Peucedanum ostruthium – ethnobotany and coumarin content in Val d'Anniviers (VS, CH) and Pyrénées-Orientales region (FR)

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ABSTRACT

Peucedanum ostruthium (L.) W. D. J. Koch is a flowering plant of the family Apiaceae endemic to the European Mountains. It is used as a medicinal plant since medieval times. The bioactivity of this plant is attributed to its coumarin content, and the rhizomes are the plant part generally used for preparations on the market. Ethnobotanical uses of *Peucedanum ostruthium* are reported for localized regions in the Alps, like in Switzerland, Austria, and Italy. Distribution maps show *Peucedanum ostruthium* abundant presence in the Alps as well as in the Pyrenees, but little is known about uses of the plant in the Pyrenees. In this study the current ethnobotanical knowledge of *Peucedanum ostruthium* in the Pyrénées-Orientales region in France and in the Val d'Anniviers valley in Switzerland is reported and compared, based on semi-structured interviews. The coumarin content of plant individuals originating from these two geographical distinct regions is analysed at a qualitative and semi-quantitative level. PCA of the HPTLC bands' luminescence in relationship to the place of origin and the sites' altitude from which the samples originate is performed on 79 plant individuals. A small subset of samples, 10 individuals, is also analysed at the genome level with DNA barcoding using the intragenic spacer region *psbA-trnH*.

Osthole presence was shown only in six individuals from the 30 individuals originating from France and no individual from Switzerland showed presence of osthole. The PCA analysis demonstrated no correlation between HPTLC fingerprint and country of origin or altitude from which the individuals were collected. The DNA barcoding showed exact same nucleotide sequence of the *psbA-trnH* region for the analysed samples, five of which were coming from the collection site in France with highest altitude and five were collected at the lowest altitude in Switzerland.

The findings presented in this study suggest that ethnobotanical knowledge of *Peucedanum ostruthium* is more prevalent in Val d'Anniviers than in the Pyrénées-Orientales region. The HPTLC analysis did not show strong qualitative or semi-quantitative divergencies in the coumarin content of the individuals coming from the two regions. The results of DNA barcoding support the hypothesis that populations of *Peucedanum ostruthium* across the Alps and Pyrenees Mountain ranges have low genetical variability.

1. INTRODUCTION

1.1. Distribution and botany of *Peucedanum ostruthium*

Peucedanum ostruthium (L.) W. D. J. Koch (**Figure 1**) is a species of the family Apiaceae from the asterids, angiosperms. This species is endemic to the central and southern European mountains (POWO 2022; Tutin et al. 1968) and the current distribution concentrates in Europe (**Figure 2** and **3**), with few points of occurrence in North America and a very dubious report in the gulf of Aden (GBIF 2022; Infoflora 2022; INPN 2022). The species was recently classified into a separate genus *Imperatoria* on the base of molecular studies, as *Imperatoria ostruthium* (Spalik, Reduron, and Downie 2004). For the present study the species is called *Peucedanum ostruthium* and abbreviated *P. ostruthium* to keep congruence with the *Flora Helvetica* (Lauber, Wagner, and Gygax 2018), the Swiss botany reference book, and other standard reference systems (POWO 2022). *Peucedanum ostruthium* is also known under the common name *masterwort* in English and is called with the vernacular name *impératoire* in French. Habitats where this plant grows in Switzerland are wet meadows, megaphorbs and along creeks. As the ecological indicator values summarised in **table 1** support, this species grows in moderately humid, nutrient rich and half shadowy habitats (Landolt et al. 2010) and can be found at montane, subalpine, and alpine altitudes (Lauber et al. 2018). In the central

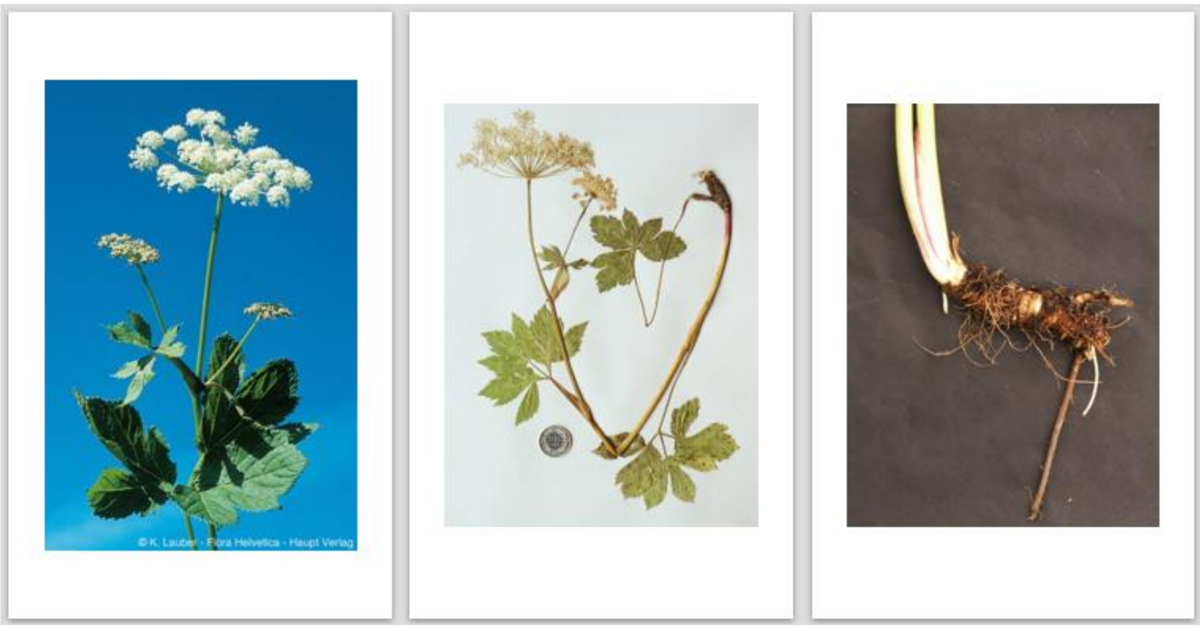

Figure 1: Peucedanum ostruthium *fresh (Lauber et al., 2018); herbal specimen of the whole dried plant and fresh rhizome (Baltisberger et al., 2022).*

alps, the montane zone is between 1300 to 1500 m and the subalpine extends between 1900 and 2400 m (Hess, Landolt, and Hirzel 1965).

Plants of specie *ostruthium* are between 40 and 100 cm tall and have three big and stalked leaflets, each one of them further divided in three differently deep divided parts (Lauber et al. 2018). The plant presents underground growing stout, nodular rhizomes (Baltisberger et al. 2022; Tutin et al. 1968) and has a strong aromatic odour (Hegi 1925).

Table 1: Some ecological indicator values of Peucedanum ostruthium *(Landolt et al. 2010)*

Climatic factors	**Value attributed and explanation**
Light value	3 penumbra
Temperature factor	2 subalpine
Continentality	3 suboceanic to subcontinental: medium relative humidity; moderate daily and annual temperature fluctuations; rather cold winter temperatures.
Soil factors	
Humidity value	3 moderately humid
Reaction value	3 pH 3.5->8.5
Nutrient value	4 nutrient rich

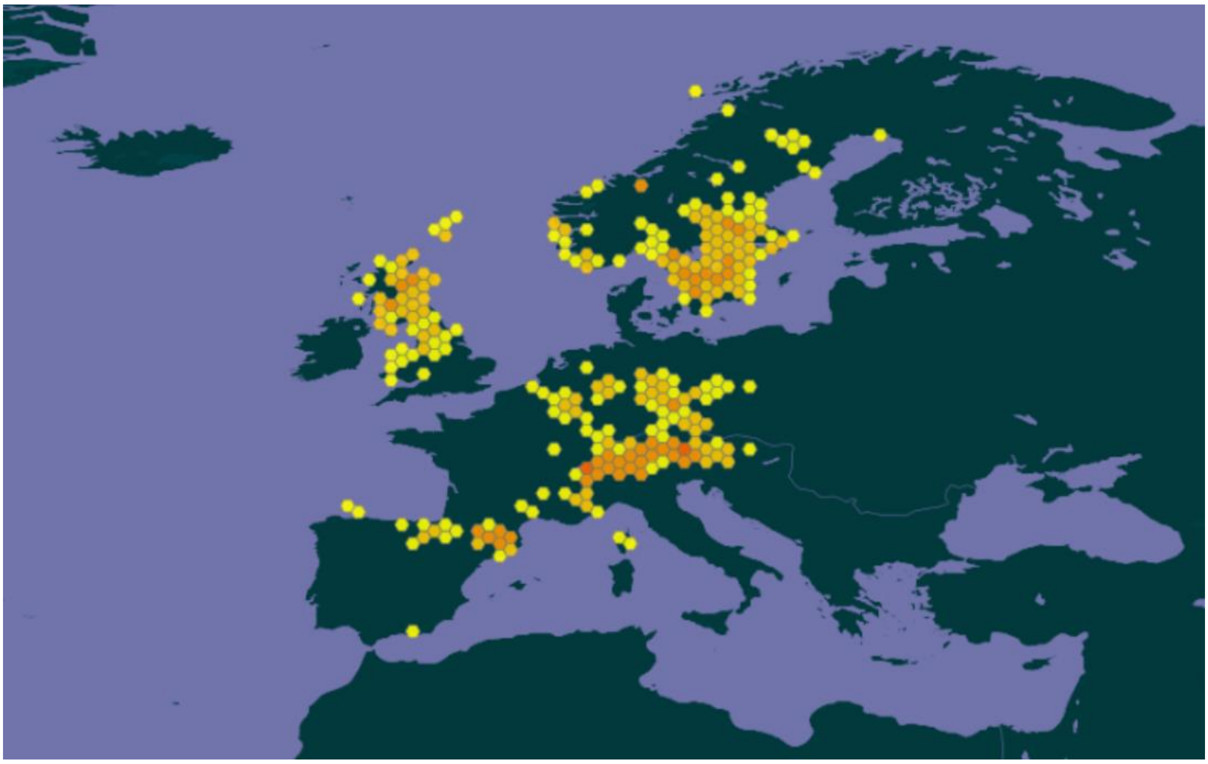

Figure 2: Peucedanum ostruthium *distribution in Europe (GBIF, 2022)*

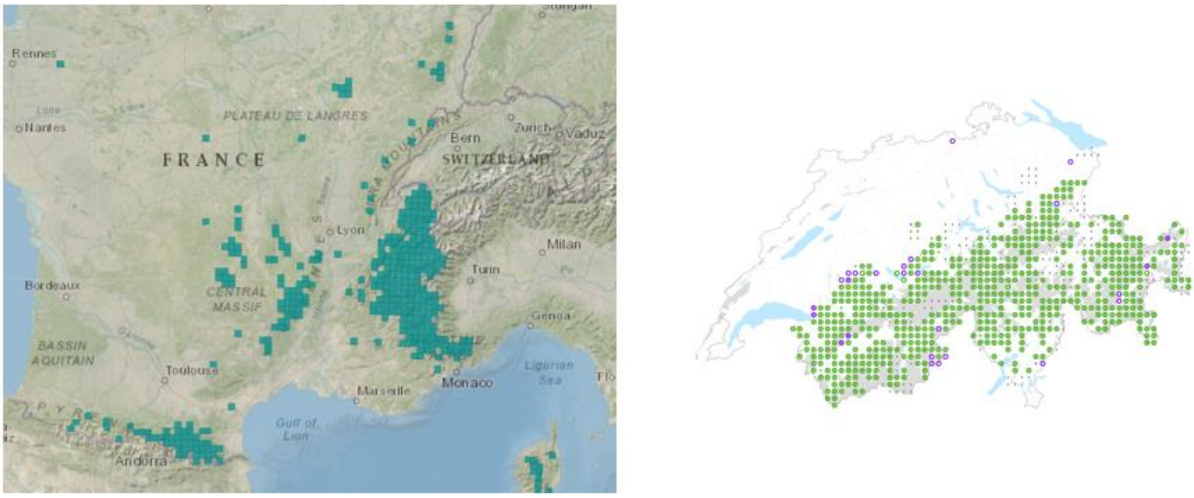

Figure 3: Peucedanum ostruthium *distribution in France (INPN, 2022) and Switzerland (infoflora, 2022)*

1.2. Ethnobotanical aspects

Peucedanum ostruthium has a long history as medicinal plant. Reports of medicinal use of this plant can be found in written form starting from the herbal work of *Macer floridus* which has been written between the 9th and the 11th century (Mayer and Goehl 2001) and Zöring (1911) also refers that *P. ostruthium* was seen as a powerful remedy since the 9th century. Traces of its use can be followed through past time periods from monastic medicine (8th to 12th Century) to the contemporary era (21st Century) (Brioschi, 2020), but it can be understood that it's use diminished over time and is nowadays only used by few people (Müller 1890). However, some recent ethnobotanical studies documented local current uses of *P. ostruthium*; in the Master thesis of Wegmann (2013), it is mentioned as the most used plant species for fumigation in Prättigau, canton Graubünden, Switzerland and in the work of Brüschweiler (2008) is the most often used wild plant in Val d'Anniviers, canton Valais, Switzerland. Also in adjacent regions to Switzerland this plant species is reported as a frequently collected plant; *P. ostruthium* is one of the most frequently listed gathered species in the Biosphere Reserve Grosses Walsertal in Austria, where it was free listed by 26% of the participants (Grasser, Schunko, and Vogl 2012). Also in the upper Varaita valley in Italy this plant species appears among the most quoted wild gathered plant species, with more than 40% of the informants who quoted it for medicinal or culinary uses (Pieroni and Giusti 2009).

The general evolution of the use of this plant species according to the literature was very nicely summarized in the Master Thesis of Camille Brioschi (Brioschi, 2020). Through the Monastic, Renaissance, Modern to contemporary eras and from recent ethnobotanical studies the rhizomes of *P. ostruthium* appear to be the most used plant part. Rhizomes and leaves find applications in gastrointestinal, dermatological, respiratory and detoxification medical fields, but they also appear for veterinary purposes rather in modern and recent times (Brioschi, 2020).

Peucedanum ostruthium appears in the list of the phytopharmaceuticals in the biggest Medication Lexicon of Switzerland, the PharmaWiki Lexicon, with the drug name from its underground part Imperatoriae rhizoma (PharmaWiki 2022). There are three herbal medicines containing this herbal drug authorized by the Swiss Agency for Therapeutic Products (Swissmedic 2022) available on the market in the dispensing category D, which in the Swiss regulation is obtainable without prescription. *Peucedanum ostruthium* or the derived drug are not mentioned in the Swiss Pharmacopoeia (Pharmacopoea Helvetica, Ph. Helv.), but naturopathic physicians in Switzerland can and do prescribe preparations from *P. ostruthium* rhizomes, such as tinctures easily obtainable from Swiss laboratories like the Schmidt-Nagel company. The situation in France looks different: pharmacies can only do preparations with plants listed on the list A of the French pharmacopoeia, which contains 365 medicinal plants used traditionally, among which *P. ostruthium* is not mentioned. This list affirms to contain many of the plants used in traditional Chinese, European and other medicine. Naturopathic practitioners in France are strongly limited by this list, knowing that prescriptions with plant preparations made with plant species outside of this list are difficult to obtain. The European Pharmacopoeia (Ph. Eur.) also does not mention *Peucedanum ostruthium*.

1.3. Phytochemistry

Several polyphenolic compounds, including phenolic acids, simple coumarins, furanocoumarins and flavonoid glycosides were identified in *P. ostruthium* crude extracts (Palmioli et al. 2019). Terpenes like the monoterpenes sabinene, 4-tepineol, β-caryophyllene, limonene, β-pinene and the sesquiterpene β-caryophyllene were identified in the essential oil from herb and rhizome of *P. ostruthium*, together with the coumarin osthole (Cisowskia et al. 2001).

