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CHECKLIST

Contribution to the knowledge of the distribution of *Parmelia* species (Parmeliaceae, Ascomycota) – New records confirmed by molecular data

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Abstract

The first records of Parmelia ernstiae from Madeira, as well as the new localities of P. encryptata from Poland and P. sulcata from Chile, are presented. All records are confirmed by molecular data, BLAST search, and haplotype network analysis of the nucITS rDNA sequences. The discussion on the morphology and secondary chemistry of all three species is provided with notes on their distribution and similar taxa. Parmelia encryptata is a rarely reported cryptic species that is morphologically indistinguishable from *P. sulcata*, and its identification requires molecular data. New localities of P. encryptata from northern Poland suggest that the taxon may be widespread in the country and not confined to large forests but also present in open areas on free-standing trees. Moreover, a new nucITS rDNA haplotype of the species is reported. Parmelia ernstiae is a near-cryptic species, morphologically and chemically very similar to P. saxatilis and P. serrana. Two specimens of the species from Madeira have sublinear lobes with marginal and laminal isidia, unlike most specimens of P. ernstiae, having short, broad lobes with laminal isidia, and thus are morphologically closer to P. saxatilis in lobe shape and to *P. serrana* in placement of isidia. However, the BLAST search and haplotype analysis of nucITS rDNA sequences placed these specimens in P. ernstiae. Parmelia sulcata, a common species in the Northern Hemisphere, is rare elsewhere, and only two records confirmed by molecular data were known from South America. The nucITS rDNA haplotype of two new and two previous records of the species represent the most common haplotype of the species known from the Northern Hemisphere.

Keywords

lichenized Ascomycota; parmelioid lichens; species distribution; morphology; molecular barcoding; haplotype network

1. Introduction

The lichen genus *Parmelia* (L.) Ach. belongs to the family Parmeliaceae and comprises foliose species with elongated or effigurate pseudocyphellae on the upper surface of the thallus (located marginal or/and laminal), and simple, branched to squarrose rhizines on the lower side (Hale, 1987; Thell et al., 2011). Atranorin is always present in the upper thallus cortex and depsides, depsidones, fatty acids, or, less frequently, dibenzofurans in the medulla (Hale, 1987; Ossowska et al.,

2018; Thell et al., 2008). To date, about 41 *Parmelia* species have been described (Crespo et al., 2020; Divakar et al., 2015), and their identification is based on morphological, chemical, and ecological data, often supported by molecular evidence (Corsie et al., 2019). Such a multifaceted approach has recently been recommended as due to the plasticity of diagnostic features in *Parmelia*, the determination of specimens based on solely the traditional taxonomic methods can lead to some identification errors (e.g. Castellani et al., 2021; Corsie et al., 2019; Haugan & Timdal, 2019; Ossowska et al.,

2018, 2019; Tsurykau et al., 2019). For example, the presence of pruina has been reported as a diagnostic feature in isidiate Parmelia species (Feuerer & Thell, 2002; Molina et al., 2004), but it has no diagnostic value as sampled studies show that individual specimens can vary in the pruinosity degree (Corsie et al., 2019; Ossowska et al., 2018). In the group of Parmelia species without vegetative propagule, the presence of lobaric acid was reported as a diagnostic (Hale, 1987; Thell et al., 2008), however, it has been shown that this secondary metabolite can be present or absent in different specimens of the same species (Ossowska et al., 2019). It proved that the identification of Parmelia on the basis of morphological and chemical characteristics alone cannot be satisfactory. In addition, the incorporation of molecular methods in the taxonomy of the genus Parmelia has contributed to the description of cryptic taxa, such as P. encryptata A. Crespo, Divakar & M. C. Molina (Molina et al., 2011; Ossowska et al., 2021) and near-cryptic, like P. rojoi A. Crespo, V. J. Rico & Divakar, within the genus (Crespo et al., 2020). As a consequence of the widespread application of the integrative taxonomy concept, the knowledge of the distribution of Parmelia species should also be updated.

Here, we report new localities of three *Parmelia* species, whose identification is supported by molecular data. These are the first localities of *P. ernstiae* Feuerer & A. Thell from Madeira, three new records of *P. encryptata* from Poland, and three new records of *P. sulcata* Taylor from Chile, the last two species being rarely reported in these countries. We also discuss the diagnostic features of these species and their differences with other similar taxa, together with notes on their distribution.

