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Crown rust fungi with short lifecycles – the *Puccinia mesnieriana* species complex

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The short lifecycle rust species *Puccinia mesnieriana* produces telia and teliospores on buckthorns (*Rhamnus* spp.) that are similar to those produced by the crown rust fungi (*Puccinia* series *Coronata*) on oats and grasses. The morphological similarity of these fungi led to hypotheses of their close relationship as correlated species. Phylogenetic analyses based on ITS2 and partial 28S nrDNA regions and the cytochrome oxidase subunit I (COI) revealed that *P. mesnieriana* was a species complex comprising four lineages within *P.* ser. *Coronata*. Each lineage was recognized as a distinct species with differentiating morphological characteristics, host associations and geographic distribution. *Puccinia mesnieriana* was restricted to a single specimen from Portugal that was morphologically similar to and shared the same provenance as the type specimen of the species, which was not sequenced. *Puccinia pseudomesnieriana,* sp. nov., included all other specimens sampled from the Mediterranean region and was closely related to *P. coronati-brevispora* and *P. coronati-longispora*. Specimens from California USA formed a monophyletic group comprising two well-supported lineages recognized as *P. digitata*, long considered a synonym of *P. mesnieriana,* and *P. pseudodigitata,* sp. nov. Descriptions, illustrations and identification keys are provided for these four microcyclic rust pathogens of *Rhamnus*.

Keywords: correlated species, paraphyly, phylogenetic species, *Puccinia* series *Coronata*, *Puccinia digitata,* 2 new species, 2 lectotypes, 2 epitypes.

The common name crown rust was coined for fungi classified in the macrocyclic, heteroecious species *Puccinia coronata* Corda, which alternate between two phylogenetically distant plant hosts to complete their lifecycles of five spore stages. Uredinial (II) and telial (III) stages are on oats and other grasses (Poaceae), basidiospores (IV) infect mainly buckthorns (*Rhamnus* L.) and produce pycnidial (0) and aecial (I) stages. There were multiple pre-molecular concepts of *P. coronata* before Liu & Hambleton (2013) used multi-locus sequence analyses to separate this complex into seven phylogenetic species and recognize it at the series level. All species in *P.* series *Coronata* share the distinctive morphological character of crown-like processes or digitations adorning the upper cell of the teliospores. Besides these macrocyclic species, the microcyclic *P. mesnieriana* Thüm., described by Thümen (1877, 1878) based on a specimen from Lusitania, an ancient province that included parts of modern Portugal and Spain, also produces teliospores with crown-like apical digitations but on buckthorns (*Rhamnus* spp.), aecial hosts of some members of the *P. coronata* complex. Dietel (1887) noted the remarkable similarity of the teliospores and hypothesized a close relationship to *P. coronata*, despite the markedly different lifecycle that lacks an alternate host and the 0–II spore stages. According to Travelbee (1914), Fischer (1898) developed a list of eight heteroecious rust species in *Puccinia* Pers., *Chrysomyxa* Unger, *Melampsora* Castagne or *Coleosporium* Lév. that could be paired with a short-cycle species producing teliospores of similar morphology on the aecial hosts. Scholler et al. (2019) recently provided an overview of the historical development of this concept of "correlated" species, first described by de Bary in 1879 in the genus *Chysomyxa*, and with molecular phylogenetic analyses

demonstrated that they had formed multiple times in the genus *Tranzschelia* Arthur.

In his monograph of rust fungi in the United States and Canada, Arthur (1934) listed over 40, or about one-third of all, microcyclic species in *Puccinia* or *Uromyces* (Link) Unger, which had been proposed as correlated with a macrocyclic or demicyclic rust species by multiple authors (e.g. Fischer 1898; Orton 1912; Travelbee 1914; Arthur 1915, 1934; Dietel 1918; Arthur & Jackson 1922; Jackson 1931). The following three conditions were considered: 1.) whether the host of the short-cycled rust is closely related to the alternate host of the long-cycled rust; 2.) whether the two rusts produce similar fruiting structures (sori), cause similar symptoms on the host and have similar geographic distributions; and 3.) whether the teliospores of the two species are morphologically similar. Relationships of most paired species have not yet been evaluated using molecular data, and although one study has tested and confirmed the relationship of *P. mesnieriana* to *P. coronata*, using nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences (Zambino & Szabo 1993), only a few members of *P.* ser. *Coronata* were included.