Peucedanum ostruthium species have a high content of coumarins. From dichloromethane extractions the content of coumarins per gram dry weight of plant material was deducted to be 6% and 78% of the total content from the extraction, the main coumarins, in decreased

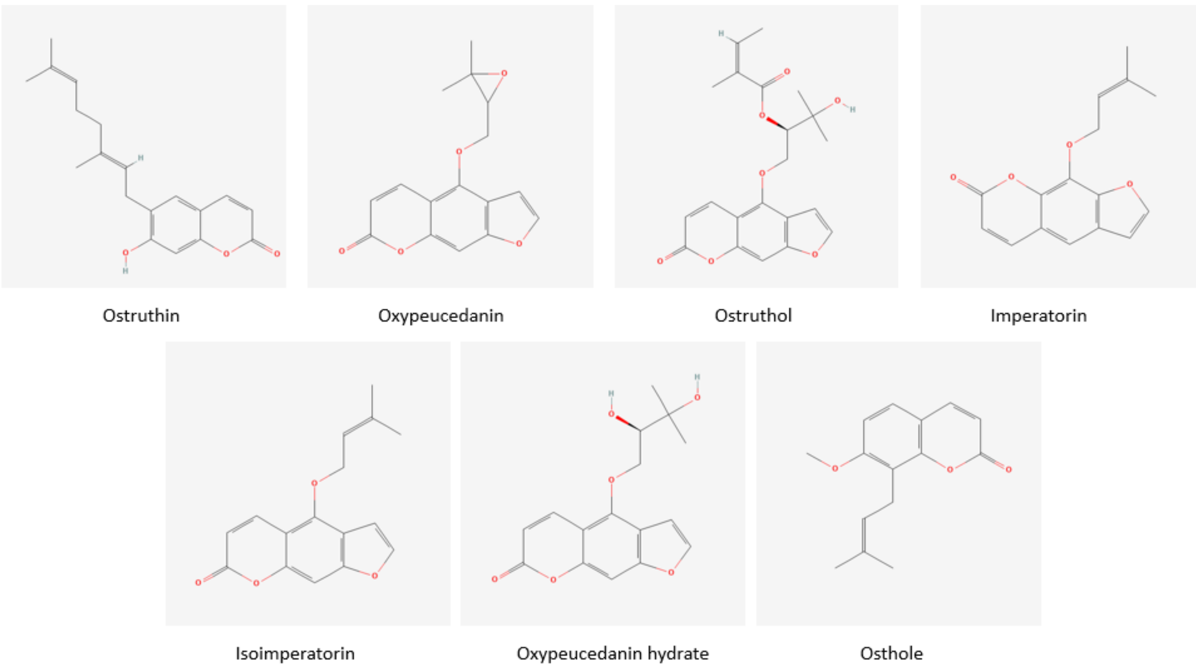

Figure 4: Coumarins identified in P. ostruthium. *From left to right found in decreasing concentration.*

order of concentration, being: ostruthin, oxypeucedanin, ostruthol, imperatorin, isoimperatorin, oxypeucedanin hydrate, and osthole, all shown in **figure 4** (Joa et al. 2011). Other than their importance in terms of amount in the plant, coumarins appear also to possess different medical-pharmacological activities (Sticher, Heilmann, and Zündorf 2015). Modern pharmacological studies showed that coumarins present in this plant have the potential to help with a variety of common and important health issues. Among others, the compound ostruthin was identified to play a role in the inhibition of vascular smooth muscle cells (VSMC) implicated in thickening of arterial walls in the atherosclerosis cardiovascular disease (Joa et al. 2011). Osthole derived amines showed anti-inflammatory activities (Zimecki et al. 2009). Extracts of *P. ostruthium* were found to inhibit acetylcholinesterase (AChE) (Urbain, Marston, and Hostettmann 2005) and to prevent neurotoxicity in neuronal cells (Palmioli et al. 2019), phenomena implied in the Alzheimer's disease. Other studies showed that coumarins present in this plant have inhibitory effect on lung and skin pathogenic bacteria and mycobacteria (Gökay et al. 2010; Schinkovitz et al. 2003).

The chemical structure of coumarins suggests lipophilicity as well as good alcohol affinity and they can be absorbed by the membranes of the digestive track and by the skin (Sticher et al. 2015).

1.4. HPTLC

High-Performance Thin Layer Chromatography (HPTLC) is a common method used in herbal drug identity tests (Attimarad et al. 2011). Thanks to previous studies on HPTLC based coumarin identification (e.g., Frommenwiler et al., 2018) and the master thesis of Brioschi (2020), the method for qualitative coumarin identification in *P. ostruthium* was already established and demonstrated as effective.

1.5. DNA barcoding

DNA barcoding is the process for species identification based on short DNA segments' nucleotide diversity (Vijayan and Tsou 2010). Plastid genome is often chosen for DNA barcoding in plants (Yuan et al. 2015). The Chloroplast DNA (cpDNA), due to its sequence variability is considered useful for intraspecific phylogeographical analysis (Honjo et al. 2004). The *psbA-trnH* intragenic marker was established as the most variable plastid region in angiosperms and was therefore suggested for angiosperm identification (Kress et al. 2005). Lately *P. ostruthium* was genetically analysed by high-resolution melting curve analysis (HRM) using, among others, the *psbA-trnH* marker to distinguish the species from closely related species (Schmiderer, Ruzicka, and Novak 2015).

1.6. Aims of the study

The main aims of this study are to investigate three different aspects of *P. ostruthium* populations in the Swiss alpine area of the Val d'Anniviers and in the Pyrénées-Orientales French area.

The first research question concerns the ethnobotanical knowledge about this species in the research areas in France and Switzerland

- Do people use *P. ostruthium* in the Pyrénées-Orientales region and in Val d'Anniviers?
    - How is the plant used?
        - How is it prepared?
        - How is it applied?
        - For which purpose is it used?

The second interest concerns the coumarin content in *P. ostruthium* individuals.

- Is there a qualitative difference in the coumarin components contained in plant samples coming from France and coming from Switzerland?
    - Is there a correlation with the place of origin?
    - Is there a correlation with the altitude level at which they were collected?

The third aspect of interest is about the genetic difference of the samples collected from France and from Switzerland.

- Does the mountain range of origin of *P. ostruthium* or the altitude gradient along which the plants grow come along with genetic differences at the level of the chloroplast DNA (cpDNA) which can be observed with DNA barcoding?

2. MATERIAL AND METHODS

2.1. Study site and plant material

The fieldwork was performed in two regions in two different mountain ranges: the Pyrenees in France and the Alps in Switzerland. The study site in France was delimited to the Pyrénés-Orientales department of the Occitanie administrative region and includes the communes Pyrénées Cerdagne and Pyrénées Catalanes. The fieldwork in Switzerland was done in the Canton of Valais and was limited to the Val d'Anniviers Alpine valley, which is within the district of Sierre. The study sites circled in violet for Switzerland and in yellow for France are illustrated in **figure 5**.

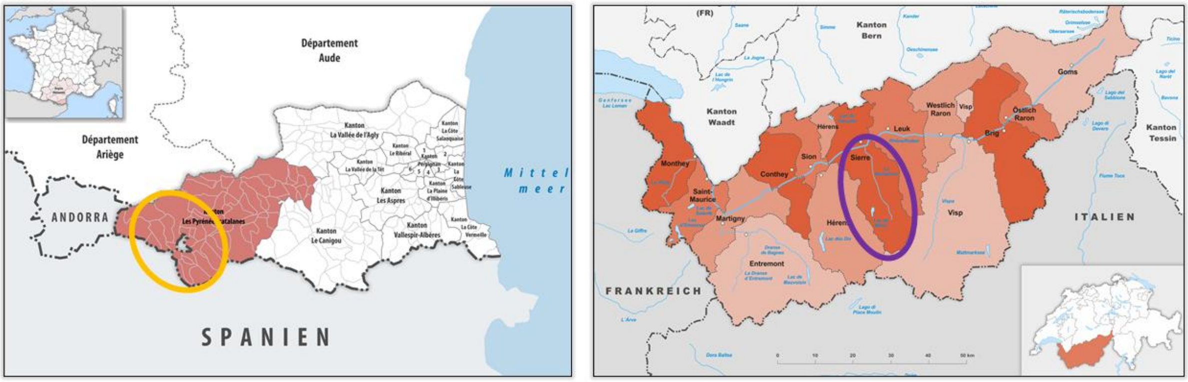

Figure 5: Study sites in Switzerland (left) and in France (right). (Modified from Wikipedia)

Within the region in France six locations (pinned in yellow on **figure 6**) were chosen and from each of them five individuals of *P. ostruthium* were collected for a total of 30 individuals from France. From the region in Switzerland 10 locations were picked (pinned in violet on **figure 7**) and from each location five individuals of *P. ostruthium* were collected, as in France, for a total of 50 plants for Switzerland. The sites name on **figures 6** and **7** refer to the ID of the sites reported on **table 2**. The locations were selected following a gradient of altitudes from the minimal to the maximal altitude at which the plant could be found and with the condition that the *P. ostruthium* populations had at least 20 individuals and five of them with at least 2 m distance from each other.

Plant material was collected between September 4th and 16th 2021 in France and between September 22nd and 27th 2021 in Switzerland.

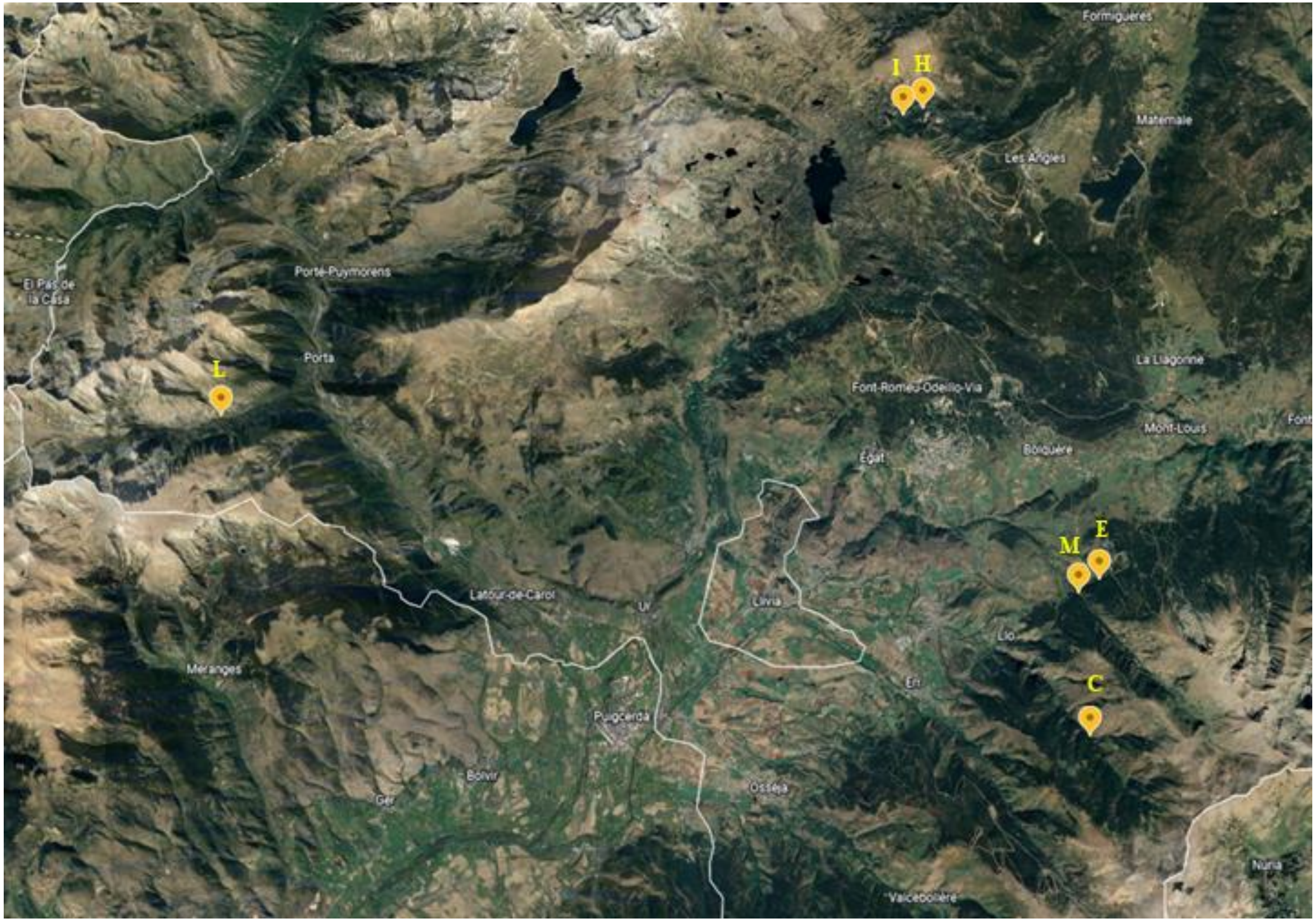

Figure 6: Collection sites in the Pyrénées-Orientales Region, pinned in orange (Google Earth modified image)

Figure 7: Collection sites in Val d'Anniviers, pinned in violet (Google Earth modified image)

Table 2: Collection sites ID and their altitude in France and Switzerland

Colleciton site ID FRANCE	Altitude (m)	Collection site ID SWITZERLAND	Altitude (m)
M) Eyne en bas	1680	CT) Tzchoudana	1718
C) Llo	1769	CO) St-Luc, resto	1756
E) Eyne en haut	1786	CU) Zinal, Cottier en bas	1800
		CH) Zinal, Cottier en haut	1875
		CI) St-Luc, prairie	1879
L) Porta	1951	CN) St-Luc, croisement riv	1936
I) Balmeta en bas	2005	CS) Petit Mountet to Zinal, Navissance	1964
H) Balmeta en haut	2049	CM) St-Luc, sentier en forêt	2064
		CR) Zinal to Petit Mountet, cabanne	2095
		CL) St-Luc, top	2254

From each collection site, the accompanying plants were also recorded, and herbal specimens were collected and are stored at the Herbaria of the University of Zürich (Herbaria Z+ZT).

From each individual plant, the leaves were collected for the genetic analysis and the rhizomes were used for the HPTLC analysis. Leaves and rhizomes of 80 individuals were collected in total. Only the rhizome of one plant in Switzerland was lost, so the total plant material available for the genetic analysis was still 80 samples, but for the HPTLC analysis 79 rhizome samples were available. Pictures of the plant material as well as the collection site were taken during collection.

Criteria for selecting the individuals, to collect the plant material from, were healthy leaves (with as few as possible pathogen or herbivore damage signs) with a leaf area of at least 2 cm^2 per leaflet, an intense green colour of the leaflets as well as robust structure of the rhizomes were favoured. Because this combination of characteristic was difficult to find and rhizomes development could be established only after digging in the ground, individuals with healthy looking leaves were selected. To collect leaf samples, three squares of approximately 2 cm long sides, one for each lobe of the leaf of one individuum were cut, put into tea bags, and stored in zipper bags containing silica gel for drying. Rhizomes were cut at the base of the stem and followed underground until a branching point or cut after a long segment without nodes. The fresh rhizomes were measured and stored in coffee filter paper and let air dry.

2.2. Interviews

Interviews were conducted between the 19th to the 29th of July 2021 in France and from the 5th to the 22nd of August 2021 in Switzerland. For recruiting local informants, the snowball network sampling method was used (Russell 2017). Thirteen informants were sampled in the Pyrénées-Orientales region and 20 in Val d'Anniviers. The initial informants were searched in medicinal plants related contexts and during fieldwork hiking and camping. Wherever possible a fresh plant individuum was presented to the informants.

The first contact point of the search for informants in Val d'Anniviers was taken at the tourism office of Grimentz, where a medicinal garden activity was listed and the person organising it was the first informant, informant 1 (I1). The rest of the sampling of informants is shown in **figure 8**.

People knowing about uses of the same plant species were considered having a general knowledge and were not considered for interviews because of the personally admitted lack of knowledge on this topic, saying that they don't know. People having directly and personally used *P. ostruthium* were considered having practical knowledge and were considered for the interviews.

Four of the informants (I5, I10, I11 and I12) in Val d'Anniviers mentioned only one practical use and the context for a more in-depth interview with them was lacking. Semi-structured interviews could take place with Informants one, two, three, four, six, seven, eight and nine.