2. Materials and methods

Taxon sampling. Fresh material (a total of 50 specimens) for this study was collected during various lichenological fieldwork in Chile in 2023, Madeira in 2022, and Poland in 2017 and 2023 by U. Schiefelbein, R. Szymczyk, and M. Kukwa, respectively. All samples are deposited in the UGDA herbarium. Morphology was examined under a stereomicroscope. Lichen substances were identified with thin-layer chromatography methods (TLC) in solvents A and C according to methods summarized by Orange et al. (2001). The distribution map was prepared using QGIS 3.30.1 software.

DNA extraction, amplification, sequencing and haplotype network. DNA was extracted, amplified, and purified using the instructions presented in previous works (Ossowska et al., 2018, 2019). Sequencing was performed in Macrogen sequencing system (http://www.macrogen.com). The newly obtained sequences were deposited in GenBank (http://www. ncbi.nlm.nih.gov/genbank) and their accession numbers are listed in Table 1. The newly generated nucITS rDNA sequences were compared using BLAST search (Altschul et al., 1990).

For haplotype network analysis, we downloaded all sequences of *Parmelia encryptata*, *P. ernstiae*, and *P. sulcata* (Table 1) from GenBank. All sequences were aligned in Seaview (Galtier et al., 1996; Gouy et al., 2010), and the terminal ends were cut. The alignment of *P. encryptata* consisted of 20 sequences, *P. ernstiae* of 123 sequences, and *P. sulcata* of 194 sequences. The TCS network (Clement et al., 2002) was created using

PopArt software (http://popart.otago.ac.nz) and modified in Inkscape (http://inkscape.org).

3. Results and discussion

3.1. *Parmelia encryptata* A. Crespo, Divakar & M. C. Molina

Parmelia encryptata is a cryptic species, morphologically and chemically identical to P. sulcata, and is characterized by sublinear lobes (Figure 1A), with marginal and laminal, elongated pseudocyphellae and soralia on the upper surface (Figure 1A, Figure 1B), the presence of salazinic acid in the medulla and black, simple to squarrose rhizines on the lower surface (Figure 1C, Figure 1D) (Molina et al., 2011). In the new specimens, we observed a predominance of simple rhizines, with squarrose rhizines being grouped only in the central part of the thalli (Figure 1C). This trait has been suggested by Ossowska et al. (2021) as a potential diagnostic feature of P. encryptata. However, the number of studied specimens of this species is still too small to unequivocally confirm its usefulness. Furthermore, very few specimens of P. sulcata (e.g., samples from Chile cited below) may show similar characteristics in the rhizines placement to studied samples of P. encryptata; nevertheless the examination of this character can help to select samples for molecular study to confirm their identification.

The nucITS rDNA sequences were obtained from three specimens (Table 1) and BLAST search shows 99% and 100% similarity to the sequence of *P. encryptata* from Switzerland (MN654571). This sequence was originally deposited by Mark et al. (2020) as *P. sulcata*, but later corrected to *P. encryptata* by Ossowska et al. (2021). Furthermore, within the new sequences, we observed six nucleotides that Molina et al. (2011) and Ossowska et al. (2021) identified as diagnostic to distinguish *P. encryptata* from *P. sulcata*.

In the haplotype network (Figure 2), the sequences of P. encryptata are represented by three haplotypes (see also Table 1). The two specimens from Poland (UGDA L-24009 and 24214), like the specimens from Białowieża Forest cited by Ossowska et al. (2021), share the same haplotype (no. 2 in Figure 2) with the specimens from Ireland, Spain and Switzerland. The specimen from Borecka Forest (UGDA L-61390, haplotype no. 1) differs from haplotype no. 2 in one site and represents a new haplotype of the species (Figure 2). The haplotype no. 3 is represented by a group of *P. encryptata* specimens from Spain (Molina et al., 2011), and a single sequence (AF159947, see Table 1) obtained by Arup and Grube (2000), for which there is no information on collection site (therefore the record is not presented in Figure 3). The sequences deposited as P. sulcata in GenBank (OQ717535) obtained from a specimen from the Czech Republic share six nucleotides characteristic of P. encryptata. This sequence also shows similarity with P. encryptata sequences in BLAST search. Unfortunately, there are no geographical coordinates for this specimen in the GenBank database. Therefore, it is not presented in Figure 3.

