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Parvoplaca nigroblastidiata, a new corticolous lichen (*Teloschistaceae*) in Europe, Turkey and Alaska

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Abstract: In a recent phylogenetic analysis of the family *Teloschistaceae* based on three molecular markers, 31 genera were newly described or resurrected. One of these genera was *Parvoplaca*, currently including four species in which anthraquinones may be present or absent in the apothecia. We have re-analyzed the genus and propose one new species, *P. nigroblastidiata* Arup, Halıcı & Vondrák, and one new combination, *P. chelyae* (Pérez-Vargas) Vondrák, Halıcı & Arup. The new species is known at present from Sweden, Turkey and Alaska. It is characterized by an endophloedal thallus, black blastidia produced in small spots and zeorin-lecanorine apothecia with an orange disc and black thalline margin. It is morphologically similar to *Caloplaca turkuensis*, but differs in the zeorine-lecanorine apothecia and the thalline margin that is dark grey-black instead of grey.

Key words: blastidiate, *Caloplaca chelyae*, ITS, molecular, phylogeny, taxonomy

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Introduction

The phylogenetic relationships within the lichen family *Teloschistaceae* were recently analyzed using three DNA loci in a combined analysis (Arup *et al*. 2013). A total of 39 genera were recognized in that study, of which 31 were newly described or resurrected. One of the new genera was *Parvoplaca* Arup *et al*., a small genus in the subfamily *Xanthorioideae*, currently including *P. athallina* (Darb.) Arup *et al*., *P. servitiana* (Szatala) Arup *et al*., *P. suspiciosa* (Nyl.) Arup *et al.* and *P. tiroliensis* (Zahlbr.) Arup *et al*. In addition, Arup *et al*. (2013) included three undescribed species, referred to as *Parvoplaca* sp. 25–27. One of these, *Parvoplaca* sp. 26, is described here as new to science. *Caloplaca chelyae* Pérez-Vargas, a recently described species from the Canary Islands (Pérez-Vargas & Pérez de Paz 2009), is shown to belong to the genus and accordingly transferred to *Parvoplaca*.

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Material and Methods

This study is based on material from BG, ERH, LD and PRA. The sequences of *Parvoplaca* sp. 1 and sp. 2 in this study correspond to *Parvoplaca* sp. 25 and sp. 27 in Arup *et al.* (2013). Unfortunately, the material of these is still too scanty for a formal description and is not elaborated further in this study. The specimens were examined by interference contrast and light microscopy. Anatomical features were measured on hand-cut sections or squash preparations mounted in water. Morphological characters were measured on dry material using a dissecting microscope (×40). Spore dimensions are average values from ten measurements per specimen. Data on spore dimensions are presented in the following way: (min. extreme) 85% of the variation (max. extreme). The measurements of anatomical and morphological characters mainly follow the guidelines of Vondrak *et al.* (2013). The term blastidia is used here *sensu* Smith *et al*. (2009), where blastidia are produced through budding from the thallus and are partly corticate whereas soredia lack any kind of cortical tissue.

PCR-amplification and sequencing

PCR amplification of the ITS regions including the 5.8S gene of the nuclear rDNA was made without an

TABLE 1. *Location, collector and GenBank accession numbers for species of* Parvoplaca *and the outgroup* Pachypeltis castellana *used in this study. Specimens collected before this study are in bold*