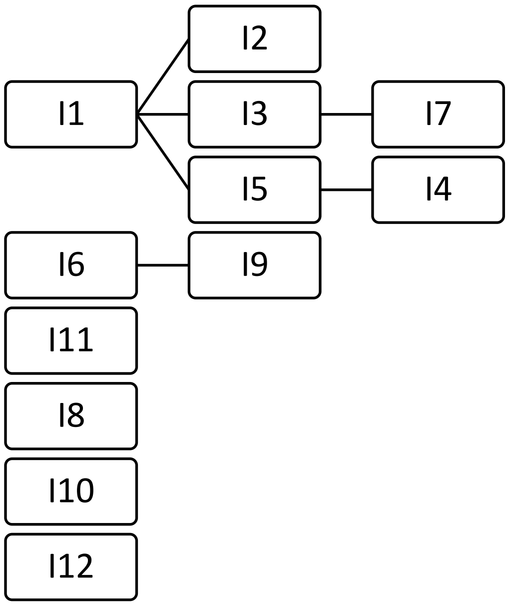

Figure 8: Snowball informants sampling in Val d'Anniviers

The informants were asked if they know the plant and if they responded positively and if there was the predisposition to conduct an interview, one was arranged. The informants were also asked to mention people who might have knowledge about uses of the *impératoire* (masterwort) plant. If the informant was suggested from a previous one, the background of the contact taking was given: who gave the contact and the reason why, in these cases an appointment was arranged, and the initial question was: how do you use the masterwort? (*Comment utilisez-vous l'impératoire?*). A short interview guideline supported the semi-structured interviewing (**Appendix B, Table 9**). The guideline had as objective to make sure specific aspects about the masterwort (*impératoire*) plant knowledge and use were covered during each interview. The order of the questions as well as the exact formulation of them depended on the interview dynamic.

From the 17 people having at least general knowledge about *P. ostruthium*, 11 were of female sex and six of male sex. From the people having practical knowledge about this species 10 were females and two males. The eight informants with which it was possible to conduct an in-depth interview, based on the amount of knowledge they had, were all women.

Informant consent to use the data from the interview and the degree of the anonymity to preserve was verbally asked and agreed upon at the beginning or at the end of the conversation. The conversations were recorded, or detailed notes were taken. Transcriptions were made with the help of the TRINT software (Trint 2022). The "Guidelines for Collecting and Recording Ethnobotanical Information" from the Book of Alexiades (Alexiades 1996) gave important inputs for establishing use report categories and skimming the interview transcriptions accordingly.

Plots were made with the RStudio software (Version 2022.02.1, © 2009-2022 RStudio, PBC)

2.2.1. Categories and use reports

People having directly and personally used *P. ostruthium* were considered having practical knowledge. People knowing about uses of the same plant species were considered having a general knowledge. Use reports (Urs) are citations of a plant part for a specific use mentioned by one person.

In this work the botanical term rhizome is used for the underground thick part of the plant, even though no informant ever used this term from proper initiative. In this sense the term rhizome and "*racine*" (root) were used and considered to be the same plant part because nobody indicated any other underground part used.

For categorising the preparations and applications of the plant parts the guidelines of Alexiades (Alexiades 1996) were very helpful; divergences from the relevant terms defined in the book and briefly summarised in **table 3** are described as follows. In this study the term tea was used for all water-based preparations from leaves because the water was reported to be always warmed (excluding the term cold infusion) and never boiled (excluding the term decoction). The term softened leaves refers to leaves that have been stored in dry form but at the moment of the preparation they are softened in water. The way of softening this leaves is similar to the one described in the results for the tea preparation. Because the application of fresh, frozen, or softened leaves were very similar and frozen leaves were reported only once, these preparations are grouped together in the fresh or softened leaves preparation form in the results.

Ways of preparing the smoke also showed differences, described mor in detail in the results. For simplicity all forms of smoke preparations were grouped together as smoke category. The term "passing smoke over affected area" (**Table 3**) was changed to "passing smoke close to affected area" because it is more inclusive toward both uses above areas (for example by directing the smoke to a skin area) and around areas (for example passing the smoke around the ears). The term balm was chosen to cover all oil, fat or lard-based preparations made with the plant parts.

For the applications, because the definition of the terms compress and poultice diverge only for the presence or absence of a bandage, they are often grouped together in this work. Direct and passive inhalation although the first one can see as an internal use and the second one as an external one, were not differentiated in the results of this study but grouped under the broader term inhalation.

Following Staub et al. (Staub et al. 2015), for the categorisation and standardisation of ailments described by the informants the ICPC-2-R Classification (WONCA 2005) were selected, but only the body system level was categorized.

Table 3: Definitions used in this study derived from Alexiades' book part I. Conducting Ethnobotanical Research, Chapter 3 Collecting Ethnobotanical Data: An Introduction to Basic Concepts and Techniques, Subsection Guidelines for Collecting and Recording Ethnobotanical Information (Alexiades, 1996: p. 81-83)

Sub-subsections Plant Resource Use: Medicinal	**Term**	**Definition**
Preparation and storage		
• Processing method	Hot infusion, tea	Boiling water is poured over the plant part. The preparation is left to steep, usually for a few minutes but sometimes longer. It may be drunk hot or cold. Infusions are often used for leaves or other nonwoody tissues of medicinal plants.
	Decoction	Plant part is boiled in water. Decoctions are often used for woody parts (root, bark) of medicinal plants.
	Cold infusion, cold extract	Plant part is steeped in cold water, often overnight, then the mixture is strained. Sometimes the plant part is crushed before or during the addition of water.
	Ointment, cream, salve	Processed plant part is mixed with lard or fat.
Route of Administration or Application		
• External	Blowing or passing smoke over affected area	

	Poultice	Plant part (usually crushed or bruises and mixed with little hot water) is applied directly over area, and area is covered with a cloth or rag.
	Compress	Plant part (usually crushed or bruises and mixed with little hot water) is applied directly to the skin.
	Rubbing	Plant part (usually crushed or mixed with water or processed as ointment or liniment) rubbed on body.

2.3. HPTLC analysis of coumarin content and PCA

To be sure that rhizomes were completely dry, they were put into a food dehydrator (Dörrgerät T2 Prima Vista) at 35-40°C until their weight remained stable, indicating no loss of additional humidity. Each dried rhizome was cleaned from earth residuals with a dish scrubber, measured in length and weighted. If the weight of the rhizome was more than 2 g, the rhizome was cut into one piece of less than 2 g and the remaining part was preserved separately. Each rhizome piece of less than 2 g was disrupted by stainless steel grinding jars of 10 ml in the TissueLyser at 30 Hz for 1 min. The obtained powder was mixed with methanol in a 1:5 (m/v) ratio for extraction. When possible 1g of rhizome powder was added to 5 ml into 15 ml falcon tubes. Each tube was shaken for 10 min at 300 rpm and then centrifuged for 5 min at 5000 rpm. The supernatants were used as test solutions.

The rest of the experiments were conducted at the CAMAG AG company, based in Muttenz, Switzerland. The reference substances xanthotoxin, imperatorin, osthol, isoimperatorin, psoralen, oxypeucedanin and oxypeucedanin hydrate were dissolved in Methanol in a 1:1 ratio and briefly vortexed. A CAMAG HPTLC system consisting of TLC Visualizer, Automatic TLC Sampler 4 (ATS4), Automatic Developing Chamber 2 (ADC2), Derivatizer, and TLC Plate Heater was used for the analysis. The method used for HPTLC analysis was the *P. ostruthium* HPTLC ATLAS / FHH (Brioschi et al., 2020), which was in turn based on HPTLC Fingerprinting of *Angelica gigas* roots (Frommenwiler et al. 2018). Images and profiles were generated by the software visionCATS (version 3.1).

From the HPTLC images, peak profiles from images (PPI) could also be derived with the visionCATS Software. PPI are peaks plotting a luminance factor in function of the RF values. The luminance function is calculated with a sum of RGB values as explained in **figure 14**.

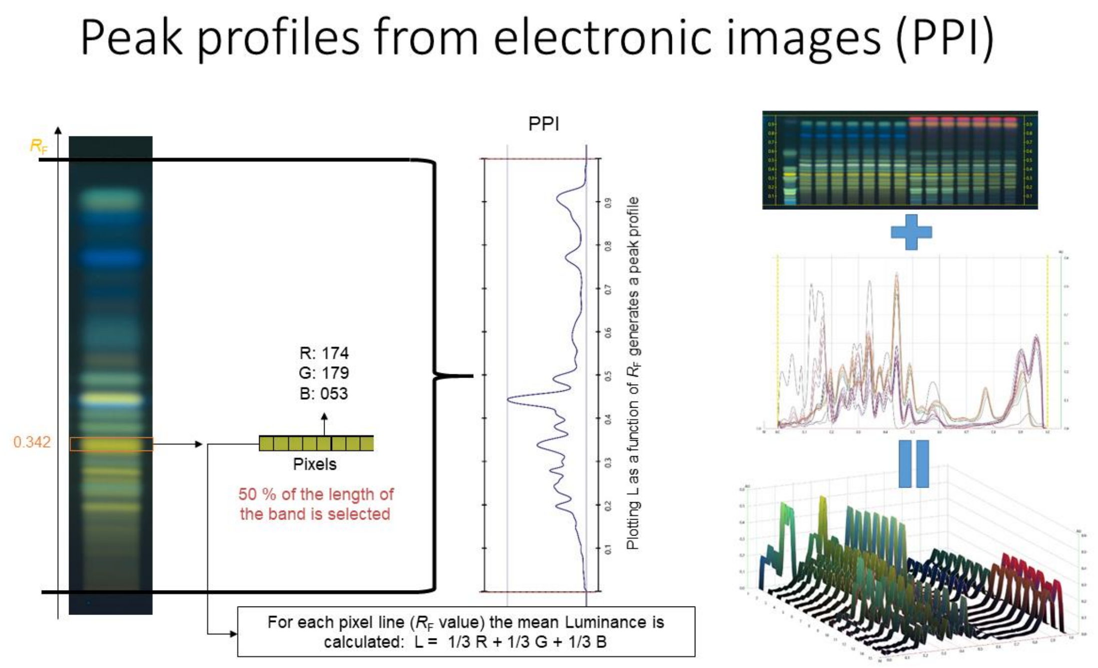

Figure 9: How PPI are generated on the visionCATS Software. Presentation slide from Do Tiên, Head of laboratory CAMAG.

The luminance factor is somewhat interpretable as the intensity of the band colour which has a positive correlation to the concentration of the compound indicated by the band. A principal component analysis (PCA) from these peak's luminance can be seen as a semi quantitative component analysis because higher concentration of the compounds will show a band with more luminance.

2.4. cpDNA barcoding

For the chloroplast DNA (cpDNA) barcoding the intergenic *trnH-psbA* region was used as molecular marker. Ten individuals from the two sites H, in France, and CT, in Switzerland, were selected for this analysis (H0101, H0102, H0103, H0104, H0105, CT0101, CT0102, CT0103, CT0104, CT0105) because of their opposite altitude: highest in France and lowest in Switzerland. For each individual one of the three silica gel dried leaf portions was taken and further segmented with a razor blade to fit a tissue disruption tube of the DNeasy Plant Pro

Kit. For the DNA isolation the protocol present in the kit's handbook, available online, was followed (QIAGEN 2019). The concentration of the extracted DNA was measured, before running the PCR, with the NanoDrop One spectrophotometer and the DNA concentrations were between 5 and 11 ng/µl. The PCR protocol, as well as the primer's sequences is shown in **table 4**.

Table 4: PCR protocol form

Marker	psbA-trnH	
Primers	psbA (forward)	5'- GTT ATG CAT GAA CGT AAT GCT C- 3'
	trnH2 (reverse)	5'- CGC GCA TGG TGG ATT CAC AAT CC- 3'

Component	**Amount**	**Final concentration**
ddH2O		
Buffer 10x	2.5 µL	1x
dNTPs	0.5 µL	200 µM
Forward primer (psbA)	1 µL	0.1-0.5 µM
Reverse primer (trnH)	1 µL	0.1-0.5 µM
Taq DNA Polymerase	0.25 µL	0.05 units/µL
Template DNA	1 µL	
Total	25 µL	

Cycles		
		95°C 15 min
	40x	95°C 45 s
		55°C 45 s
		72°C 90 s
		72°C 9 min

Southern blotting with agarose gel electrophoresis was performed to check the presence of nucleic acid sequences. The DNA of the individual H0101 could not be detected with the southern blot and was therefore excluded from the further procedure, reducing the number of samples sequenced for the *psbA-trnH* region to nine.

After the southern blot, the PCR product was purified with the QIAquick PCR Purificaiton Kit and the Quick-start protocol was followed, also available online (Quick-Start 2018).

The cleaned product was sent to Microsynth AG for the Sanger sequencing of the fragments.

To compare the sequences the MEGA 11 software was used (Version 11.0.10).

3. RESULTS

3.1. Current local knowledge and uses of *Peucedanum ostruthium*

3.1.1. In the Pyrénées-Orientales region

From the thirteen people asked in France only five had heard about the *Impératoire* plant before. Two of them being botanists, one a naturopath and one person known for her knowledge of plants and stones in the region. Nobody of them used the plant personally. Only one person, met at the restaurant of the natural reserve *Valle d'Eyne*, mentioned how preparations with this species can be made. The preparation mentioned were: an infusion with the leaves "for the digestion", an oil for "tonifying the body" with the rhizomes. This woman gathers often plants and defined herself as "*cueilleuse*" (gatherer) but never did these preparations herself; she therefore was not considered to have practical knowledge about *Peucedanum ostruthium* and the interview was very short.

3.1.2. In the Val d'Anniviers region

In Val d'Anniviers the plant *impératoire* is more commonly known. From the 20 people asked, 17 had at least heard about *l'impératoire* (masterwort). Five of them mentioned general knowledge, knowing that it is, or it was, used in a certain way and could describe roughly and with incertitude the use. Twelve of the 17 persons had practical knowledge with *P. ostruthium*, meaning that they used the plant at least once personally.

Figure 10 shows the plant part uses in relationship to the preparations made with them mentioned by informants in Val d'Anniviers through the extensive interviews. The plant parts commonly used are leaves and rhizomes, with 33 use reports for leaves and 13 use reports for rhizomes (all as smoke), only one use of the flowers was mentioned by informant 6 for food decoration purposes. Informants who bought ready to use balm preparations were not considered for this count because the processing of fresh material did not happen in person, and they did not know with which part of the plant the preparation was made.

Beside one culinary use type and the decorative one mentioned before, this plant species in Val d'Anniviers is mostly used for medicinal purposes. Four veterinary use reports were also mentioned, 1 Ur in the digestive, 1 Ur in the musculoskeletal, and 2 Urs in the skin related ailment categories. The ailment categories of the veterinary uses correspond to the most mentioned medical ones and informants reported often to use the same flow of procedure (preparation, application, and ailment category) for humans and animals. Therefore, the veterinary and the medicinal use reports are shown in the results without distinction between them.

