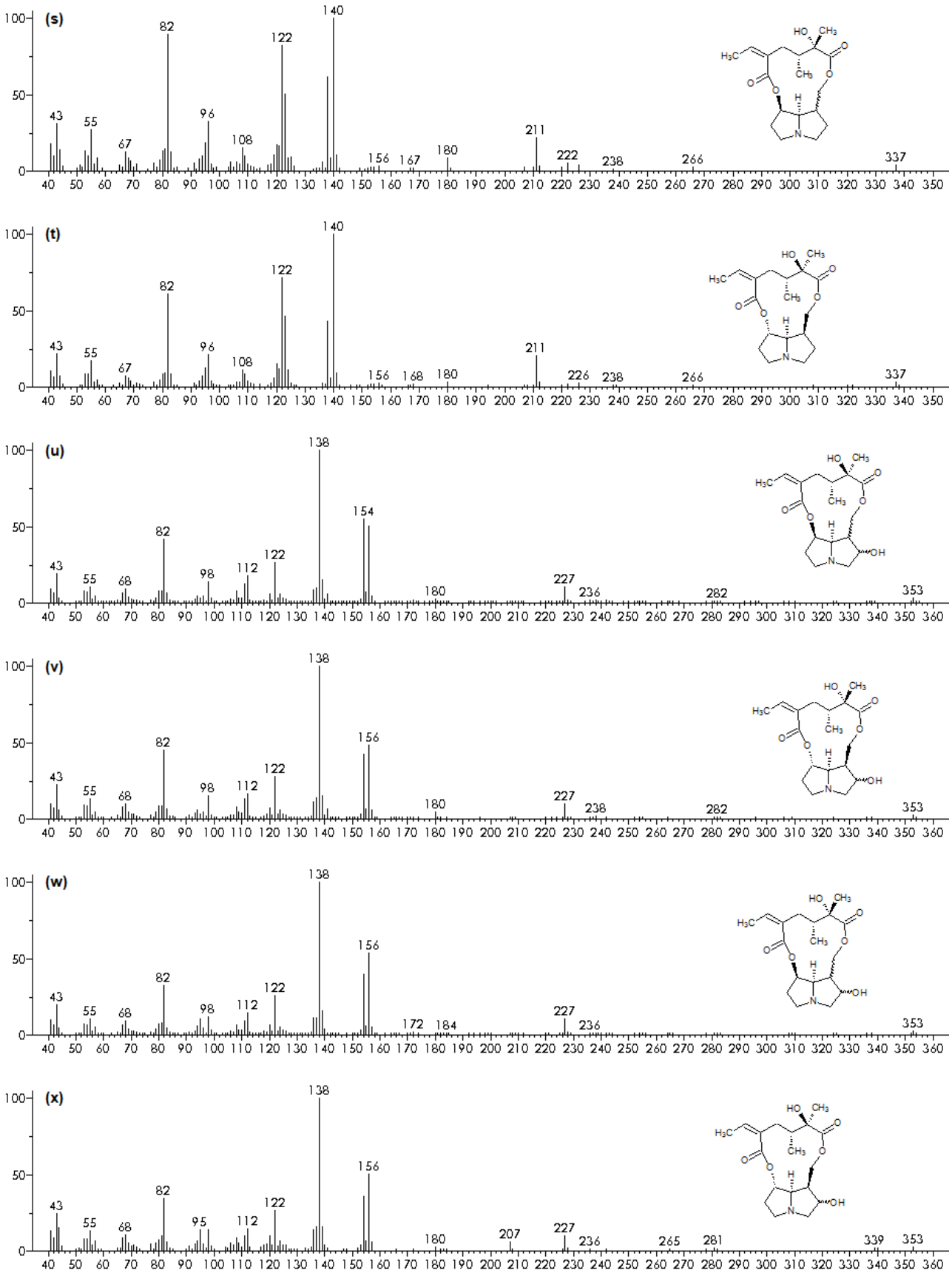


Fig. S1 (cont.)


(s)
140
82
122
43
55
67
96
108
211
156
167
180
222
238
266
337
(t)
140
82
122
43
55
67
96
108
211
156
168
180
226
238
266
337
(u)
138
154
82
122
43
55
68
98
112
227
180
236
282
353
(v)
138
156
82
122
43
55
68
98
112
227
180
238
282
353
(w)
138
156
82
122
43
55
68
98
112
172
184
227
236
353
(x)
138
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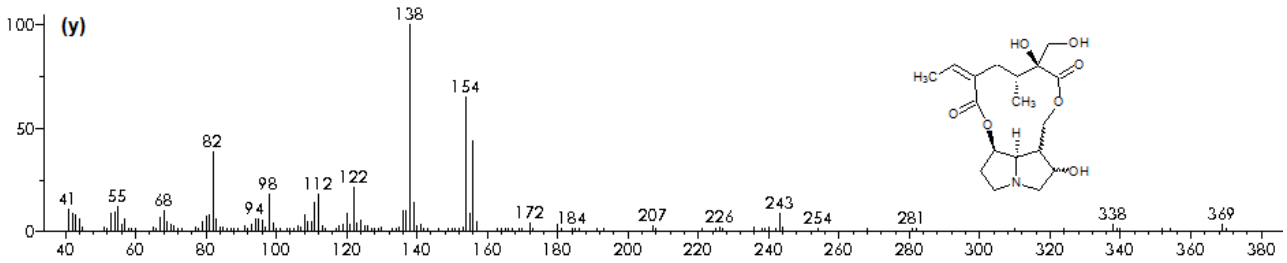
353

Fig. S1 (cont.)


(y)
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0
138
154
82
41
55
68
94
98
112
122
172
184
207
226
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338
369
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
HO
OH
H3C
CH3
O
O
O
O
H
N
OH

Fig. S2


Pop.
Euclidean distances
A01 A02 A03 A04 A05 A06 A07 A08 A09 A10 A11 A12 A13 E01 E02 E03 E04 E05 E06 E07 E08 E09 E10 E11 E12 S01 S02 S03 S04 S05 S06 S07 S08 S09 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19
Similarity
Dissimilarity