Species	Location and collector	GenBank Accession Number
P. athallina	**Antarctica, *Søchting* 11393 (C)**	**KC179111**
P. nigroblastidiata 1	Turkey, *Vondrák* JV10617 (PRA)	KT161978
P. nigroblastidiata 2	Turkey, *Halıcı* CL 0.098 (ERH)	KT161979
P. nigroblastidiata 3	Sweden, *Arup* L02344 (LD)	KT161980
P. nigroblastidiata 4	Sweden, *Arup* L02345 (LD)	KT161981
P. nigroblastidiata 5	USA, Alaska, *Tønsberg* 42983 (BG)	KT161982
***P. nigroblastidiata* 6**	**Sweden, *Arup* L10208 (LD)**	**KC179113**
P. nigroblastidiata 7	Sweden, *Jonsson* FU9356 (LD)	KT161983
P. nigroblastidiata 8	Sweden, *Jonsson* FU9449 (LD)	KT161984
P. nigroblastidiata 9	Sweden, *Nordin* FU8788 (LD)	KT161985
P. nigroblastidiata 10	Sweden, *Jonsson* FU5958 (LD)	KT161986
P. nigroblastidiata 11	Sweden, *Jonsson* FU7584 (LD)	KT161987
P. nigroblastidiata 12	Sweden, *Jonsson* FU5959 (LD)	KT161988
P. aff. nigroblastidiata	Turkey, *Halıcı* CL 0.096 (ERH)	KT162001
***P. suspiciosa* 1**	**Russia, *Hermansson* 16839 (LD)**	**KC179115**
P. suspiciosa 2	Russia, *Urbanavichene* 201-1 (H)	KT161989
P. suspiciosa 3	Sweden, *Hermansson* 18005 (LD)	KT161990
***P. servitiana* 1**	**Greece, *Spribille* 16225 (PRA)**	**JN641778**
***P. servitiana* 2**	**Greece, *Spribille* 13700 (PRA)**	**JN641779**
P. tiroliensis 1	Sweden, *Arup* L03354 (LD)	KT161991
***P. tiroliensis* 2**	**Sweden, *Arup* L02364 (LD)**	**KC179116**
P. tiroliensis 3	Norway, *Arup* L03372 (LD)	KT161992
P. chelyae 1	Turkey, *Halıcı* CL 0.828 (ERH)	KT161993
P. chelyae 2	Tenerife, *Vondrák* JV13093 (PRA)	KT161994
P. chelyae 3	La Palma, *Vondrák* JV13094 (PRA)	KT161995
P. chelyae 4	Turkey, *Vondrák* JV13092 (PRA)	KT161996
P. chelyae 5	Turkey, *Halıcı* CL 0.005 (ERH)	KT161997
P. chelyae 6	Turkey, *Halıcı* CL 0.237 (ERH)	KT161998
P. chelyae 7	Turkey, *Halıcı* CL 0.353 (ERH)	KT161999
P. chelyae 8	Turkey, *Halıcı* CL 0.508 (ERH)	KT162000
***Parvoplaca* sp. 1**	**USA, Oregon, *McCune* 26523 (priv. hb.)**	**KC179112**
***P.* sp. 2**	**USA, Oregon *McCune* 28337 (priv. hb.)**	**KC179114**
Pachypeltis castellana	**Greenland, *Søchting* 10500 (C)**	**KC170105**

extraction step. Direct PCR as described by Arup (2006) was used where no DNA extraction is needed prior to the PCR as pieces of either thalli, apothecia or soredia/blastidia are put directly into the PCR tube. The quantity of tissue used can be adjusted according to its age. Usually the procedure works well with 1–2 hand-cut slices of apothecia (0·1–0·2 mm thick), less then 1 mm^2 of thallus tissue or 10–20 soredia/blastidia when the material (within *Teloschistaceae*) is up to five years old. More material can be used in older collections if needed, but normally not beyond the age of 20–25 years. Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The PCR settings used followed the manufacturer's recommendations (Five Prime). In each PCR tube we added: 21 µl of water, 2·5 µl of buffer, 0·5 µl of dNTP, 0·4 µm of each primer and 0·125 µl of polymerase. PCR products were electrophoresed in a 1% agarose gel and visualized using GelRed™ (Biotium). Products were cleaned using a Cycle Pure Kit (Qiagen or Five Prime). The primers used for the PCR were also used in the sequencing reaction; sequencing was carried out by Macrogen Inc., Korea.