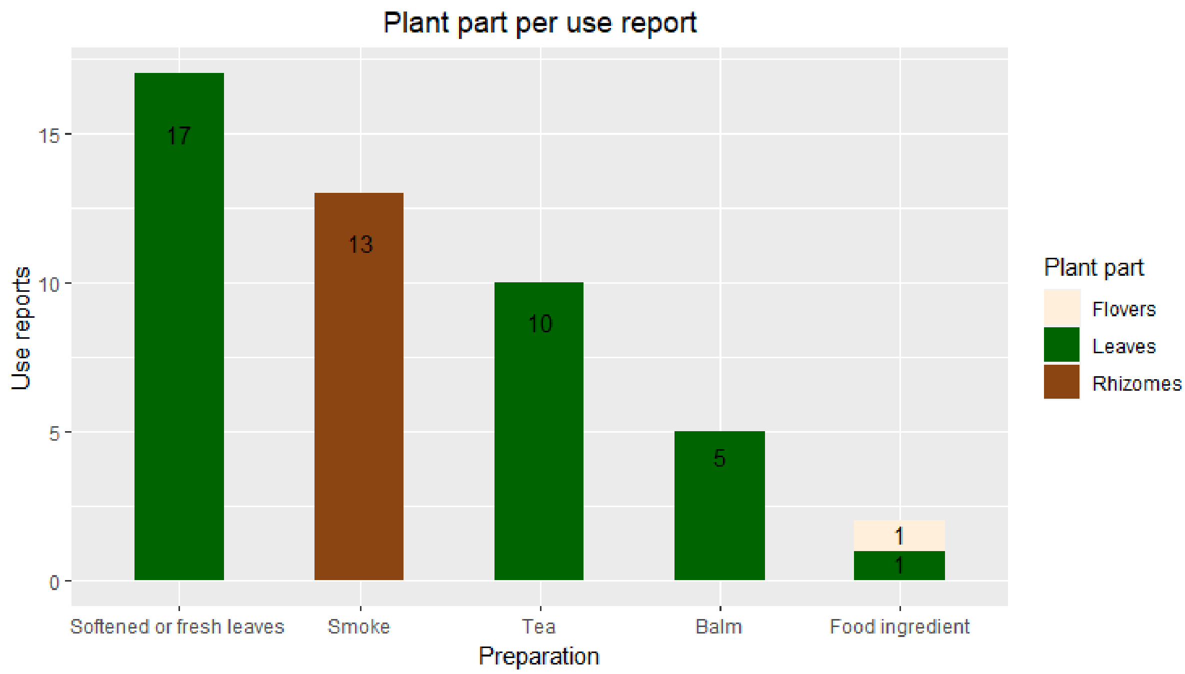

Figure 10: P. ostruthium *part uses and preparations in Val d'Anniviers*

For the preparation of tea and softened leaves, the leaves were usually put in a pan with cold water and then warmed on the stove. The lengths of the presence of the leaves in the water was variable. If the preparation of interest was the tea or the liquid of the leaves, factors mentioned as criteria were a nice green colour of the water. Leaves were used fresh or dry depending on the availability of the fresh leaves. Another storage possibility mentioned by one informant was the freezing of the leaves in aluminium sheets and the conservation of them in the freezer. If the preparation of interest was the softened leaves, factor determining how long the leaves stay in the water continuously heating is more about the consistency of the leaves. Softened leaves were usually removed from the water when their consistency was soft, resembling the softness of fresh collected leaves. The preparation time for softened leaves is therefore generally shorter than the tea preparation. The softened leaves are always cooled down to allow a direct skin application. For the smoke preparation, the dry rhizomes were sometimes directly lightened up with a lighter or a candle and the smoke directly inhaled or passed around affected areas, sometimes small rhizomes pieces were cut and put on the bottom of a bee smoker together with ashes. Informants mentioned this way to be more effective for directing the smoke in the wished direction and allowing the smoke to last longer.

The application ways of the mentioned preparation forms are shown in **figure 11**. The most mentioned application is a compress or poultice from softened or fresh leaves, with 15 Urs. One chewing and spitting application was mentioned and softened or fresh leaves were also mentioned one time as part of an herbal bath application. Herbal bath, having in total 3 Urs are otherwise made with the tea preparation, which has 10 Urs. Tea preparations are used also for drinking (6 Urs) or wound washing (2 Urs). Smoke, the second most mentioned preparation as shown in the previous figure, is mostly used for inhalation, or to pass the smoke close to affected areas. All the balm preparations are applied by rubbing it onto the skin, as external application and the food ingredient are used as decoration or for internal consumption.

The ailments for which *P. ostruthium* is used in Val d'Anniviers, correlated with the applications mentioned before, are represented in **figure 12**. The ailments for which the plant is most commonly used are for skin related issues, for which the compress or poultice is the favourite application. Next are musculoskeletal related ailments, for which the same application is common. Continuing in decreasing Urs order are respiratory ailments for which mostly the rhizome's smoke is inhaled and only one report for this ailment category concerned tea made with leaves of *P. ostruthium*. The fourth more common ailment for which the plant is reported to be applied are digestive disorders, with 4 Urs of the leafy parts, 3 as internal use, drinking, and one as external, chewing and spitting. There are also two Urs about ear related ailments for which the rhizomes are mentioned to be used as smoke passing it around the ears.

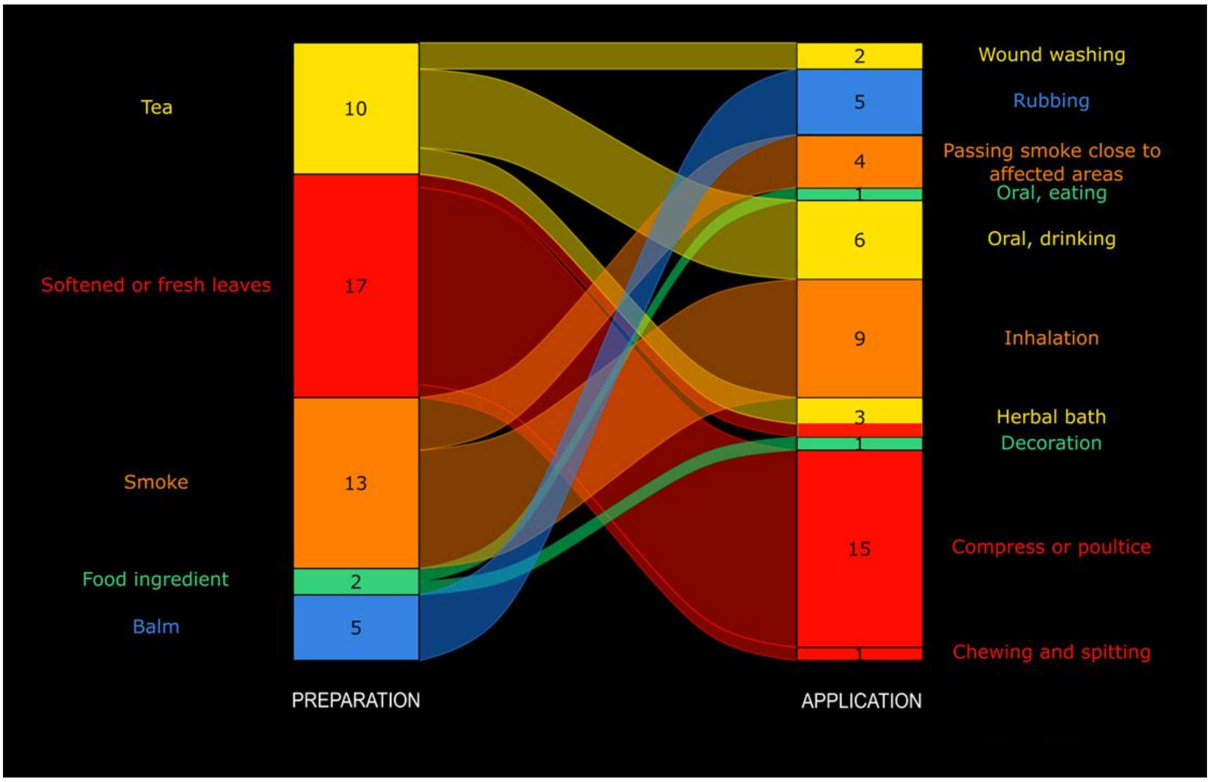

Figure 11: Application ways from the preparation forms. Numbers indicating the respective use reports

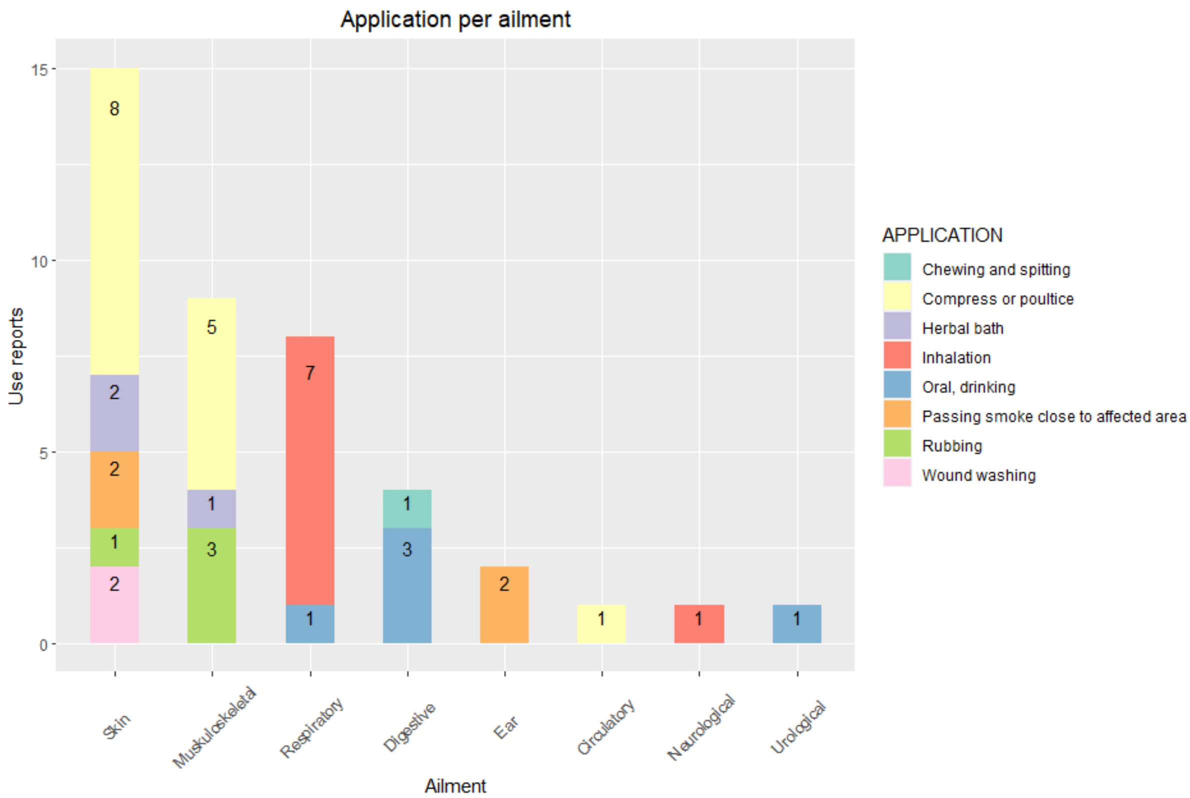

Figure 12: Ailment ICPC categories for which applications of P. ostruthium *are used. Numbers indicating the respective use reports.*

For an overview of the most common uses of *P. ostruthium* the plant parts, preparations, applications, and ailments which were mentioned more than three times are summarised in **figure 13**. This figure allows to follow with the different colours the path each informant takes through the different use levels: from which plant part the informant mentioned to use to which preparation is made from them, how these are applied and for which ailment. On top of the same figure the singular histograms for each level are shown. The flows without data were, as in **figure 12**, omitted form this plot.

Raw data, interview data that could not be consequently compared as well as notes from the interview transcripts are summarized in a use report table available upon request (miniature in **figure 17**, **appendix B**).

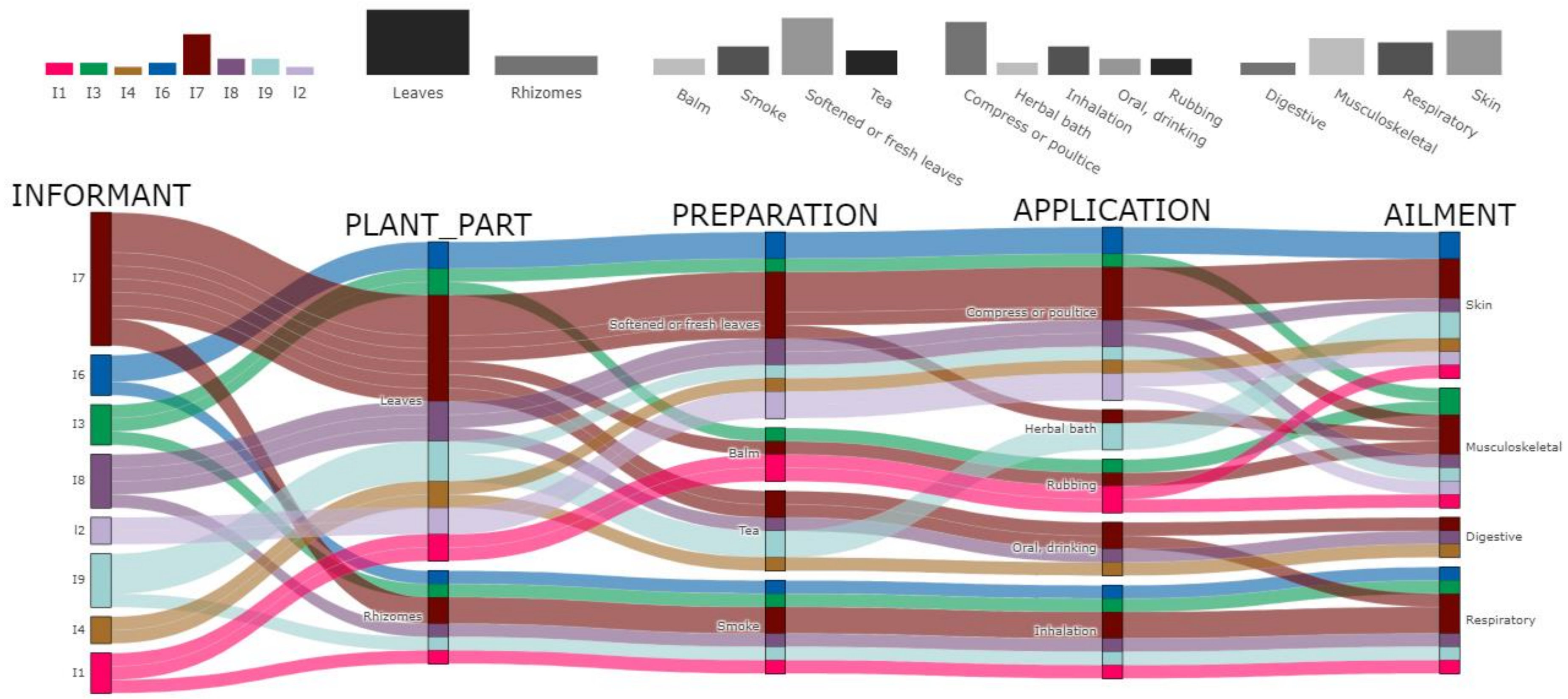

Figure 13: Flow of P. ostruthium uses through informant, plant part, preparation, application, and ailment levels of uses. Colour per informant and separate histograms per each level.

