


Short Communication

An ITS sequence of a specimen from the probable *locus classicus* of *Ramalina peruviana* and its consequences

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(Accepted 11 May 2023)

Ramalina peruviana Ach. was described in the early days of lichen classification (Acharius 1810). In those days, only a limited set of distinguishing characters was known, resulting in short descriptions with limited information (Fig. 1). Today, microscopy, TLC and phylogenetic analyses provide much more data, allowing a much more detailed and phylogenetically supported classification (see e.g. Spjut *et al.* 2020). However, as a result, it can be problematic finding the correct application of names such as *Ramalina peruviana*. Spjut *et al.* (2020) thus proposed the hypothesis that records of *R. peruviana* from Southern Europe and Macaronesia, far away from Peru, belong to a separate species with a type from the region, *Ramalina crispans* Werner, described from Morocco.

To test this hypothesis, we looked for a specimen from the *locus classicus* that would allow extraction of the ITS sequence. The age of the type specimen makes it unsuitable for simple sequencing and we needed a fresh specimen. Unfortunately, this *locus classicus* is not very precisely indicated in the original description (Fig. 1), just given as Peru ('in Peruvia'). However, in the English translation the complete second paragraph of the first description says: 'Lives in Peru, together growing with *Borrera villosa* and *Borrera epebea*. Lagasca.'. These associated lichen species, now named *Seiropora villosa* (Ach.) Frödén and *Tornabea scutellifera* (With.) J. R. Laundon, restrict the *locus classicus* greatly because they occur in South America only along the Pacific coast from Peru to northern Chile. Here, the Humboldt Current in the Pacific Ocean causes frequent fog in the otherwise desert-like, cool, coastal area and provides a suitable climate for these species. The presence of these two species along the Pacific coast of Peru, and in particular near Lima (Peru), is confirmed in the revisions of Hillmann (1930) and Nimis & Tretiach (1997).

A further, rather cryptic indication for the origin of the type specimen of *Ramalina peruviana* is the addition 'Lagasca'. This refers to Mariano Lagasca y Segura (1776–1839), a leading botanist in Spain. Apparently Acharius received the specimen from him. Lagasca is not known to have visited Peru himself and must have obtained the specimen from somebody else. Krog & Swinscow (1976), who identified the holotype of *R. peruviana* in the Acharius herbarium

in Helsinki (abbreviation H-ACH), give the name as 'Lagasta', apparently a misinterpretation of the unusual characters used in the book. Remarkably, the picture of the holotype on the internet (JSTOR 2022) lacks the name of this botanist and instead has 'Cavanilles' written in pencil and probably added later. This represents Antonio J. Cavanilles (1745–1804), another leading botanist in Spain at that time, who also did not visit Peru himself. In view of this contradictory information, it is believed that the specimen was collected during the Expedición Malaspina in 1789–1794, most probably by the botanist of the team, Thaddäus Haenke, a prolific plant collector in South America. His visit to Lima (Callao) during this expedition is certain because he left the expedition there to explore the Andes. In those days, a trained botanist travelling to Lima and being prepared to collect botanical specimens was still very unusual, so it is unlikely that somebody else did collect the material of *Ramalina peruviana*. After the expedition, Malaspina, the expedition leader, fell into disrepute and was put in jail (Wikipedia, accessed 4 October 2022; https://es.wikipedia.org/wiki/Alejandro_Malaspina). For this reason, the evaluation of his botanical collections was taken over by Spanish botanists. This would explain why the names of Lagasca and Cavanilles both show up in connection with the type specimen of *R. peruviana*. Summarizing all available evidence, the type specimen of *R. peruviana* most probably came from fog oasis-like vegetation near Lima in Peru. Such vegetation is still present there in the National Reserve of Lachay, and that seems to be a suitable place to obtain ITS sequences of *Ramalina peruviana* in the original sense (Fig. 2).