An alignment was produced with 33 sequences (Table 1) of *Parvoplaca* and *Pachypeltis castellana* (Räsänen) Arup *et al.* as outgroup, since this genus has been shown to be closely related to *Parvoplaca* (Arup *et al.* 2013). A suitable model of molecular evolution was selected using the Bayesian Information Criterion (BIC) as implemented in jModeltest ver. 2.1.4 (Guindon & Gascuel 2003; Darriba *et al.* 2012), evaluating only the 24 models available in MrBayes 3.2.0 (Ronquist *et al.* 2012). The SYM+G model was found to be optimal. Bayesian tree inference was carried out using Markov chain Monte Carlo (MCMC) as implemented in MrBayes 3.2.4. The number of discrete categories used to approximate the gamma distribution was set to 4. The following priors were used: beta (1, 1) on the transition-transversion rate, fixed on the state frequencies, uniform (0·001, 200) for the gamma shape parameter, and all trees *a priori* equally likely. The prior on branch lengths for the analyses was set to an exponential with mean 1/10. Three parallel

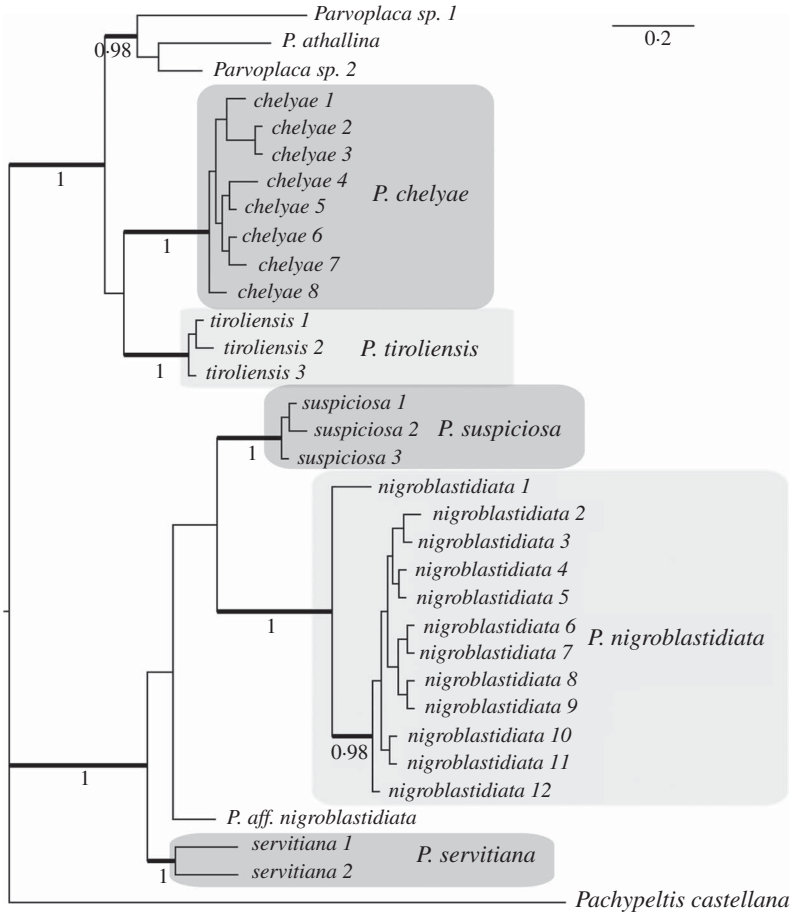

FIG. 1. Majority-rule consensus tree for all compatible groups of *Parvoplaca* used in this study. Average branch lengths are based on a Bayesian posterior tree sample comprising 2238 trees. Bayesian posterior probabilities are displayed at nodes. *Pachypeltis castellana* was used as the outgoup.

runs were performed, each with 6 chains, 5 of which were incrementally heated with a temperature of 0·10. Analyses were diagnosed every 100 000 generations and automatically halted when convergence was reached. Convergence was defined as a standard deviation of splits (with frequency b ≥0·1) between runs below 0·01. Every 1000th tree was sampled and the first 50% of runs were removed as burn-in. FigTree 1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CS4 were used to construct and illustrate a phylogenetic consensus tree.