The species is only known in Europe from Ireland, Poland, Spain, Switzerland (Ossowska et al., 2021) (Figure 3), and the Czech Republic (see above). In Poland, it was previously reported only from two localities in the Białowieża Forest (Ossowska et al., 2021). Taking into account all confirmed localities of this taxon, Ossowska et al. (2021) suggested that

Table 1 List of sequences used in haplotype network. Newly obtained sequences are in bold.					
Species	Haplotype number	Sequences	Country		
P. encryptata	1	OR509530	Poland		
	2	MN387037, MN387038, OR509531, OR509532	Poland		
		MN654571	Switzerland		
		EU788037, EU788036	Ireland		
		AY579455, AY579456, MZ557928, MZ557929,	Spain		
		MZ337930	Croch Dopublic		
	3	A E150047	No information		
	5	M7557024 M7557027 AV570440	Spain		
D ornetiao	1	WIL257724-WIL257727, AI 577447	Jatria		
r. ernstute	1	ON800467	Luvombourg		
	L	01809407 M72559021	Eranco		
		MZ558021	France		
		KU845670, M1581504	Latvia		
		MK56/160, MK56/162	Norway		
		KU845668	Poland		
		KT625494, EF421713, AY247007	Sweden		
		KT625495	Ireland		
		EF611286	Bosnia and Herzegovina		
		EF611291	Estonia		
		EF406113, EF406114	Denmark		
		OR509536, OR509534	Portugal (Madeira)		
		OQ717532, EF611289	Czech Republic		
		MZ558020, MH039612-MH039691, AY350041	UK		
		AY295110	Spain		
		AF410833, EF611292, AF410834	Germany		
	3	OQ717996, OQ717531	Czech Republic		
		MK778629	Russia		
		KT625493	Turkey		
		EF611290	Germany		
		OR509533, OR509535	Portugal (Madeira)		
		MZ558018, MZ558019, MT580478, MT580479, MT580480, MT580481, MT580482, MT580483	Spain		
		KU845674, KU845681	Poland		
P. sulcata	1	KU845646	Poland		
	2	KU845644	Poland		
	3	KU845650	Poland		
	4	HM017012	Spain		
	5	IN118581 IN118582	Slovakia		
	6	IN118584	India		
	7	FU788031 HM017009	Turkey		
	-	EU788029	France		
		NR119704 EU788026 EU788027 M7557923	Ireland		
		0.0918728 0.0717537 KU845622	Czech Republic		
		MW703518 MT200727 MT200740	Italy		
		$\frac{1}{1} \frac{1}{1} \frac{1}$	Dolond		
		KU845629-KU845637, KU845639-KU845643,	Poland		
		к ∪845645, K ∪845647–K U845649, K U845653–K U845659, MN 387107–MN 387112			

Table I Continue

Species	Haplotype number	Sequences	Country
		MW793516, MN654563-MN654565, MN654568	Estonia
		MG676382, MG676383	Finland
		KU845652	Latvia
		KU845628, AF451773	Denmark
		KX961402, AF410840	Sweden
		GU994574	New Zealand
		AY579453, OK491792, AY579454, AF410839	Germany
		AF451774, AF410838, OR509537 , OR509538	Chile
		OQ622242, AY581083, FR799253-FR799255	UK
		MZ557869–MZ557883, MZ557885–MZ557891, MZ557893, MZ557895–MZ557919, HM017007, HM017008, HM017010, HM017019, HM017020, EU788020, AY579445, AY579447, AY580313, AY579452, EU788021, EU788022	Spain
		MZ557921, MK811938, MK812065, MK812088, MK812475, MK812499	Norway
		MZ557922, KT625521, KT625523, KT625524, HM017011, HM017013–HM017018, EU788024, EU788025	USA
		MZ557884	Romania
		MZ557920, KT625525, KT695399	Canada
		MN654566, MN654567, MN654569, MN654571, MN654572, KX132926	Switzerland
		EU266084	Hungary
	8	KT625520	Canada
	9	EU788033	USA
	10	EU788032	Norway
		AY036981	Russia
	11	OQ717536	Czech Republic
	12	MZ557892, MZ557894	Spain
	13	EU788030	UK
		EU788028	Ireland
	14	EU788023, HM017006	Spain
	15	MN654562	Estonia
	16	AY036980	No information
	17	JN118583	Slovakia

it prefers large forest ecosystems. However, new record of the species from the cemetery indicate that *P. encryptata* may also be present in other habitats as well.