3.2. HPTLC of coumarin content

3.2.1. Qualitative analysis of coumarins and origin of samples

The qualitative HPTLC analysis of the collected samples enabled to distinguish one group of fingerprints differing from the general phytochemical pattern. Fingerprints of this group, represented in **figure 14** as Group 1 and on **table 5** shaded in grey, show on the fingerprints the presence of a band just above 0.4 R_F corresponding to the reference substance osthole. On tracks number 2, 4,5,6,7,8 and 9 in **figure 14** this band is visible in blue at UV 254 nm (A), in turquoise at UV 366 nm (D) and in pink colour under white light after derivatization. The

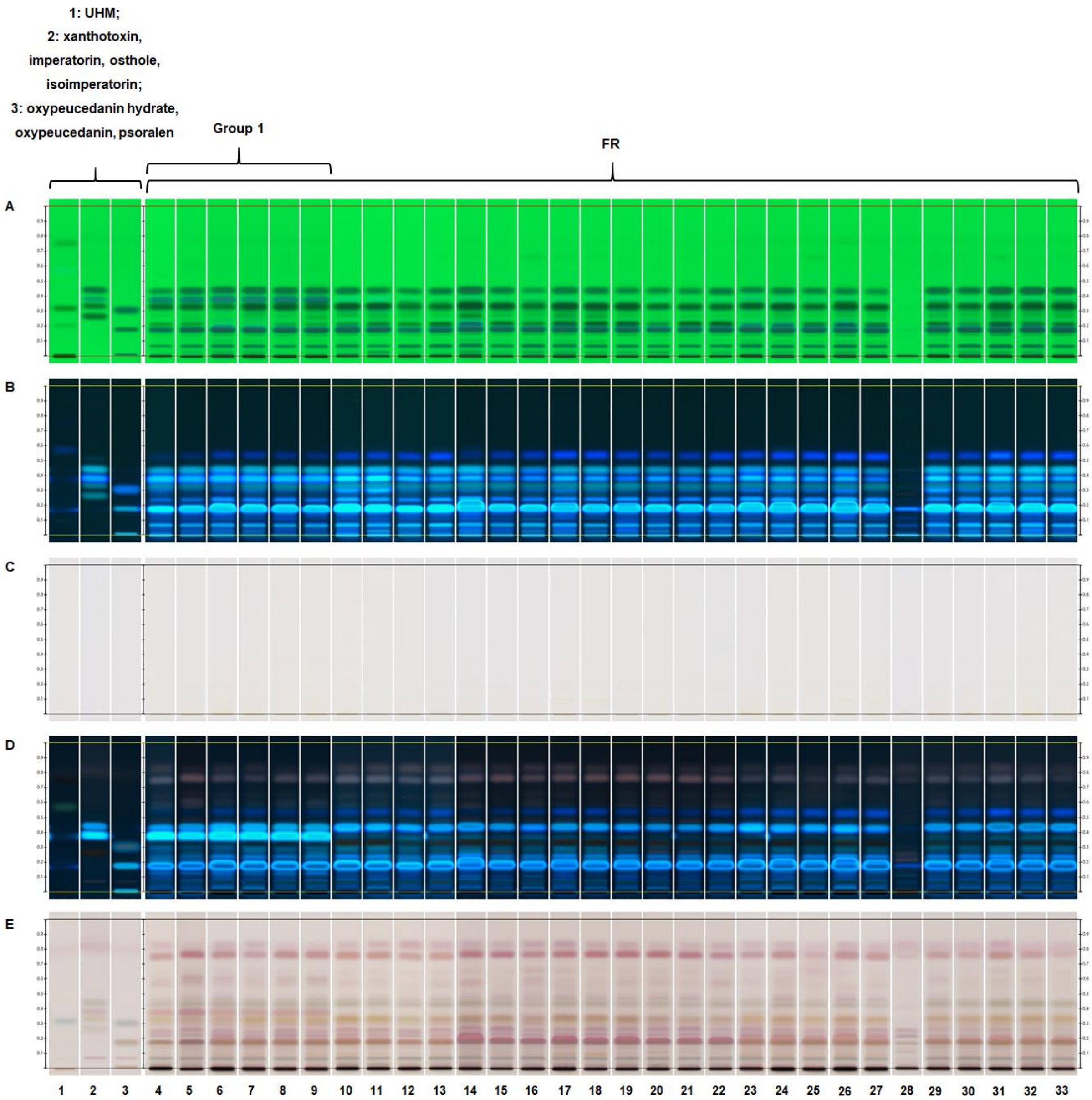

*Figure 14: HPTLC fingerprints UV 254 nm (**A**), UV 366 nm (**B**) and white light (**C**) prior to derivatization; UV 366 nm (**D**) and white light (**E**) after derivatization.*

osthole containing samples, tracks 2, 4,5,6,7,8 and 9, are six out of the 79 analysed samples and they were all collected in France, as shown in **table 5**. The tracks within Group 1 and the tracks from 10 to 33 are ordered by increasing altitude and sample ID number as well in **figure 14** as in **table 5**. In none of the samples collected in Switzerland, 49 samples, it was possible to detect presence of osthole for this study. Apart from the group with osthole presence it was not possible to see any other qualitatively different fingerprint pattern in the analysed samples. **Figure 14** only shows the samples fingerprints from France, because the difference in fingerprint pattern was visible on these samples, the fingerprints of the Swiss samples can be seen in **figure 18 appendix C**.

Table 5: Tracks content explanation of Figure 14. Gray shading highlighting samples of the Group 1

Track	Sample content	Origin (Country)	Collection site ID	Altitude (m)	Sample ID
1	UHM- Universal HPTLC Mix	-	-	-	-
2	Xanthotoxin, imperatorin, osthole, isoimperatorin with increasing R_F	-	-	-	-
3	Oxypeucedanin hydrate, oxypeucedanin, psoralen with increasing R_F	-	-	-	-
4	*P. ostruthium* – powdered rhizome	France (FR)	M) Eyne en bas	1680	M0104
5	*P. ostruthium* – powdered rhizome	France (FR)	E) Eyne en haut	1786	E0103
6	*P. ostruthium* – powdered rhizome	France (FR)	L) Porta	1951	L0102
7	*P. ostruthium* – powdered rhizome	France (FR)	L) Porta	1951	L0103
8	*P. ostruthium* – powdered rhizome	France (FR)	L) Porta	1951	L0104
9	*P. ostruthium* – powdered rhizome	France (FR)	L) Porta	1951	L0105
10	*P. ostruthium* – powdered rhizome	France (FR)	M) Eyne en bas	1680	M0102
11	*P. ostruthium* – powdered rhizome	France (FR)	M) Eyne en bas	1680	M0103
12	*P. ostruthium* – powdered rhizome	France (FR)	M) Eyne en bas	1680	M0104
13	*P. ostruthium* – powdered rhizome	France (FR)	M) Eyne en bas	1680	M0105
14	*P. ostruthium* – powdered rhizome	France (FR)	C) Llo	1786	C0101
15	*P. ostruthium* – powdered rhizome	France (FR)	C) Llo	1786	C0102
16	*P. ostruthium* – powdered rhizome	France (FR)	C) Llo	1786	C0103
17	*P. ostruthium* – powdered rhizome	France (FR)	C) Llo	1786	C0104
18	*P. ostruthium* – powdered rhizome	France (FR)	C) Llo	1786	C0105
19	*P. ostruthium* – powdered rhizome	France (FR)	E) Eyne en haut	1786	E0101
20	*P. ostruthium* – powdered rhizome	France (FR)	E) Eyne en haut	1786	E0102

21	*P. ostruthium* – powdered rhizome	France (FR)	E) Eyne en haut	1786	E0104
22	*P. ostruthium* – powdered rhizome	France (FR)	E) Eyne en haut	1786	E0105
23	*P. ostruthium* – powdered rhizome	France (FR)	L) Porta	1951	L0101
24	*P. ostruthium* – powdered rhizome	France (FR)	I) Balmeta en bas	2005	I0101
25	*P. ostruthium* – powdered rhizome	France (FR)	I) Balmeta en bas	2005	I0102
26	*P. ostruthium* – powdered rhizome	France (FR)	I) Balmeta en bas	2005	I0103
27	*P. ostruthium* – powdered rhizome	France (FR)	I) Balmeta en bas	2005	I0104
28	*P. ostruthium* – powdered rhizome	France (FR)	I) Balmeta en bas	2005	I0105
29	*P. ostruthium* – powdered rhizome	France (FR)	H) Balmeta en haut	2049	H0101
30	*P. ostruthium* – powdered rhizome	France (FR)	H) Balmeta en haut	2049	H0102
31	*P. ostruthium* – powdered rhizome	France (FR)	H) Balmeta en haut	2049	H0103
32	*P. ostruthium* – powdered rhizome	France (FR)	H) Balmeta en haut	2049	H0104
33	*P. ostruthium* – powdered rhizome	France (FR)	H) Balmeta en haut	2049	H0105

3.2.1. PCA analysis based on HPTLC bands' luminance and origin of samples

The samples with origin in France and origin in Switzerland overlap in the PCA of the fingerprints images took at 254 nm, 366 nm after derivatization and white light after derivatisation, for all three most variable principal components (PCs). The PCA analysis at UV 254 for the two most variable PCs discerned by country of origin is represented in **figure 15** and discerned by collection site in **figure 16** the same analysis for UV 366 and white light after derivatisation are presented in **appendix C** (**Figures 19** and **20**).

The PPI similarity for the HPTLC fingerprints bands do not seem to cluster neither with the different collection sites nor with the altitude. The five samples from site H and four samples from site CT are grouped together in the upper right part of **figure 16**, even though samples H are from France highest site altitude, 2049m, and CT samples come from the lowest Swiss sample altitude, 1718m.

On the PCA plots two groups one upper left and one down right for the light at 254 nm and one left and one right for both 366 nm and white light seem to cluster. The PCA analysis was repeated based on the other measured variables: rhizome's length and weight. Both of these factors also do not correlate with the two groups, as possible to see in **figure 21, 22** and **23** in **appendix C**. It was also tested for correlation with the order number in which the samples have been grinded to investigate if residuals of previous samples in the jars contaminated the next samples. This problem could be excluded based on the same plots of **appendix C**.

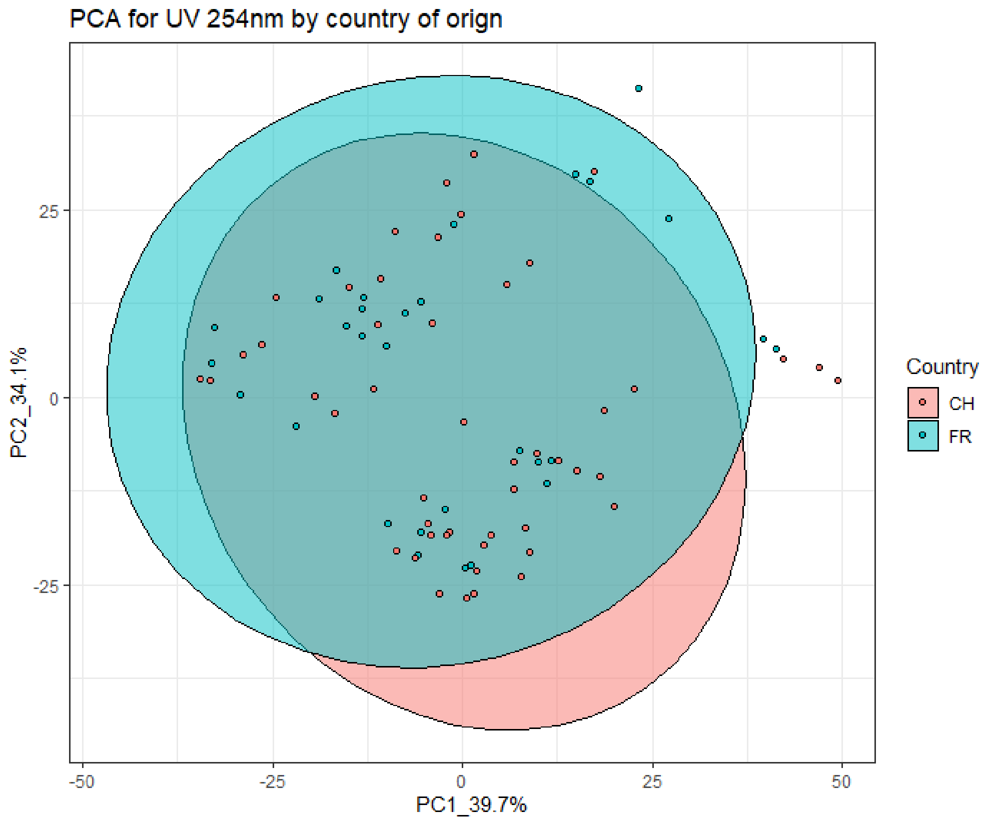

Figure 15: PCA analysis for UV 254 nm after derivatization with the two most variable PCs based on HPTLC band luminance. Blue for samples from France (FR) and red for samples from Switzerland (CH)

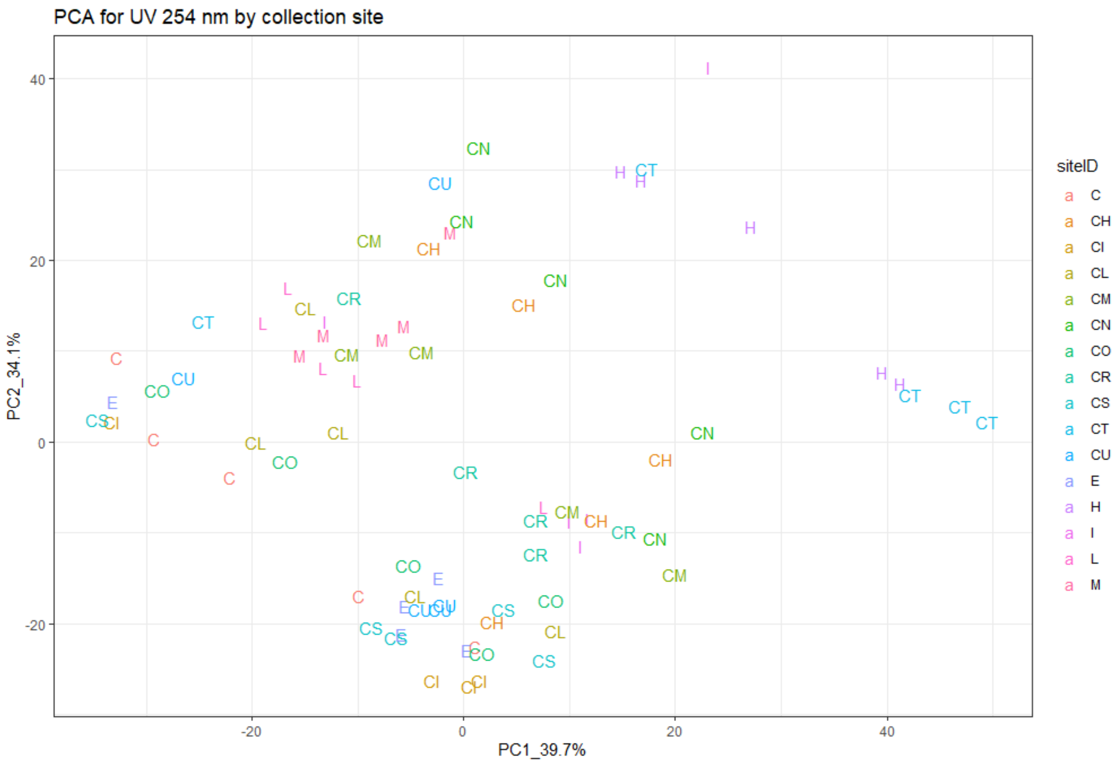

Figure 16: PCA analysis with the two most variable PCs based on HPTLC band luminance. Coloured by Collection site ID following table 5.

3.3. cpDNA barcoding

The chloroplast DNA barcoding showed the same exact sequence of nucleotides, with no differences at any site, in all the nine individuals sequenced for the *psbA-trnH* region, four coming from the site H and five coming from the site CT. The length of the fragment for which all nucleotides overlapped was of 163 bp, with additional noisy terminal regions.

4. DISCUSSION

4.1. Current local knowledge and uses of *Peucedanum ostruthium*

This study documented and compared the current local knowledge about *P. ostruthium* uses in Val d'Anniviers and in the Pyrénées-Orientales region. Knowledge about *P. ostruthium* uses is more abundant in the Swiss Valais than in the French Pyrenees region. A limitation to the study sites delimitation, which may be relevant in the discussion of these findings, is the socio-political context of the two regions. In Val d'Anniviers the villages were closer to the collection sites, in contrast to the situation in the French Pyrenees, where the distance from one village to a *P. ostruthium* population was bigger (additional information in **table 8**, **appendix A**). In addition, the demographic situation of the communes in which the samples were collected in France look different than in Switzerland. The municipality of Anniviers has a demography of 12.2 habitants per[1] quadrat kilometre (hab/km^2), whereas the Eyne, Llo, Porta and Les Anglescommunes have a mean of 6.72 hab/km^2 (DataFrance 2012; OFS 2020). The almost half proportion of the demography in the French region compared to the Swiss one, might influence the difficulty on finding people with plant use knowledge. The regulation about the plants allowed to be given in the pharmacies play probably another big role in the distribution of ethnobotanical knowledge about *P. ostruthium*. In France this plant species is not on the List A of the medicinal plants used traditionally and its distribution as preparation is not allowed in pharmacies, whereas in Switzerland products containing *P. ostruthium* are easily available.