Secondary chemistry

The secondary metabolite pattern was identified using HPLC and analyzed separately for thallus and apothecia. The relative composition of the secondary metabolites was calculated based on absorbance at 270 nm, according to Søchting (1997). Pigment nomenclature for non-anthraquinones follows Meyer & Printzen (2000).

Results and Discussion

A 50% majority-rule consensus tree from the post burn-in tree samples is presented in Figure 1. The ingroup split into two major clades, one including *P. athallina*, *P. chelyae* and *P. tiroliensis* together with two undescribed species, and the other including *P. suspiciosa*, *P. servitiana*, *P. nigroblastidiata* and a specimen similar to *P. nigroblastidiata*, but separate from the core group of that species.

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The new species forms a fully supported clade with some minor genetic variation within it. It appears to be sister to *P. suspiciosa*, but this relationship is not supported in the analysis. *Parvoplaca* aff. *nigroblastidiata* is placed outside this sister relationship and has greater genetic similarity with *P. servitiana*.

In the other clade, *P. athallina* and the two undescribed species form a supported clade (PP = 0·98) with *P. tiroliensis* and *P. chelyae* as sister species (PP = 1·0). The clades representing these species are fully supported. *Parvoplaca chelyae* is morphologically very similar to *P. tiroliensis*, but seems to be well separated genetically. The two species are also ecologically distinct and it seems reasonable to recognize *P. chelyae* as a separate species in *Parvoplaca*. However, *P. tiroliensis* is a more complex species than shown here (U. Arup & J. Vondrák, unpublished data) and requires further study.

Key to the known species of *Parvoplaca*, including some morphologically similar species of *Caloplaca*

1 Blastidia present, but sometime inconspicuous 2
Thallus not blastidiate 4

2(1) Blastidia in ± crater-formed cups, up to 0·15 mm diam . . . . . **Caloplaca ahtii**
Blastidia in ± flat spots to diffuse 3

3(2) Apothecia lecanorine-zeorine; apothecium margin and blastidia dark grey-black; blastidia usually in thin spots, 0·1–0·5 mm wide . . . . . **P. nigroblastidiata**
Apothecia lecanorine; apothecium margin and blastidia pale to dark grey; blastidia usually in slightly thicker layer and diffuse, usually in rather extensive patches, but sometimes in smaller spots . . . . . **Caloplaca turkuensis**

4(1) Apothecium disc greenish-yellow to blackish-yellow, with anthraquinones; muscicolous, lignicolous or on plant debris 5
Apothecium disc brownish-black to black, without anthraquinones; corticolous . . . 7

5(4) Antarctic species . . . . . **P. athallina**
Northern Hemisphere species 6

6(5) Arctic-alpine species growing mainly in humid, limestone dominated areas; muscicolous, lignicolous or on plant debris . . . . . **P. tiroliensis**
Alpine species growing in dry, mainly siliceous areas; muscicolous . . . . **P. chelyae**

7(4) In Northern Europe and Asia; hymenium up to 65 µm; apothecia 0·5 mm diam . . . . . **P. suspiciosa**
In SE Europe; hymenium up to 80 µm; apothecia 0·8 mm diam . . . . **P. servitiana**

Parvoplaca nigroblastidiata Arup, Halıcı & Vondrák sp. nov.

MycoBank No.: MB 812855

Similar to *Caloplaca turkuensis*, but with zeorine-lecanorine apothecia, a darker thalline margin, endophloedal thallus and black blastidia in small spots, usually darker and less extensive than in *C. turkuensis*.

Type: Sweden, Jämtland, Nyhem par., SE-facing slope of Bodberget, on *Populus tremula*, alt. 415 m, 30 June 2012, *F. Jonsson* FU9356 (LD—holotype; UPS—isotype).