For the sequencing, the following sample from the *locus classicus* was used: Peru, Prov. Lima, district Huacho, National Reserve of Lachay (Resolución Jefatural 007-2018), elev. 350 m, 11°21.3'N, 77°21.85'W, 15 Dec. 2018, A. M. Ramírez Ordaya, 17 dic. 2018 (USM, B 60 0176943). A fragment was sent to the Botanical Museum in Berlin (B), where the chemistry was investigated by TLC (Orange *et al.* 2001; solvents A, B and C). DNA sequencing of the ITS region was performed by P. Alvarado (Alvalab, Spain; <http://www.alvalab.es/>) using the extracted material remaining after TLC, and the methods outlined in van den Boom & Alvarado (2021). In addition, using the same methods, new sequences were obtained from other specimens of *R. peruviana* and five related species (Table 1).

For the phylogenetic analysis presented in Fig. 3, all available ITS sequences in GenBank of *Ramalina peruviana* and the

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Cite this article: Sipman HJM and Ramírez Ordaya Á (2023) An ITS sequence of a specimen from the probable *locus classicus* of *Ramalina peruviana* and its consequences. *Lichenologist* 55, 437–440. <https://doi.org/10.1017/S0024282923000324>



4. RAMALINA peruviana.
*R. thallo plano-compresso ramofo cinerascete, ramis angustatis undulatum tor-
 tis margine inaequalibus apice lacero-fubdigitatis.*
 Habitat in Peruvia in confortio crescens cum Borrera villosa et B. ephre-
 bea. *Logofsa.*

Figure 1. The first description of *Ramalina peruviana* (Acharius 1810). In colour online.

probable relatives *R. crispans*, *R. hyrcana* Sipman and *R. montagnei* De Not. were used. We also added four sequences each of related species present in fig. 9 of Spjut *et al.* (2020). All sequences were trimmed according to the shortest sequence (MN811427), and sequences shorter than this were omitted. The sequences were aligned with Muscle (<https://www.ebi.ac.uk/Tools/msa/muscle/>; accessed 12 September 2022) (Madeira *et al.* 2022) with some manual corrections. A maximum likelihood (ML) tree was calculated with IQ-TREE (Trifinopoulos *et al.* 2016; <http://iqtree.cibiv.univie.ac.at/>) using default settings (number of bootstrap alignments 1000, maximum iterations 1000, minimum correlation coefficient 0.99, perturbation strength 0.5 and IQ TREE stopping role 100). The tree was visualized with FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) and rooted in accordance with fig. 9 of Spjut *et al.* (2020). Maximum parsimony and maximum likelihood values are presented in Fig. 3.

Ramalina peruviana clusters with *R. crispans*, *R. hyrcana* and *R. montagnei* (Fig. 3) and forms a well-supported clade. Sister is *R. polymorpha* (Lilj.) Ach., as in fig. 9 of Spjut *et al.* (2020). Within the clade, all sequences except those from East Asia are on a well-supported branch. On this branch, *R. montagnei* holds a distinct position and appears to deserve recognition as a separate species. It differs morphologically because it is a non-sorediate, regularly fertile taxon. In the alignment it deviated by two base pairs from all other samples (Fig. 4). Otherwise it agrees with *R. peruviana* s. str., except for one base pair which it shares with the East Asiatic samples.

Further samples on this branch, previously identified as *Ramalina peruviana*, *R. crispans* and *R. hyrcana*, have completely identical sequences, which supports their treatment as a single species. The earliest name available for this subclade

Table 1. Newly generated ITS sequences from specimens of *Ramalina* species, including voucher information and GenBank Accession numbers.

| Name | Country/ region | Specimen | GenBank Accession no. |
|-----------------------------|--------------------|---------------|--------------------------|
| <i>Ramalina canariensis</i> | Greece | Berger 33103 | OQ695772 |
| <i>R. farinacea</i> | Greece | Sipman 60734 | OQ695768 |
| <i>R. farinacea</i> | Greece | Sipman 62173 | OQ695769 |
| <i>R. fraxinea</i> | Greece | Sipman 62225 | OQ695770 |
| <i>R. fraxinea</i> | Greece | Sipman 62509 | OQ695771 |
| <i>R. hyrcana</i> | Iran | Kasemi 7942 | OQ695773 |
| <i>R. hyrcana</i> | Iran | Kasemi 7970 | OQ695774 |
| <i>R. peruviana</i> | Azores | Boom 55163 | OQ695775 |
| <i>R. peruviana</i> | Azores | Boom 57046 | OQ695776 |
| <i>R. peruviana</i> | Brazil | ISE 42753 | OQ695777 |
| <i>R. peruviana</i> | Brazil | Aptroot 78432 | OQ695779 |
| <i>R. peruviana</i> | Peru | Ramirez 17 | OQ695778 |