Comparing the present findings with the ones of Camille Brioschi (2020) the ethnobotanical knowledge in Switzerland about *Peucedanum ostruthium* seems very heterogenous, presenting one region, the Val d'Anniviers, where this species is well known for its medicinal properties and another region, the Saastal, at only 76.5 km distance of road, where the plant species is poorly known for ethnobotanical uses. To better understand local knowledge about *P. ostruthium* in general and in Switzerland more research is needed, but informants in Val d'Anniviers had tendency to agree upon the fact that Val d'Anniviers is particularly rich in

[1] https://www.atlas.bfs.admin.ch/maps/13/fr/12614_75_3501_70/20695.html

knowledge about this plant and that other regions do not use it that much. Only Val d'Heréns was also mentioned in this regard for ethnobotanical knowledge about *P. ostruthium*.

In summary the results of the in-depth interviews showed that people in Val d'Anniviers tend to use more the leaves than the rhizomes of the plant and that leaves are mostly used in a softened or fresh state as compress or poultice and applied for skin related ailments. When these findings are compared to the present situation in Switzerland, where *P. ostruthium* is mostly prepared as tincture from the underground part drug imperatoria rhizoma and applied orally for digestive issues, some questions about the exact development and fundamentals of these procedures and knowledge emerge. Cisowski et al. (2001) mentioned the presence of four compounds found by hydrodistillation present in the leaves and not in the rhizomes but for the rest of the compounds the differences in leaves and rhizomes of this plant were quantitative more than qualitative. Publications about why are rhizomes more investigated than leaves were not found and this gives realm for new research and explanations on this topic. Moreover, the actual consumption and prescriptions from medical practitioners are difficult to follow and would be wished in the frame of ethnobotanical research. Findings in this study contribute to bring to the surface the interesting discordance between ethnobotanical uses of *Peucedanum ostruthium* in Val d'Anniviers and the actual pharmacological preparations in Switzerland.

4.1.1. Limitations with fieldwork and interviews

Although the distribution of *P. ostruthium* in Val d'Anniviers and in the Pyrénées-Orientales seemed comparable in terms of number of sites where the plant was observed, the number of individuals per site was notably different. In the Pyrénées-Orientales (PO) region it was much more difficult to find populations of *P. ostruthium* with at least 20 individuals and finding populations of this plant in general was a more difficult task than in Val d'Anniviers. This is the reason why the study sites in France are more spatially distributed than the ones in Switzerland. The scarce availability of *P. ostruthium* in the French region also gave restrictions about the altitude gradient along which the plant samples for this study could be collected and this situation also explains the difference of number of samples from France and Switzerland. For a more congruent comparison, the study field in France could have been also restricted to a valley; unfortunately, this was not possible. Val d'Eyne has a natural reserve on the territory and for collecting samples inside that area a permission had to be requested before - and not knowing the difficulties finding *P. ostruthium* in the rest of the PO region, this could have been a good alternative. Other valleys like the Val Llo only had one population where the criteria for collection were met.

Collecting leaves and rhizomes from the same plant was challenging in terms of quality and quantity standards: big enough green leaves to subtract 2 cm^2 presenting fully developed rhizomes were hard to find. The size of the rhizomes could not be derived before underground examination, and this put an additional criterion in study sites where *P. ostruthium* populations were already hard to find.

The biggest challenge when it comes to ethnobotanical interview analysis is the intricate qualitative to quantitative transformation of data. The high personal value people give to a plant are not easily convertible in numbers and quantitative plots. Qualitative interviews have a high degree of complexity and the intent of summarizing these data in quantitative forms is limiting in itself. What also was not considered are situation dependent divergencies in the preparations and consecutive treatments with different preparations: the informants might choose other patterns of treatment depending on the gravity or the specific scenario, and the application of preparations might not be linear but consecutive, for example compress followed by passing smoke close to affected area. These scenarios are not considered because of their complexity, but they offer an interesting realm to further investigate.

Another limitation to the fieldwork research conducted was the type of interview performed. As Bernard Russell (2011: 156-158) describes, interviews are a continuum of situations depending on “the amount of control we try to exercise over people’s responses” but depends also on the background of the informant. It is therefore quite difficult to find an exact way of interviewing that can be applied to all informants. The lack of time for establishing proper trust and understanding within the community, added to the unknown outcomes of the interviews, made the interview lean toward a very open approach. A more structured approach would have produced quicker quantitative results but, as Bernard (2011: 156-158) writes, with some informants, to conduct formal interviews just feels unnatural and “unstructured interviewing is better for building initial rapport”.

4.2. Coumarin content in *Peucedanum ostruthium* and spatial correlation

The correlation between coumarin presence in *P. ostruthium* individuals and their distribution in two different mountain ranges and at different altitude gradients was further investigated. Qualitatively only one group of six samples coming from France showed presence of osthole could be identified as different from the general fingerprint patter. No samples from Switzerland showed presence of osthole in this study. This is an interesting finding if compared to the results of Camille Brioschi (2020), in which only one sample out of the 120 samples, all coming from Switzerland, showed the presence of osthole with the same analysis method as this study. Four of the six rhizome samples showing osthole presence in this study are from the same site, suggesting that for this qualitative feature the origin of the samples could play some kind of role, but this result is too weak to make such an assumption. Furthermore, no other qualitative difference of the coumarins was observed in correlation to the mountain range or the altitude level of the origin of the samples, and the PCA analysis based on the HPTLC fingerprint patterns luminance also did not show correlation between these factors. In the PCA analysis even the samples coming from the same site did not cluster together, indicating that other site-dependent variables do not contribute to a difference in the luminance pattern of the fingerprints. Morphological traits of the rhizomes were also embedded in the analysis in relationship to the origin and altitude of the sample sites and also morphological characters do not seem to contribute. These findings contribute to the hypothesis that morphological trait of *Peucedanum ostruthium* do not influence the coumarin content quality, as shown in the work of Camille Briosci (2020).

The PCA results do show two groups clearly separated but the reason for this pattern is neither origin, altitude of sites nor morphological traits of the rhizomes and the possibility of laboratory procedure contamination were also excluded. The reason for this grouping stays unclear at the present time.

4.3. cpDNA barcoding

The cpDNA barcoding with the *psbA-trnH* intragenic marker showed common nucleotide sequence between the samples from site H and CT, which were collected at different altitudes in different mountain range. The sequence overlapped also with the sequence published by Schmiderer et al. (2015) on the Genebank platform and samples coming both from Austria and Italy shared the same sequence with a query cover of 100%. It is therefore possible to infer that sample origin do not play a role in the nucleotide sequence difference at the *psbA-trnH* region. To investigate intraspecific and phylogenetic differences in this species, other genetical markers or microsatellites mapping should be used, as suggested by Yuan et al. (2015).

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APPENDIX A: Botany and sites information

Table 6: Accompanying flora per site at least at the family level. Individuum number 1 for Peucedanum ostruthium. More information about each site in table 7.

Site	Individuum number	Country	Place	Genus	Species	Autor	Family
C	1	FR	Llo	Peucedanum	ostruthium	(L.) W. D. J. Koch	Apiaceae
C	2	FR	Llo	Rubus	idaeus	L.	Rosaceae
C	3	FR	Llo	Hypericum	perforatum	L.	Hypericaceae
C	4	FR	Llo	Pinus	uncinata	(DC.) Domin	Pinaceae
C	5	FR	Llo	Urtica	dioica	L.	Urticaceae
C	6	FR	Llo	Heracleum	sphondylium	L.	Apiaceae
C	7	FR	Llo	Dactylis	glomerata	L.	Poaceae
C	11	FR	Llo	Rhododendron	ferrugineum	L.	Ericaceae
E	1	FR	Eyne	Peucedanum	ostruthium	(L.) W. D. J. Koch	Apiaceae
E	2	FR	Eyne	Heracleum	sphondylium	L.	Apiaceae
E	3	FR	Eyne	Rosa	pendulina	L.	Rosaceae
E	4	FR	Eyne	Aconitum	lycoctonum	L.	Ranunculaceae
E	5	FR	Eyne	Geranium	pratense	L.	Geraniaceae
E	6	FR	Eyne	Angelica	sylvestris	L.	Apiaceae
E	8	FR	Eyne	Vicia	cracca	L.	Fabaceae
E	9	FR	Eyne	Urtica	dioica	L.	Urticaceae
E	11	FR	Eyne	Knautia	n.d	L.	caprifoliaceae
E	12	FR	Eyne	Mentha	longifolia	(L.) Huds.	Lamiaceae
H	1	FR	Balmeta en haut	Peucedanum	ostruthium	(L.) W. D. J. Koch	Apiaceae
H	2	FR	Balmeta en haut	Rhododendron	ferrugineum	L.	Ericaceae
H	3	FR	Balmeta en haut	Vaccinium	myrthillus	L.	Ericaceae
H	4	FR	Balmeta en haut	Prenanthes	purpurea	L.	Asteraceae
H	5	FR	Balmeta en haut	Pinus	uncinata	(DC.) Domin	Pinaceae
H	6	FR	Balmeta en haut	Veratrum	album	L.	Melanthiaceae
H	7	FR	Balmeta en haut	Aconitum	napellus	L.	Ranunculaceae
H	8	FR	Balmeta en haut	Gentiana	lutea	L.	Gentianaceae
I	1	FR	Balmeta en bas	Peucedanum	ostruthium	(L.) W. D. J. Koch	Apiaceae
I	2	FR	Balmeta en bas	Adenostyles	alliariae	(Gouan) A. Kern.	Asteraceae
I	3	FR	Balmeta en bas	Rhododendron	ferrugineum	L.	Ericaceae
I	4	FR	Balmeta en bas	Sorbus	aucuparia	L.	Rosaceae
I	5	FR	Balmeta en bas	Pinus	uncinata	(DC.) Domin	Pinaceae
I	6	FR	Balmeta en bas	Oxalis	acetosella	L.	oxalidaceae
I	7	FR	Balmeta en bas	Aconitum	napellus	L.	Ranunculaceae
I	8	FR	Balmeta en bas	Achillea	millefolium	L.	Asteraceae
I	9	FR	Balmeta en bas	Veratrum	album	L.	Melanthiaceae
I	10	FR	Balmeta en bas	Prenanthes	purpurea	L.	Asteraceae
L	1	FR	Porta	Peucedanum	ostruthium	(L.) W. D. J. Koch	Apiaceae
L	2	FR	Porta	Betula	pubescens	Ehrh.	Betulaceae
L	3	FR	Porta	Aconitum	napellus	L.	Ranunculaceae

L	4	FR	Porta	Rhododendron	ferrugineum	L.	Ericaceae
L	5	FR	Porta	Veratrum	album	L.	Melanthiaceae
L	6	FR	Porta	Oxalis	acetosella	L.	oxalidaceae
L	7	FR	Porta	Astrantia	major	L.	Apiaceae
L	8	FR	Porta	Angelica	sylvestris	L.	Apiaceae
M	1	FR	Eyne en bas	P	ostruthium	(L.) W. D. J. Koch	Apiaceae
M	2	FR	Eyne en bas	Sorbus	aucuparia	L.	Rosaceae
M	3	FR	Eyne en bas	Urtica	dioica	L.	Urticaceae
M	4	FR	Eyne en bas	Aconitum	napellus	L.	Ranunculaceae
M	5	FR	Eyne en bas	oxalis	acetosella	L.	oxalidaceae
M	6	FR	Eyne en bas	Angelica	sylvestris	L.	Apiaceae
M	7	FR	Eyne en bas	Prenanthes	purpurea	L.	Asteraceae
M	8	FR	Eyne en bas	Pinus	sylvestris	L.	Pinaceae
CH	1	CH	Zinal, Cottier en haut	Adenostyles	alliariae	(Gouan) A. Kern.	Asteraceae
CH	2	CH	Zinal, Cottier en haut	Alnus	glutinosa	L.	Betulacea
CH	3	CH	Zinal, Cottier en haut	Chaerophyllum	hirsutum	L.	Apiaceae
CH	4	CH	Zinal, Cottier en haut	Heracleum	sphondylium	L.	Apiaceae
CH	5	CH	Zinal, Cottier en haut	Oxalis	acetosella	L.	Oxalidaceae
CH	6	CH	Zinal, Cottier en haut	Sorbus	aucuparia	L.	Rosaceae
CH	7	CH	Zinal, Cottier en haut	Larix	decidua	Mill.	Pinaceae
CH	8	CH	Zinal, Cottier en haut	Silene	dioica	(L.)	Caryophyllaceae
CH	9	CH	Zinal, Cottier en haut	Rumex	alpestris	Jacq.	Polygonaceae
CI	1	CH	St-Luc, prairie	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CI	2	CH	St-Luc, prairie	Epilobium	angustifolium	L.	Onagraceae
CI	3	CH	St-Luc, prairie	Larix	decidua	Mill.	Pinaceae
CI	4	CH	St-Luc, prairie	Rubus	idaeus	L.	Rosaceae
CI	5	CH	St-Luc, prairie	Geranium	sylvaticum	L.	Geraniaceae
CI	6	CH	St-Luc, prairie	Lotus	corniculatus	L.	Fabaceae
CI	7	CH	St-Luc, prairie	Silene	vulgaris	(Moench) Garcke	Caryophyllaceae
CI	8	CH	St-Luc, prairie	Urtica	dioica	L.	Urticaceae
CL	1	CH	St-Luc, top	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CL	2	CH	St-Luc, top	Rumex	alpestris	Jacq.	Polygonaceae
CL	3	CH	St-Luc, top	Vaccinium	myrtillus	L.	Ericaceae
CL	4	CH	St-Luc, top	Plantago	alpina	L.	Plantaginaceae
CL	5	CH	St-Luc, top	Campanula	scheuchzeri	Vill.	Campanulaceae
CL	6	CH	St-Luc, top	Phyteuma	n.d	n.d	Campanulaceae
CL	7	CH	St-Luc, top	Gymnadenia	nigra	L.	Orchidaceae
CL	8	CH	St-Luc, top	Trifolium	n.d	n.d	Fabaceae
CL	9	CH	St-Luc, top	Epilobium	angustifolium	L.	Onagraceae
CM	1	CH	St-Luc, sentier en fôret	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CM	2	CH	St-Luc, sentier en fôret	Hieracium	n.d	n.d	Asteraceae

CM	3	CH	St-Luc, sentier en fôret	Larix	decidua	Mill.	Pinaceae
CM	4	CH	St-Luc, sentier en fôret	Pinus	sylvestris	L.	Pinaceae
CM	5	CH	St-Luc, sentier en fôret	Rumex	alpinus	L.	Polygonaceae
CM	6	CH	St-Luc, sentier en fôret	Myosotis	alpestris	F. W. Schmidt	Boraginaceae
CM	7	CH	St-Luc, sentier en fôret	Epilobium	angustifolium	L.	Onagraceae
CM	8	CH	St-Luc, sentier en fôret	Rhododendron	ferrugineum	L.	Ericaceae
CM	9	CH	St-Luc, sentier en fôret	Geranium	sylvaticum	L.	Geraniaceae
CM	10	CH	St-Luc, sentier en fôret	Sorbus	aucuparia	L.	Rosaceae
CN	1	CH	St-Luc, croisement rivières	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CN	2	CH	St-Luc, croisement rivières	Epilobium	angustifolium	L.	Onagraceae
CN	3	CH	St-Luc, croisement rivières	Silene	vulgaris	(Moench) Garcke	Caryophyllaceae
CN	4	CH	St-Luc, croisement rivières	Alnus	glutinosa	(L.) Gaertn.	Betulacea
CN	5	CH	St-Luc, croisement rivières	Geranium	sylvaticum	L.	Geraniaceae
CN	6	CH	St-Luc, croisement rivières	Lotus	corniculatus	L.	Fabaceae
CN	7	CH	St-Luc, croisement rivières	Trifolium	n.d	n.d	Fabaceae
CN	8	CH	St-Luc, croisement rivières	Thymus	serpyllum	n.d	Lamiaceae
CO	1	CH	St-Luc, restaurant	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CO	2	CH	St-Luc, restaurant	Piceae	abies	L.	Pinaceae
CO	3	CH	St-Luc, restaurant	Larix	decidua	Mill.	Pinaceae
CO	4	CH	St-Luc, restaurant	Sorbus	aucuparia	L.	Rosaceae
CO	5	CH	St-Luc, restaurant	Urtica	dioica	L.	Urticaceae
CO	6	CH	St-Luc, restaurant	Adenostyles	alliariae	(Gouan) A. Kern.	Asteraceae
CO	7	CH	St-Luc, restaurant	Oxalis	acetosella	L.	Oxalidaceae
CO	9	CH	St-Luc, restaurant	Geranium	sylvaticum	L.	Geraniaceae
CO	10	CH	St-Luc, restaurant	Hieracium	n.d	n.d	Asteraceae
CO	11	CH	St-Luc, restaurant	Campanula	n.d	n.d	Campanulaceae
CO	12	CH	St-Luc, restaurant	Epilobium	angustifolium	L.	Onagraceae
CO	13	CH	St-Luc, restaurant	Rubus	fruticosus	L.	Rosaceae
CR	1	CH	Zinal to Petit Mountet, cabanne	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CR	2	CH	Zinal to Petit Mountet, cabanne	Petasites	paradoxus	(Retz.) Baumg.	Asteraceae
CR	3	CH	Zinal to Petit Mountet, cabanne	Trifolium	badium	Schreb.	Fabaceae
CR	4	CH	Zinal to Petit Mountet, cabanne	Alchemilla	n.d	n.d	Rosaceae
CR	5	CH	Zinal to Petit Mountet, cabanne	Silene	dioica	(L.)	Caryophyllaceae
CR	6	CH	Zinal to Petit Mountet, cabanne	Adenostyles	alliariae	(Gouan) A. Kern.	Asteraceae
CR	7	CH	Zinal to Petit Mountet, cabanne	Rumex	alpinus	L.	Polygonaceae
CR	9	CH	Zinal to Petit Mountet, cabanne	Geranium	sylvaticum	L.	Geraniaceae
CR	10	CH	Zinal to Petit Mountet, cabanne	Silene	vulgaris	(Moench) Garcke	Caryophyllaceae
CS	1	CH	Petit Mountet to Zinal, Navissance	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae