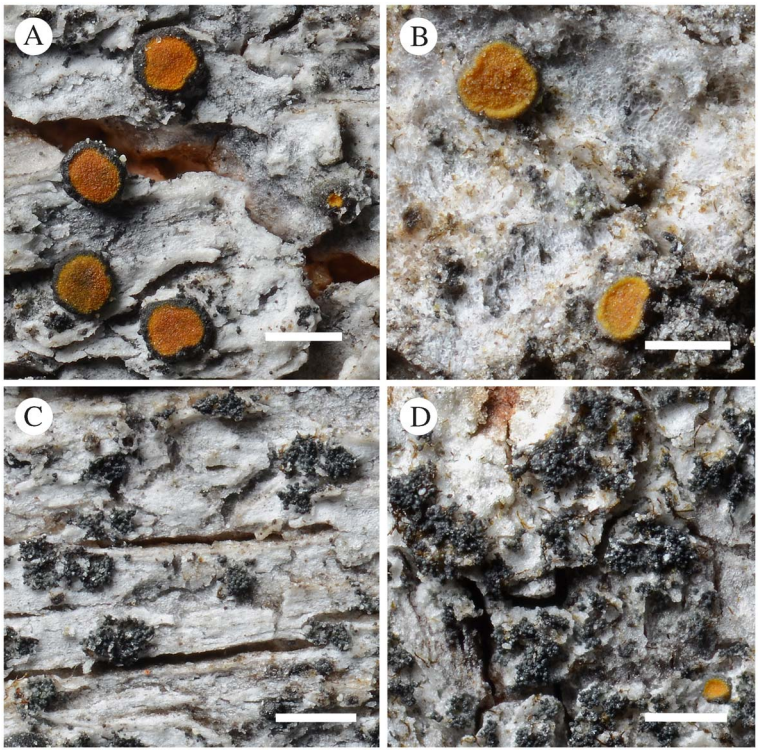

FIG. 2. *Parvoplaca nigroblastidiata*. A, typical apothecia with some blastidia on the thalline margin (*Jonsson* FU5959); B, morphotype common in Turkey where a proper margin is present (*Nordin* FU8788); C, typical soralia with dark blastidia (*Jonsson* FU5959); D, (holotype) well-developed soralia with one young apothecium. Scales = 0·5 mm.

(Fig. 2A–D)

Thallus endophloeodal, sometimes staining the substratum dark grey, often covering several cm^2; prothallus not observed. *Blastidia* present, sometimes very sparse, (grey-)dark grey to black, sometimes with bluish or greenish tinge, as scattered, 0·1–0·5 mm large, thin, flat irregular spots, sometimes coalescing to 1 mm width, (15–)20–30(–35) µm (n = 70), orbicular, covered by a paraplectenchymatous cortex, sometimes forming conblastidia.

Apothecia often present and sometimes abundant, but always scattered, immersed at first but soon adnate to sessile, round to irregular, (zeorine-)lecanorine, 0·3–0·5(–0·8) mm diam.; *disc* slightly concave to slightly convex, yellow-orange or rarely darker; proper margin usually absent, but occasionally present and very thin, yellow; *thalline margin* slightly raised above or level with disc, dark grey-black, sometimes paler or with brownish tinge, K+ violet, N+ red, 25–60(–75) µm, smooth or partly granular from blastidia, with fairly well-delimited paraplectenchymatous cortex, 12–35 µm thick; *epihymenium* yellowish orange, granular inspersed; *hymenium* (50–)65–80(–110) µm thick, hyaline; *hypothecium* 50–100 µm thick, hyaline or pale brown; *paraphyses* simple and lax, sometimes weakly branched above, 2·0–2·5 µm broad with upper cells hardly wider, up to 3·5(–4·5) µm; *asci* cylindrical, 40–45 × 12–13 µm, 8-spored; *spores* polaribilocular, ellipsoid to broadly ellipsoid, (10·0–)10·5–15·0(–17·5) × (4–)5–8(–9) µm, septum (2·5–)3·0–5·0(–5·5) µm (n = 75), ratio of spore length/width (1·47–)1·67–2·70(–3·50), ratio of septum/spore length (0·22–)0·25–0·38(–0·42).