is *Ramalina peruviana*. For *R. crispans*, no morphological difference from *R. peruviana* is known (Spjut *et al.* 2020) and it is best considered as a synonym of *R. peruviana*. *Ramalina hyrcana* is said to be different from *R. peruviana* because the lobes are more flattened and the soralia are downturned which give the thallus lobes a dorsiventral aspect (Lumbsch *et al.* 2011; fig. 21E & F). However, observation of specimens in herbarium B suggests that these differences are not constant, and the species is considered here to be also a synonym of *R. peruviana*.

The position of the nine East Asian specimens in Fig. 3 indicates that they deserve a separate classification. They deviate in the alignment from *R. peruviana* as defined above by six base pairs forming two groups of sequences containing three and six specimens (Fig. 4). *Ramalina intermediella* Vain., described from Japan and reported as a synonym of *R. peruviana* by Stevens & Kashiwadani (1987), appears to be the earliest

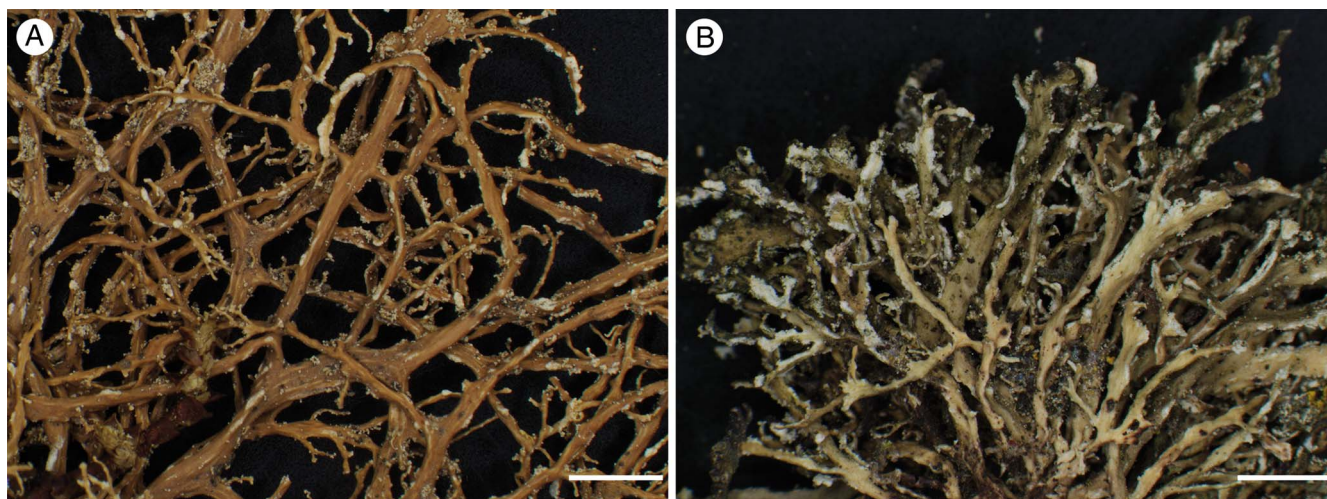


Figure 2. Specimens of *Ramalina peruviana* from the probable *locus classicus*, Lachay near Lima, Peru. A, well-developed specimen, collected in 1960 (Mattick 25, B 60 0016401). B, specimen used for sequencing, collected in 2018 (B 60 0176943), dwarfed probably by air pollution. Scales: A & B = 2 mm. In colour online.