Specimens examined: Poland, (1) Pojezierze Ełckie, Puszcza Borecka Forest, 54.11662°N, 22.07067°E, ATPOL grid square Bf 03, deciduous forest dominated by oaks and hornbeam, on *Tilia cordata*, 18 March 2023, leg. R. Szymczyk, det. E. A. Ossowska (UGDA L-61390; GenBank OR509530); (2) Pojezierze Dzierzgońsko-Morąskie, Postolin, 53.873889°N, 19.052222°E, ATPOL grid square Bd 43, cemetery with free standing trees, on *Tilia cordata*, 15 Apr. 2017, leg. M. Kukwa 19243, det. E. A. Ossowska (UGDA L-24009; Gen-Bank OR509531); (3) Wysoczyzna Polanowska, Skotawskie Łąki nature reserve, by unnamed lake (N of Lipieniec lake), 54.261944°N, 17.553056°E, ATPOL grid square Ac 93, beech forest, on *Fagus sylvatica*, 27 June 2017, leg. M. Kukwa 19830, det. E. A. Ossowska (UGDA L-24214; GenBank OR509532).

3.2. Parmelia ernstiae Feuerer & A. Thell

Parmelia ernstiae was previously separated from other isidiate species mainly on the basis of the presence of pruina, placement of isidia and the production of fatty acids and lobaric acid (Feuerer & Thell, 2002; Molina et al., 2004; Ossowska, 2021; Ossowska et al., 2018). However, according to Corsie et al. (2019), *P. ernstiae* is a near-cryptic species, very similar in morphology and secondary chemistry to *P. saxatilis* and *P. serrana* A. Crespo, M. C. Molina & D. Hawksw., and their



Figure 1 Morphology of *Parmelia encryptata*. (**A**) lobes with marginal and laminal, elongated pseudocyphellae (UGDA L-61390); (**B**) lobes with soralia (UGDA L-24009); (**C**) rhizines (UGDA L-24009); (**D**) sorediate lobes with rhizines visible at the edges (UGDA L-61390). Scale bars: 1 mm.



Figure 2 Haplotype network showing relationships among ITS haplotypes of *Parmelia encryptata*. The new sequences from Poland are marked as red dots.

correct differentiation, therefore, requires the use of a combination of molecular and phenotypic characters. Variation in morphological characters was also observed in specimens from Madeira. In specimens UGDA L-61375 and 61386, we found a set of features that Corsie et al. (2019) and Ossowska et al. (2018) described as most common in *P. ernstiae* – a short, broad lobes with laminal isidia, which are present predominantly in central parts of thalli (Figure 4C). On the other hand, in specimens UGDA L-61382 and 61384 the lobes were longer and broader, and sublinear (Figure 4A, Figure 4B) as in *P. saxatilis*. In addition, the isidia were laminal and marginal, as in *P. serrana* (Figure 4A). The common features for all specimens were non-overlapping lobes very often with lobules, laminal and marginal, linear pseudocyphellae (Figure 4A–D), and simple to furcate rhizines (Figure 4D). The shape of lobes and distribution of isidia are characteristics that may vary between samples, as shown by Corsie et al. (2019). All specimens produce lobaric and fatty acids as reported in previous works, but specimens without these substances are also known from the species (Corsie et al., 2019; Ossowska et al., 2018).

The nucITS rDNA sequences were obtained from four specimens (Table 1) and show 97% (UGDA L-61375 & 61384) and 100% (UGDA L-61386 & 61382) of similarity to the sequences of P. ernstiae deposited in GenBank (OQ717996 and MK567162). In the haplotype network, three haplotypes of P. ernstiae were found (Figure 5). The samples from Madeira represent two haplotypes (no. 2 and 3), which differ from each other by two sites (Figure 5). The haplotype no. 2 seems to be very common, as it is represented by more than one hundred P. ernstiae sequences. Most of these were obtained from specimens collected in Great Britain (Corsie et al., 2019), but nevertheless, this haplotype was observed in specimens collected from fifteen countries (Table 1). The haplotype no. 3, in addition to two samples from Madeira, is also represented by sequences from the Czech Republic, Germany, Poland, Russia, Spain, and Turkey (see also Table 1).