Pycnidia not observed.

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Chemistry. The apothecial disc and true exciple contain parietin as a major compound, and small amounts of fallacinal, emodin, teloschistin and parietinic acid, which corresponds to chemosyndrome A of Søchting (1997). The thallus and thalline exciple do not contain anthraquinones but instead contain the pigment Sedifolia-grey which reacts K+ violet and N+ brownish red.

Habitat and distribution. In Sweden this species usually grows on trunks of *Populus tremula*. In Turkey it has been collected at high altitudes on *Juniperus excelsa* and *Abies cilicica*. It seems to grow in both shady and open conditions. The known distribution comprises Sweden, Turkey and Alaska. In the Mediterranean it is probably confined to higher altitudes. There is also one record from Alaska, where it was found on *Populus*.

Remarks. This species is characterized by an endophloeodal thallus with scattered blackish blastidia and small, scattered yellow to orange apothecia with a grey to black outer margin. Morphologically it is very similar to *Caloplaca turkuensis* (Vain.) Zahlbr., with which it has been previously confused. Šoun *et al.* (2011) had already pointed out the presence of the new species and its similarity with *C. turkuensis*. It differs from *C. turkuensis* in the generally darker blastidia and thalline margin, thinner and more discrete blastidia and, in some specimens, the presence of zeorine apothecia. *Parvoplaca nigroblastidiata* can also be confused with *Caloplaca ahtii* Søchting, which differs in that the blastidia appear in crater-formed, dark bluish grey formations and in the more colourful apothecia with a less pronounced grey margin. Another possible risk for confusion is *Caloplaca borealis* (Vain.) Poelt, but this species lacks asexual propagules and has biatorine apothecia, usually with a faint grey (rarely almost black) outer portion of the proper margin.

Phylogenetically, the new species is well established in *Parvoplaca* on the basis of a three-gene analysis (see Arup *et al.* 2013, as *Parvoplaca* sp. 26). Within *Parvoplaca* it is most closely related to *P. suspiciosa*, but differs from this in several aspects. *Parvoplaca nigroblastidiata* has endophloeodal thalli normally several cm wide, scattered apothecia, anthraquinones in the epihymenium and proper exciple, a hypothecium 65 µm or thicker and often brownish, and usually a thalline margin with a well-delimited cortex while *P. suspiciosa* has small and thin epiphloedal thalli normally 5–10 mm wide with small groups of apothecia, no anthraquinones in the apothecia, a hypothecium that is hyaline and normally below 60 µm thick, no vegetative propagules, and a thalline margin with a poorly delimited cortex.

The quantity of blastidia varies within and among specimens and they are sometimes hardly visible, especially in Turkish specimens. The colour of the blastidia and the thalline margin is usually very dark but can occasionally be pale grey, and in fresh material there is often a green-blue tinge. A proper margin is usually not present in the Nordic and American material, but in Turkish specimens it is often present (Fig. 2B).

Selected specimens examined. **Sweden:** *Dalarna*: Särna par., Mt. Hornberget, on *Populus tremula*, elev. 560 m, 2002, *Arup* L02344, L02345 (LD). *Gästrikland*: Hamrånge par., between Svartsjön and Romsån, on *Populus tremula*, alt. 72 m, 2012, *Nordin* FU8788 (LD). *Jämtland*: Borgvattnet par., SW of Bergflon, on *Populus tremula*, 2009, *Jonsson* FU5958, FU5959 (LD); Kall par., NW of Berge, elev. 500 m, on *Populus tremula*, 2010, *Arup* L10208 (LD); Bräcke par., E of Rövarstenen, on *Populus tremula*, alt. 402 m, 2012, *Jonsson* FU9449 (LD). *Östergötland*: Malexander par., N. Dalberga, on *Populus tremula*, 2012, *Jonsson* FU7584 (LD).—**Turkey:** *İçel*: Gülnar-Silifke highway, on *Juniperus excelsa*, 40·23666°N, 36·549566°E, alt. 1000–1020 m, 2012, *Halıcı* CL0.098 (ERH); Taurus Mts., Çamlıyayla, alt. 1350 m, 37·177500°N, 34·583889°E, on *Abies cilicica* in lit forest, 2012, *Halıcı & Vondrák* JV10617 (PRA).—**USA:** *Alaska*: Katmai, on *Populus*, 2013, *Tønsberg* 42983 (BG).