CS	2	CH	Petit Mountet to Zinal, Navissance	Adenostyles	alliariae	(Gouan) A. Kern.	
CS	3	CH	Petit Mountet to Zinal, Navissance	Phleum	alpinum	L.	Poaceae
CS	4	CH	Petit Mountet to Zinal, Navissance	Betula	n.d	n.d	Betulacea
CS	5	CH	Petit Mountet to Zinal, Navissance	Larix	decidua	Mill.	Pinaceae
CS	7	CH	Petit Mountet to Zinal, Navissance	Urtica	dioica	L.	Urticaceae
CS	8	CH	Petit Mountet to Zinal, Navissance	n.d	n.d	n.d	Asteraceae
CS	9	CH	Petit Mountet to Zinal, Navissance	n.d	n.d	n.d	Poaceae
CT	1	CH	Tzchoudana	Peucedanum	ostuthium	(L.) W. D. J. Koch	Apiaceae
CT	2	CH	Tzchoudana	Epilobium	angustifolium	L.	Onagraceae
CT	3	CH	Tzchoudana	Rubus	idaeus	L.	Rosaceae
CT	4	CH	Tzchoudana	Fragraria	vesca	L.	Rosaceae
CT	5	CH	Tzchoudana	Achillea	millefolium	L.	Asteraceae
CT	6	CH	Tzchoudana	Alnus	viridis	(Chaix) DC.	Betulaceae
CT	7	CH	Tzchoudana	Aconitum	napellus	L.	Ranunculaceae
CT	8	CH	Tzchoudana	Adneostyles	alliariae	(Gouan) A. Kern.	Asteraceae
CT	9	CH	Tzchoudana	Leucantemum	vulgare	Lam.	Asteraceae
CT	10	CH	Tzchoudana	Rumex	scutatus	L.	Polygonaceae
CT	11	CH	Tzchoudana	Urtica	dioica	L.	Urticaceae
CT	12	CH	Tzchoudana	Sorbus	aucuparia	L.	Rosaceae
CU	1	CH	Zinal, Cottier en bas	Adenostyles	alliariae	(Gouan) A. Kern.	Asteraceae
CU	2	CH	Zinal, Cottier en bas	Alnus	glutinosa	L.	Betulacea
CU	3	CH	Zinal, Cottier en bas	Chaerophyllum	hirsutum	L.	Apiaceae
CU	4	CH	Zinal, Cottier en bas	Heracleum	sphondylium	L.	Apiaceae
CU	5	CH	Zinal, Cottier en bas	Oxalis	acetosella	L.	Oxalidaceae
CU	6	CH	Zinal, Cottier en bas	Sorbus	aucuparia	L.	Rosaceae
CU	7	CH	Zinal, Cottier en bas	Larix	decidua	Mill.	Pinaceae
CU	8	CH	Zinal, Cottier en bas	Silene	dioica	(L.)	Caryophyllaceae
CU	9	CH	Zinal, Cottier en bas	Rumex	alpestris	Jacq.	Polygonaceae

Table 7: Sites information

Country	Site	Closest village	Latitude	Longitude	Hight (m)	Collection date
FR	C	Llo	42°25'43.517''N	2°5'33.546''E	1769	24.07.21
FR	E	Eyne	42°28'8.219''N	2°5'44.368''E	1786	27.07.21
FR	H	Les Angles	42°35'19.902''N	2°2'3.329''E	2049	08.09.21
FR	I	Les Angles	42°35'13.406''N	2°1'38.586''E	2005	08.09.21
FR	L	Porta	42°30'38.144''N	1°47'28.413''E	1951	10.09.21
FR	M	Eyne	42°27'55.433''N	2°5'19.211''E	1680	15.09.21
CH	CH	Zinal	46°8'20.537''N	7°38'4.09''E	1875	06.08.21
CH	CI	St-Luc	46°12'57.813''N	7°37'8.748''E	1879	07.08.21
CH	CL	St-Luc	46°12'36''N	7°37'50''E	2254	08.08.21
CH	CM	St-Luc	46°12'47''N	7°37'28''E	2064	08.08.21
CH	CN	St-Luc	46°12'53''N	7°37'17''E	1936	08.08.21
CH	CO	St-Luc	46°13'4.391''N	7°36'44.447''E	1756	09.08.21
CH	CR	Zinal	46°5'47.391''N	7°38'5.207''E	2095	12.08.21
CH	CS	Zinal	46°5'42.84''N	7°38'16''E	1964	12.08.21
CH	CT	Zinal	46°7'23.994''N	7°37'54.971''E	1718	12.08.21
CH	CU	Zinal	46°8'19''N	7°37'56''E	1800	13.08.21

Table 8: Minimum distance between a P. ostruthium *site in this study and a village.*

Country	Site	Village name	Distance (m)	Number habitants (Hab)	Surface (km²)	Height of Village (m)
FR	C	Llo	3,695.50	171	28.44	1,319
FR	H	Les Angles	3,507.83	553	17.77	1,531
FR	M	Eyne	1,221.26	141	20.36	1,470
FR	L	Porta	3,134.48	115	65.19	1,354
CH	CO	St-Luc	1,277.60	300	31.9	1,655
CH	CU	Zinal	557.86	200	0.537	1,675

APPENDIX B: Interviews

Table 9: Semi-structured interview guidelines.

Category	French	English translation
Opening question	Comment utilisez-vous l'impératoire?	How do you use the masterwort?
Plant part	• Avec quelle partie de la plante ?	• With which plant part ?
Collection, who	• Qui cueille la plante ?	• Who collects the plant ?
Collection, when	• Quand ?	• When ?
Form/ storage	• Et puis vous faitez quoi ? (Vous l'utilisez fraiche ou vous la séchez ?)	• And then what do you do? (Do you use it fresh, or you dry it
Preparation	• Vous la préparez comment exactement ?	• How do you prepare it exactly?
Ailment/ reason	• Et vous utilisez ça pour quelle raison/ quand ?	• And what do you use this for?
Source of knowledge	Vous avez appris comment à l'utiliser ? (C'était quand la première fois ?)	How did you learn to use it? (When was the first time?)

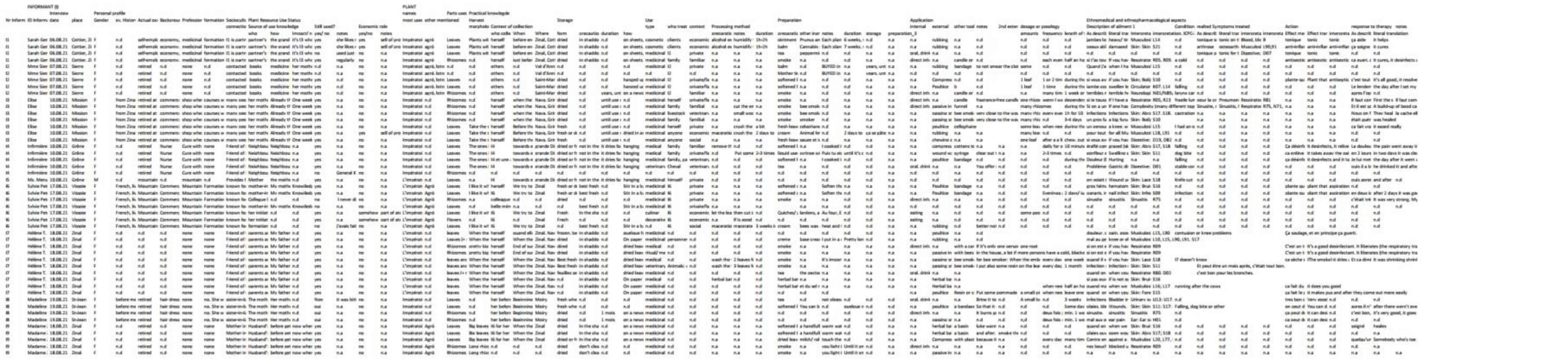

Figure 17: Raw interview results miniature table, available upon request in xls format

Appendix C: HPTLC and PCA

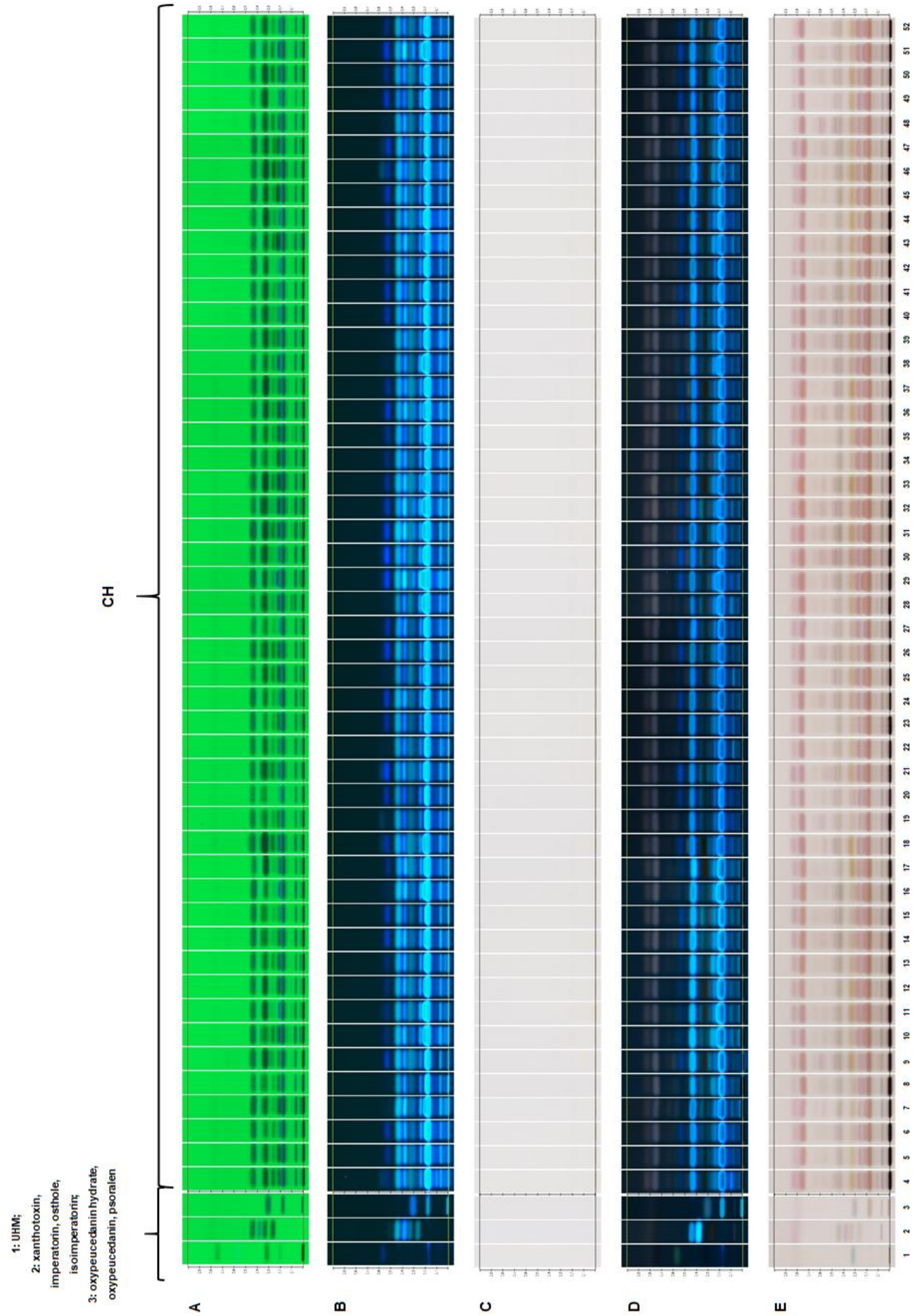

Figure 18: HPTLC Fingerprints of the 49 powdered rhizome methanol extracted samples from Val d'Anniviers, Switzerland (CH).

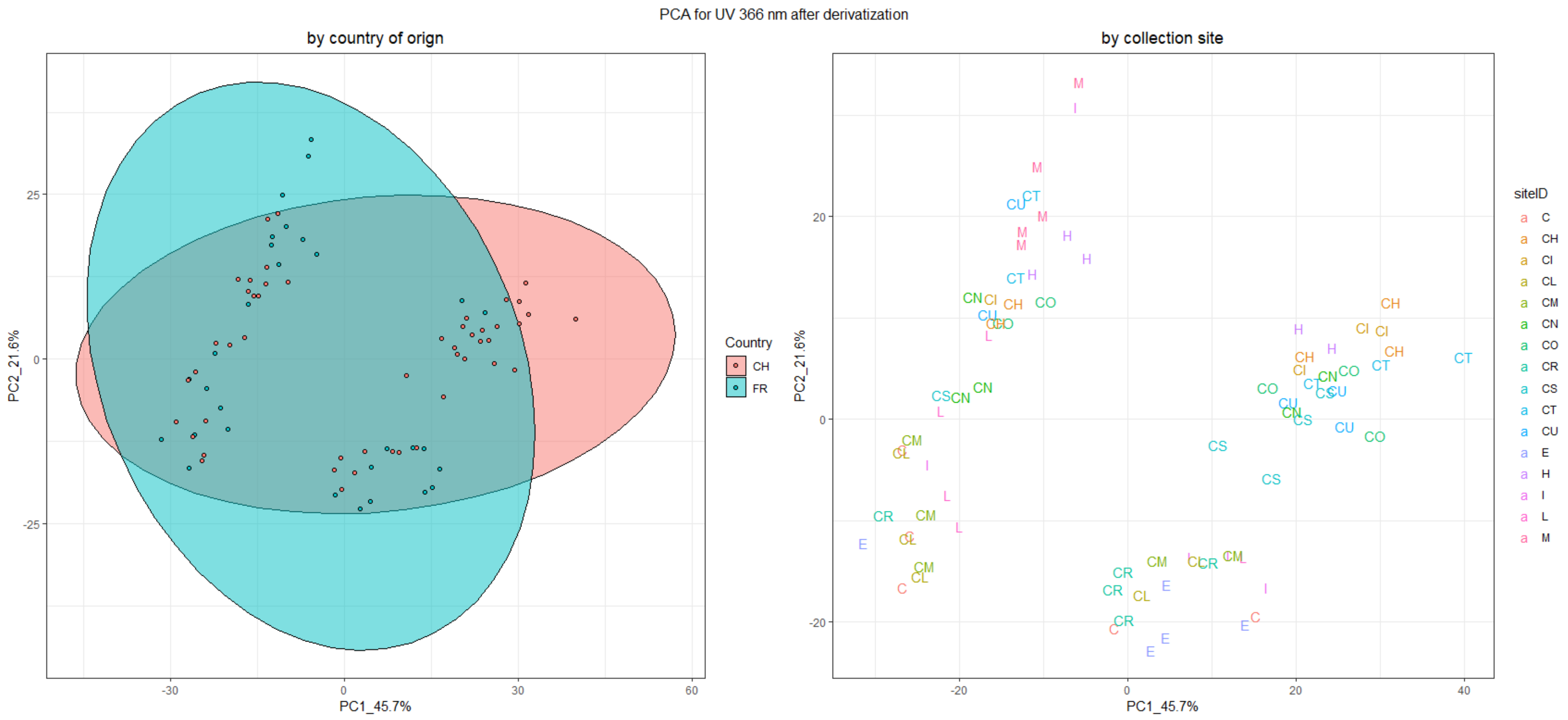

Figure 19: PCA analysis for UV 366 after derivatization with the two most variable PCs based on HPTLC band luminance. Coloured by country (left) and by Collection site (right) following table 5