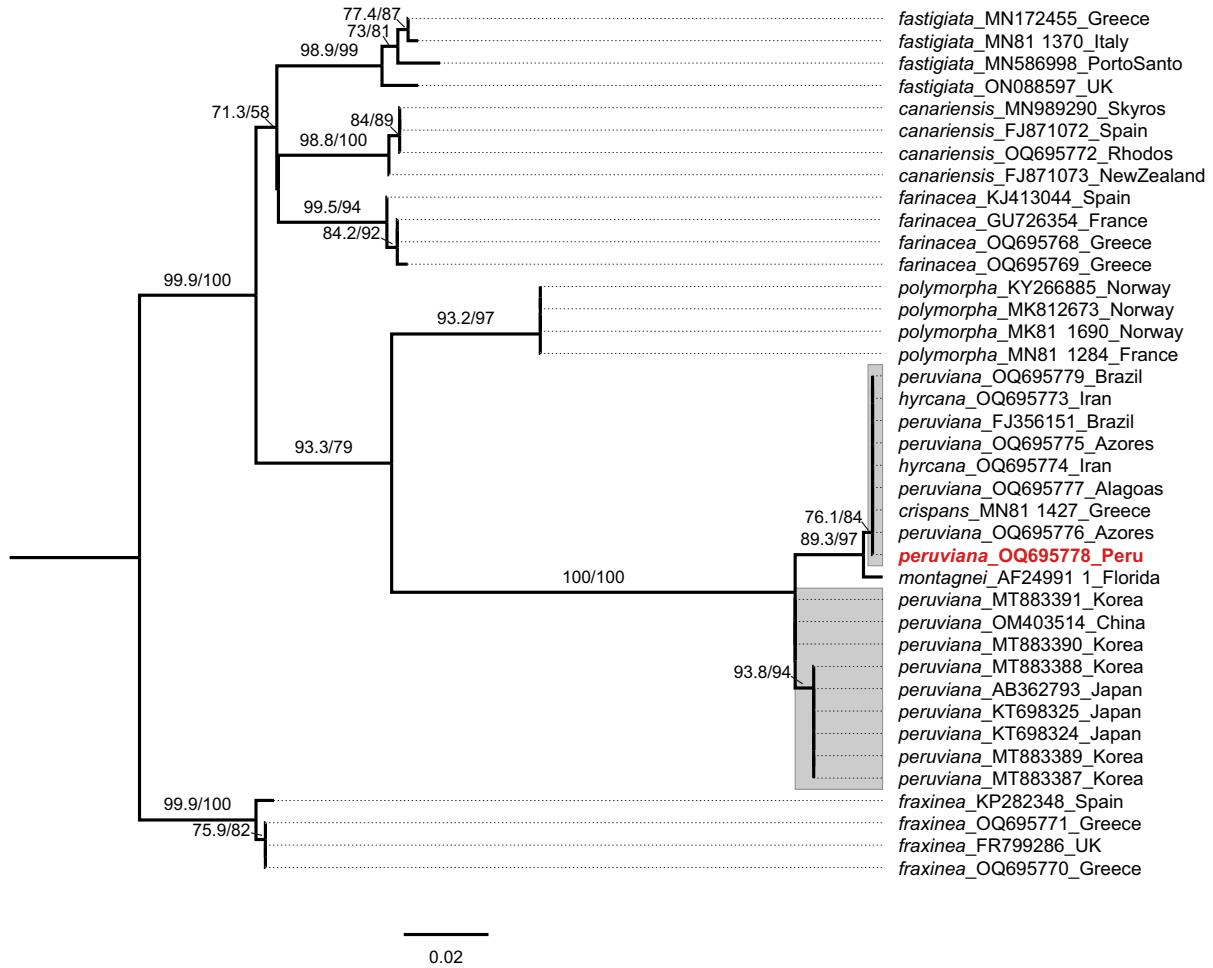


Figure 3. Maximum likelihood (ML) tree of all available ITS sequences of the *Ramalina peruviana* complex, compared with four selected sequences each of *R. fraxinea* (L.) Ach., *R. polymorpha*, *R. fastigiata* (Pers.) Ach., *R. canariensis* J. Steiner and *R. farinacea* (L.) Ach. The sequence from the locus classicus is in bold red font; the clades of *R. peruviana* and *R. intermediella* are indicated with grey boxes and *R. montagnei* is shown outside these clades. Bootstrap values (maximum parsimony/maximum likelihood) > 50 are indicated. In colour online.

name available for East Asiatic samples, which are currently treated as *R. peruviana*, for example in Kashiwadani *et al.* (2006).