Figure 3 Distribution map of *Parmelia encryptata*. Blue dots – localities according to literature data, red dots – new localities in Poland (base map provided by Natural Earth, www.naturalearthdata.com).



Figure 4 Morphology of *Parmelia ernstiae* specimens from Madera. (**A**) and (**B**) sublinear lobes with marginal and laminal isidia (UGDA L-61382); (**B**) lobes with marginal and laminal pseudocyphellae (UGDA L-61382); (**C**) short and broad lobes (UGDA L-61384); (**D**) lobes with lobules (UGDA L-61384). Scale bars: 1 mm.



Figure 5 Haplotype network showing relationships among ITS haplotypes of *Parmelia ernstiae*. The new sequences from Madeira are marked as red dots.

Parmelia ernstiae is widely distributed in Europe (Castellani et al., 2021; Corsie et al., 2019; Feuerer & Thell, 2002; Hawksworth et al., 2008, 2011; Kukwa et al., 2012; Tsurykau et al., 2019). It is worth noting that not all records are supported by phylogenetic evidence. Outside Europe, the species has been reported from Africa (Algeria and the Canary Islands), as well as from the Asian part of Russia (Hawksworth et al., 2008, 2011). Here, we present the first records of *P. ernstiae* from Madeira (Portugal). Specimens examined: Portugal, Madeira, (1) Near the road to Abrigo do Poiso village, 32.71917°N, 16.88806°W, forest with *Picea* sp., on the bark of *Picea* sp., 30 May 2022, leg. R. Szymczyk, det. E. A. Ossowska (UGDA L-61382 & 61384; GenBank OR509534 & OR509535); (2) Riberio Frio village, edge of deciduous forest, 32.738889°N, 16.886944°W, road-side trees, on the bark of *Quercus* cf. *robur*, 30 May 2022, leg. R. Szymczyk, det. E. A. Ossowska (UGDA L-61375; GenBank OR509533); (3) Miradouro do Rabaçal, 32.756111°N, 17.131111°W, road in *Laurel* and *Juniper* forest, saxicolous, 02 June 2022, leg. R. Szymczyk, det E. A. Ossowska (UGDA L-61386; GenBank OR509536).

3.3. Parmelia sulcata Taylor

Parmelia sulcata is characterized by sublinear lobes (Figure 6A, Figure 6B), with marginal and laminal soralia (Figure 6B, Figure 6C), simple to squarrose rhizines (Figure 6D), and the presence of salazinic acid in the medulla (see also Hale, 1987; Ossowska, 2021; Thell et al., 2011). Morphologically and chemically similar species are P. barrenoae Divakar, M. C. Molina & A. Crespo, P. encryptata and P. asiatica A. Crespo & Divakar (Divakar et al., 2005; Lumbsch et al., 2011; Molina et al., 2011). From P. encryptata the species differs only in the nucITS rDNA sequence (see notes under that species). The other two taxa differ from P. sulcata in the distribution of the soredia, the lobe shape and width and, the rhizines. Parmelia barrenoae has broad and overlapping lobes, soralia are laminal and like fissures in the upper cortex and rhizines are simple to furcate (Divakar et al., 2005; Hodkinson et al., 2010). In P. asiatica, on the other hand, soralia are predominantly circular and semicircular, terminal,



Figure 6 Morphology of *Parmelia sulcata* from Chile. (A) and (B) sublinear lobes with marginal and laminal soralia (6190 & 6643); (C) marginal and laminal soredia (6643); (D) lobes with abundant, squarrose rhizines (6190). Scale bars: 1 mm.



Figure 7 Haplotype network showing relationships among ITS haplotypes of *Parmelia sulcata*. The new sequences from Chile are marked as red dots.

or marginal, while the lobes are narrow and sublinear (Lishtva et al., 2013; Lumbsch et al., 2011). Both taxa have rarely been observed, although some recent data suggest that they may be more common (Ossowska, 2023).