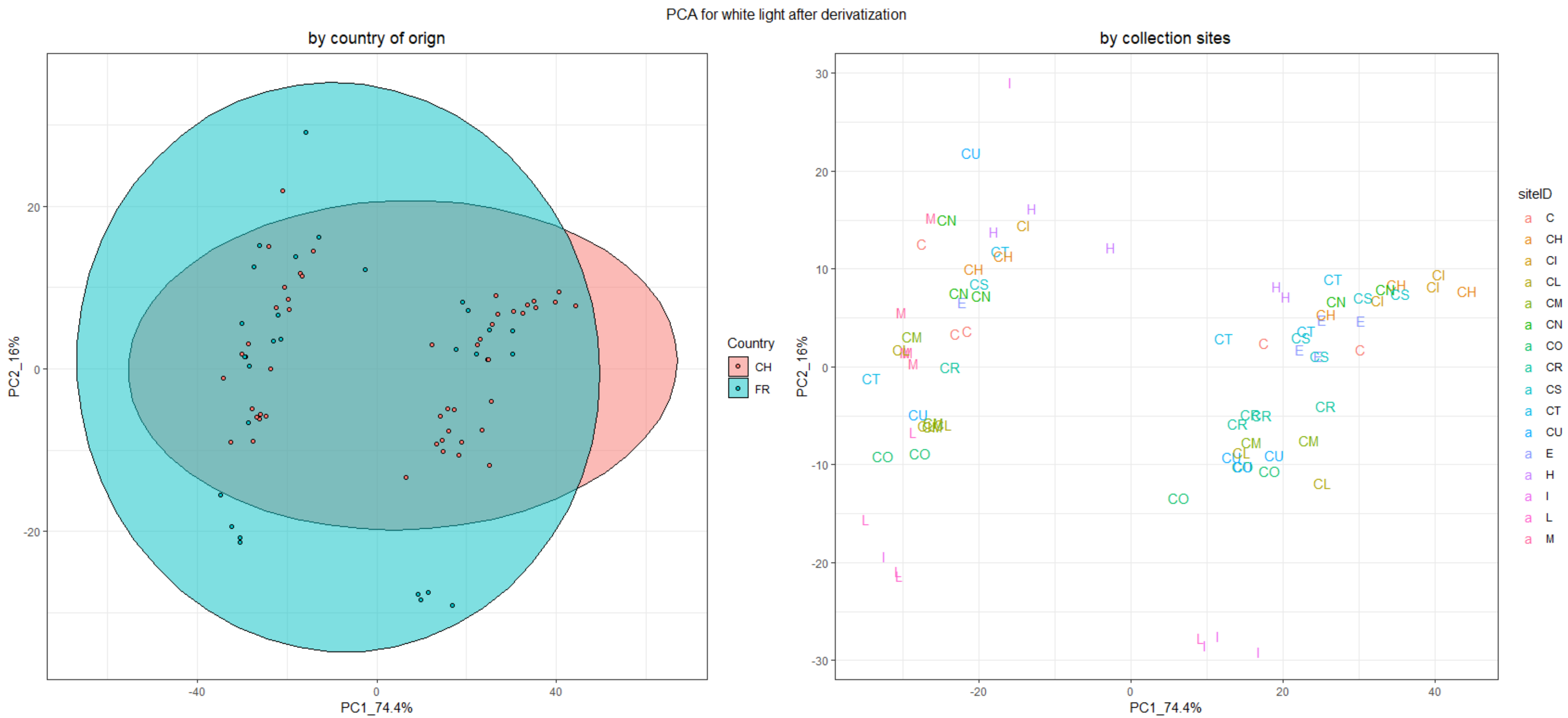

Figure 20: PCA analysis for white light after derivatization with the two most variable PCs based on HPTLC band luminance. Coloured by country (left) and by Collection site (right) following table 5

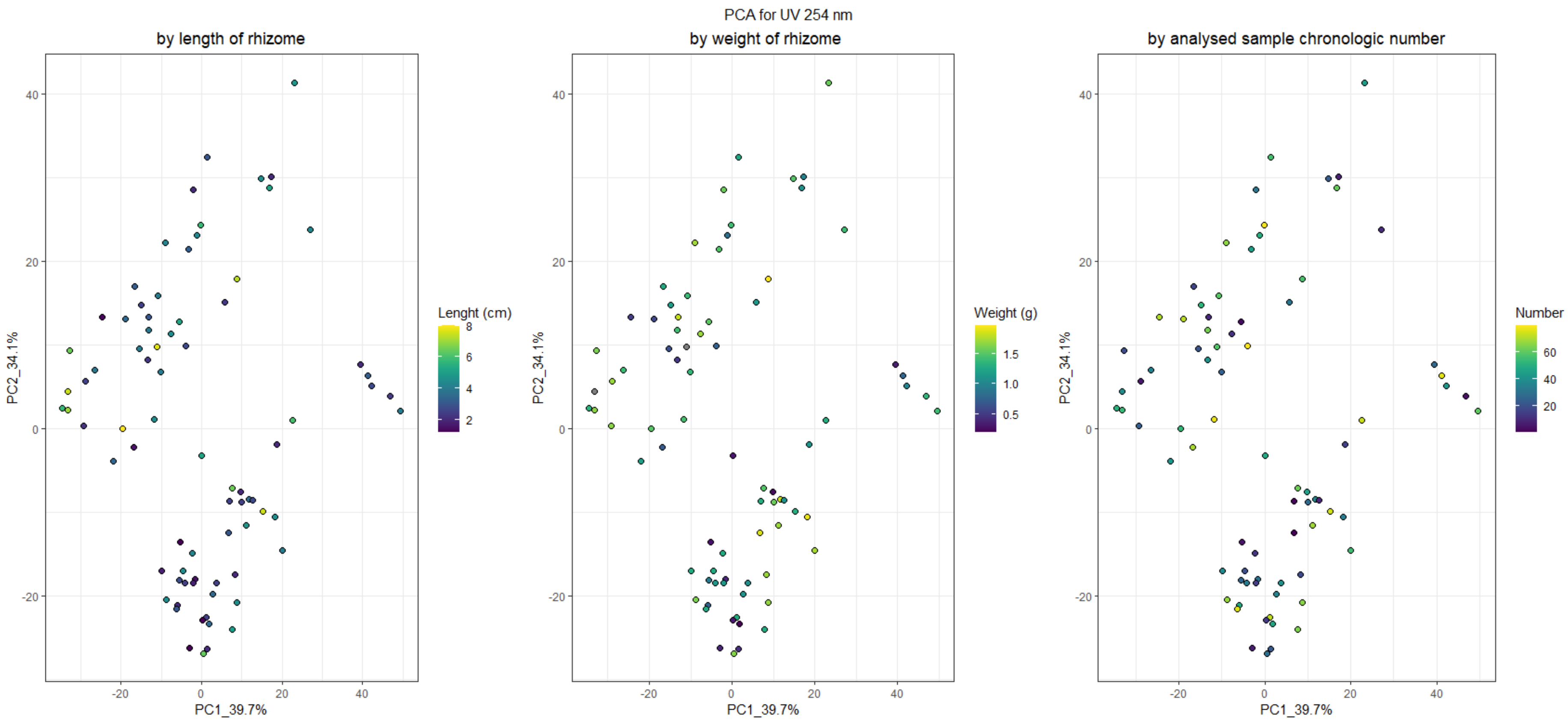

Figure 21: PCA analysis for UV 254 nm with the two most variable PCs based on HPTLC band luminance. Coloured by length (left) and weight (centre) of rhizomes and order in which the sample grinding was proceeded (right).

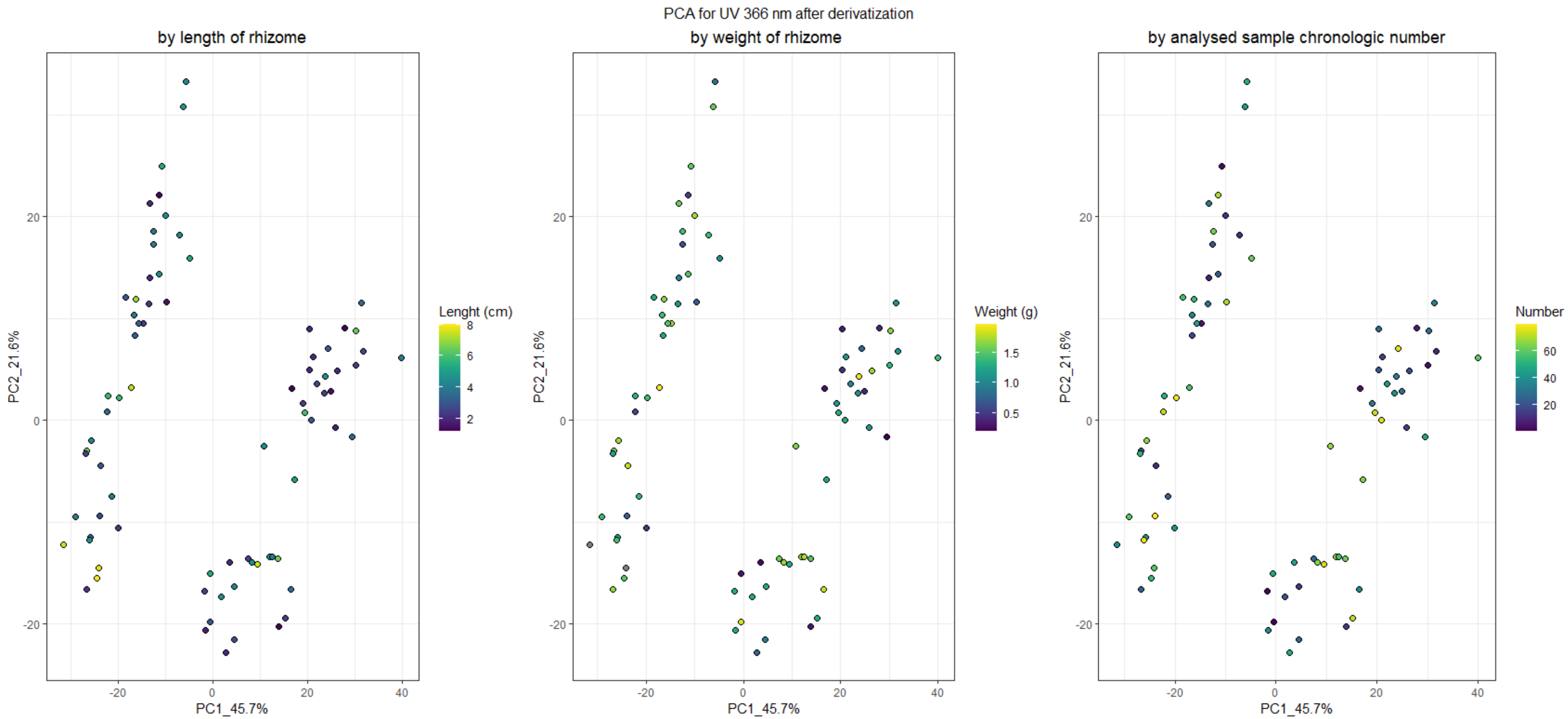

Figure 22: PCA analysis for UV 366 nm after derivatization with the two most variable PCs based on HPTLC band luminance. Coloured by length (left) and weight (centre) of rhizomes and order in which the sample grinding was proceeded (right).

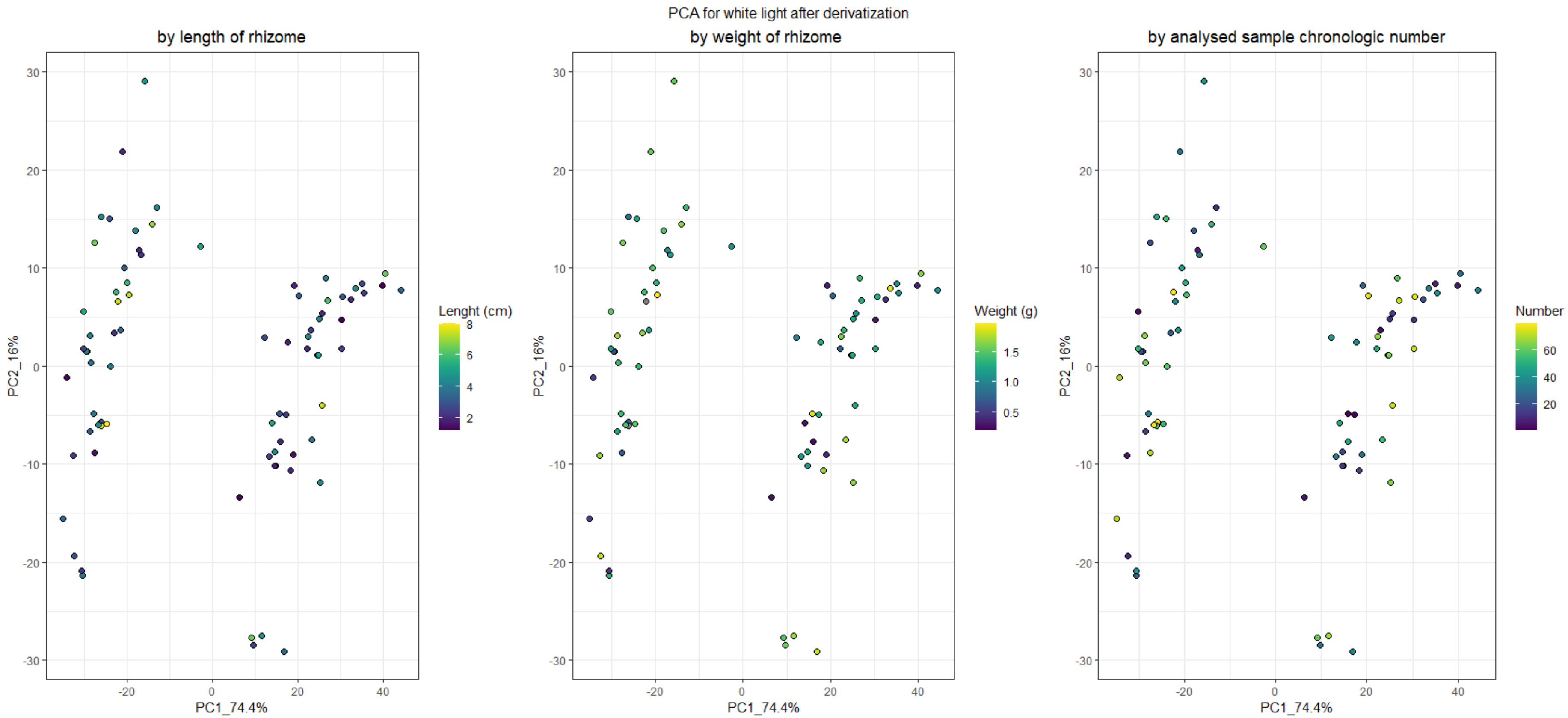

Figure 23: PCA analysis for white light after derivatization with the two most variable PCs based on HPTLC band luminance. Coloured by length (left) and weight (centre) of rhizomes and order in which the sample grinding was proceeded (right).