Taxonomic Treatment

Ramalina peruviana Ach.

Lich. Univ., 599 (1810); type: PERU (‘Peruvia’). Comm. Cavanilles (H-ACH 1801—holotype).

Syn.: *Ramalina crispans* Werner, *Scientific Annals of the School of Agriculture and Forestry, Aristotelian University, Thessaloniki IH’-B*, 1 (1977); type: Morocco, Forêt de Mamora près Rabat, sur Quercus suber, 01.02.1936, Werner s. n. (BC—holotype).

Syn.: *Ramalina hyrcana* Sipman, in Lumbsch *et al.*, *Phytotaxa* **18**, 100 (2011); type: Iran, Golestan, Gonbad-e-Kavus District, along road from Khan Bebin to Shirabad Waterfall, 120 m, deciduous forest with *Parrotia persica* and *Carpinus*, on *Pterocarya* trunk, October 2007, Sipman *et al.* 55201 (IRAN—holotype; B, hb. Sohrabi 9432—isolotypes).

Morphologically and chemically, *Ramalina peruviana* fits the descriptions provided by Kashiwadani (1987) and Stevens (1987). The secondary chemistry is dominated by homosekikaic and sekikaic acids. These are easily recognizable since they form two clearly separate spots in solvent B and stay close together in A and C (Elix 2018). The additional substances 4’-O-methylnorhomosekikaic, 4’-O-demethylsekikaic and

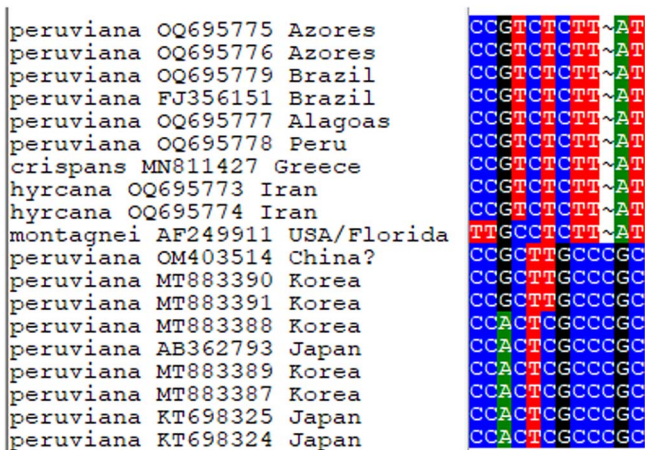


Figure 4. The variable base pairs in the ITS sequence of the *Ramalina peruviana* s. lat. cluster. In colour online.

ramalinolic acids, reported for example by Kashiwadani (1987) and Stevens & Kashiwadani (1987), were not visible on our TLC plates with the sample from the *locus classicus*, and seem to be present only in trace amounts. It remains to be seen if specimens with other substances, such as protocetraric, hypoprotocetraric and boninic acids, reported by Marcano *et al.* (2021), belong to the same species.

Ramalina intermediella Vain.

Bot. Mag., Tokyo **35**, 46 (1921); type: Japan, Prov. Inaba, leg. A. Yasuda 150, 1917 (TUR-V 1558—holotype).


Since *R. intermediella* was described from Japan, the only known genotype from there must be attributed to it. We refrain from proposing a name for the second genotype because we have not seen a specimen.

Ramalina montagnei De Not.

Giornale Botanico Italiano **2**(1.1), 218 (1846); type: 'Nelle Antille'.

The absence of soralia and frequency of apothecia make this species easily recognizable.

Acknowledgements. Thanks are due to the staff of the National Reserve of Lachay for providing a collecting permit for *Ramalina peruviana*, and to A. Aptroot and P. van den Boom, who sent specimens for verification.

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Competing Interests. The authors declare none.

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