Parvoplaca aff. *nigroblastidiata*. **Turkey:** *Konya*: Taşkent, Gevne valley, Eşekkırıldı, on *Juniperus excelsa*, alt. 1530 m, 36·796619°N, 32·416348°E, 2010, *Halıcı* CL0.096 (ERH).

Parvoplaca chelyae (Pérez-Vargas) Vondrák, Halıcı & Arup comb. nov.

MycoBank No.: MB 812856

Basionym: *Caloplaca chelyae* Pérez-Vargas, *Bryologist* **112:** 840 (2009); type: Spain, Canary Islands, Tenerife, El Teide

National Park, Montana de Los Pinos, basaltic rocks, *C. Hernández-Padrón & P. L. Pérez de Paz* (TFC Lich 6247).

This species was described recently from the Canary Islands, Tenerife and La Palma (Pérez-Vargas & Pérez de Paz 2009), where it grows on bryophytes in the dry alpine zone. However, it is not endemic to these islands, as it has recently been recorded from Turkey (Vondrák *et al*. 2012). The species is probably strictly muscicolous and restricted to siliceous bedrocks in dry alpine or dry continental sites.

The morphology of the species is described in Pérez-Vargas & Pérez de Paz (2009) and Vondrák *et al*. (2012). Although it can hardly be distinguished from *P. tiroliensis* by morphology, the two species are ecologically distinct. *Parvoplaca tiroliensis* is an arctic-alpine species, that is restricted to more humid limestone areas in its alpine localities.

Additional specimens examined. **Spain:** *Canary Islands*: Tenerife, Corona forestal, Mt Montaña del Cascajo, 28° 24'35"N, 16°25'15"W, alt. 1600 m, 2013, *Vondrák* 13093 (PRA); La Palma, Roque de los Muchachos, alt. 2400 m, 28°45'15"N, 17°53'5"W, 2014, *Vondrák* 13094 (PRA).— **Turkey:** *Giresun*: Şebinkarahisar, Eğribel Geçiti'nin güneyi, silisli kayalar, 40·461355°N, 38·403021°E, alt. 2276 m, on mosses on siliceous overhanging rocks in alpine zone, *Vondrák* 13093 (PRA); Şebinkarahisar, south of Eğribel Pass, on mosses, 40°27'41"N, 38°24'11"E, alt. 2276 m, 30 viii 2012, *Halıcı et al.* (CL 0.508). *Kayseri*: western slope of Mount Ali, Çaybağları Position, on mosses, 38°40'N, 35°32'E, alt. 1240 m, 26 v 2008, *Halıcı* (CL-0.005). *Bursa*: Uludağ, above the Hotel area, 40° 06'12"N, 29°09'01"E, alt. 1800–2000 m, 24 v 2012, *Halıcı* (CL 0.237). *Malatya*: Hekimhan, north-east of Güzelyayla Village, 38°44'13"N, 37°50'54"E, alt. 1830 m, 20 v 2010, *Halıcı* (CL 0.353). *Ankara*: Kızılcahamam, north-west of Dereiçi Village, on mosses, 40°36'44"N, 32°31'39"E, alt. 1380 m, 21 vii 2012, *Halıcı & Candan* (CL 0.828).

A list of specimens from East Turkey was published by Vondrák *et al*. (2012).

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