A diagnostic feature that has been suggested by various authors as crucial in the identification of sorediate Parmelia species is the shape of rhizines (Divakar et al., 2005; Molina et al., 2011; Ossowska & Kukwa, 2016), as P. barrenoae has simple to furcate rhizines while P. sulcata, P. asiatica, and P. encryptata simple to squarrose. However, according to Hodkinson et al. (2010) and Ossowska (2021), the rhizine shape may be confusing, which agrees with our observations on specimens from Chile. In one specimen (leg. Schiefelbein 6615), we observed a dominance of simple rhizines, with only a few squarrose, distributed in the central part of the thallus. At first sight, we assumed that this specimen represented P. encryptata or P. barrenoae. However, the molecular data confirmed that the sample represents P. sulcata. Therefore, caution is needed in the examination of rhizines in sorediate Parmelia specimens, and they need to be checked in several thallus parts, not only in marginal areas.

We obtained nucITS rDNA sequences from three specimens collected in Chile, two of which were identical to *P. sulcata* sequences (MN387108 and MN654572) deposited by Singh et al. (2019) and Mark et al. (2020). One specimen (leg. Schiefelbein 6615) showed only 97% similarity to the *P. sulcata* sequence (MN654566). Unfortunately, this sequence was of poor quality, with too many unspecific positions, so we did not include it in further analyses.

To better understand the sequence variation from Chile with other *P. sulcata* samples, we constructed a haplotype network (Figure 7). To do this, we used most of the nucITS rDNA sequences of *P. sulcata* deposited in GenBank (Table 1). In total, we found seventeen *P. sulcata* nucITS rDNA haplotypes (Figure 7), with differences usually in one or two nucleotide positions. The exception is sequence KT625520 from Canada (as haplotype no. 8 in Figure 7). On the tree presented by Molina et al. (2017), this sequence forms a separate clade, sister to *P. sulcata*. It may represent a new species, but this hypothesis requires further study.

All *Parmelia sulcata* sequences from Chile represent the most common haplotype (no. 7, Figure 7), also found in specimens from Europe, Asia, and North America (Table 1).

Parmelia sulcata belongs to the widespread *Parmelia* species and is common, especially in Europe (see Ossowska, 2021 and literature cited therein), although not all records are molecularly confirmed. In Chile this taxon was reported by Hale (1987) from the Magallanes and Stenroos (1991) from Tierra del Fuego, however, the molecular evidence for the occurrence of this species there was only provided by Thell et al. (2002) and Feuerer and Thell (2002) from the department Magallanes y Antártica Chilena. Here, we present a further three records of *P. sulcata* from Chile supported by molecular data (Figure 8).

Specimens examined: Chile, (1) Dept. Magallanes y Antártica Chilena, Prov. De Última Esperanza, Torres del Paine, E of national park Torres del Paine, Camino Laguna Azul, 50.856111°S, 72.71056°W, saxicolous, 18 Jan. 2023, leg. U. Schiefelbein 6590, det. E. A. Ossowska (UGDA; GenBank



Figure 8 Distribution map of *Parmelia sulcata* in South America (only records confirmed by molecular data). Blue dots – localities according to literature data, red dots – new localities (base map provided by Natural Earth, www.naturalearthdata.com).

OR509537); (2) Seno Ultima Esperanza, Estancia Perales, 51.542778°S, 72.84361°W, 19 Jan. 2023, leg. U. Schiefelbein 6615, det. E. A. Ossowska (UGDA); (3) Prov. De Magallanes, Punta Arenas, Parrillar Laguna National reserve, forest NW of the camping place along the hiking trail, 53.40444°S, 71.26944°W, 21 Jan. 2023, leg. U. Schiefelbein 6643, det. E. A. Ossowska (UGDA; GenBank OR509538).

4. Conclusion

The genus *Parmelia* includes lichens with large, easily visible thalli, however, their identification can be confusing due to the morphological and chemical similarity between species as well as intraspecific variability. In addition, several new *Parmelia* species have been described in recent years that can be considered as cryptic or near-cryptic and whose differentiation on the basis of phenetic characteristics may not be correct. We therefore recommend that the identification of *Parmelia* should be based on the concept of integrative tax-

Acta Mycologica / 2023 / Volume 58 / Article 175356 Publisher: Polish Botanical Society onomy. As we show here, quick tools such as a haplotype network or a BLAST search are sufficient for simple taxonomic identification.

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