In 1884, Ellis & Harkness (1884) described another microcyclic species on *Rhamnus crocea* Nuttall ex Torrey & A. Gray from California USA, *P. digitata*. They noted that the species differs from *P. coronata* by having larger and more prominent sori and teliospores with shorter apical digitations (Ellis & Harkness 1884). Due to the similarities in host and morphology, *P. digitata* was considered a synonym of *P. mesnieriana* in multiple publications (Sydow & Sydow 1904, Arthur 1934, Anikster & Wahl 1985), with a combined distribution of southern Europe, western Asia, northern Africa and southern California USA. To the best of our knowledge, *P. mesnieriana*, including *P. digitata*, is the only taxon recorded as producing *P. coronata*-like teliospores on *Rhamnus* spp. However, given the vast geographic distance between California and the Mediterranean region, it is reasonable to investigate further whether the two species are conspecific.

In this study, we evaluated the following questions using molecular phylogenetic analyses combined with morphological and distributional data. 1.) Given that recent molecular phylogenetic studies have recognized seven phylogenetic species in the *P. coronata* complex and others that were unnamed (Liu & Hambleton 2013), what is the precise relationship of *P. mesnieriana* to these fungi? 2.) Does the taxon of California origin, *P. digitata*, differ from the one of Mediterranean origin, *P. mesnieriana*, genetically? and 3.) Are there cryptic taxa within *P. mesnieriana*?

Materials and methods

Specimens sampled for DNA analysis and morphology study

The 58 specimens included in this study are listed in Tab. 1 with host, origin, year collected and GenBank accession numbers for the sequences analyzed. Seventeen *P. mesnieriana* and two *P. digitata* herbarium specimens from California, Greece, Portugal and Spain were borrowed from four fungaria and examined for morphological features: Canadian National Mycological Herbarium, Ottawa, Canada (DAOM), New York Botanical Garden, New York, USA (NY), Real Jardín Botánico, Madrid, Spain (MA) and U.S. National Fungus Collections, Beltsville, USA (BPI). In addition, two fresh teliospore samples of *P. mesnieriana* were harvested from *R. palaestina* Boiss. in Israel by YA (ISRt). All were processed for DNA extraction and analysis except a specimen of *P. digitata* listed as authentic material by the New York Botanical Garden (NY 1840381) and the type specimen of *P. mesnieriana*, BPI 085572, because the collections were not plentiful. DNA sequencing failed for the second *P. digitata* specimen, DAOM replicate of Ellis North American Fungi No. 1466 (DAOM 710570). Also included in the DNA analyses were 37 cereal or grass rust specimens sampled in previous studies (Liu & Hambleton 2010, 2012, 2013; Liu et al. 2013), representing 16 taxa including *P. chunjii* M. Liu, C.J. Li & Hambl., seven species of *P.* ser. *Coronata*, *P. graminis* Pers., *P. hordei*, *P. poae-nemoralis* G.H. Otth, *P. poarum* Nielsen, *P. recondita* Roberge ex Desm., and *P. striiformis* Westend.

DNA extraction, PCR amplification and sequencing

The amount of infected leaf tissue sampled per specimen depended on the sizes of lesions and degree of infection. Infected leaf tissue samples, or when possible pure spores, were excised with tweezers or scalpels, or with 1 mm or 2 mm biopsy punches. Most often, two 2 mm biopsy punches were used to excise infected regions of plant tissue. Methods for genomic DNA extraction were as described in Demers et al. (2017) using an OmniPrep™ for Fungi bench kit (G-Biosciences, St. Louis, MO USA), or a Macherey-Nagel NucleoMag® 96 Trace kit (Macherey Nagel GmbH & Co. KG, Düren, Germany) and a KingFisher Flex magnetic particle

Tab. 1. Specimens included in the morphological or molecular analyses, with voucher numbers, host, provenance, year collected and GenBank accession numbers. (‡ indicates a type specimen; * indicates a pure spore collection; n.d. = no data)

Voucher no.	Rust species	Host species	Country	Year	GenBank ITSII28S	GenBank COI
BPI 085602‡	*P. digitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1935	KX036376	KX036372
BPI 085606	*P. digitata*	*Endotropis crocea* subsp. *pirifolia*	CA, USA	1931	KX036375	n.d
BPI 085616	*P. digitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1920	KX036374	n.d
DAOM 36609	*P. digitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1938	KF661256	n.d
DAOM 710570	*P. digitata*	*Endotropis crocea*	CA, USA	–	n.d	n.d
NY 1840381‡	*P. digitata*	*Endotropis crocea*	CA, USA	–	n.d	n.d
BPI 085567‡	*P. mesnieriana*	*Rhamnus alaternus*	Portugal	1932	KF661260	KF661275
BPI 085572‡	*P. mesnieriana*	*Rhamnus alaternus*	Portugal	1876	n.d	n.d
BPI 085603	*P. pseudodigitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1957	KF661261	KF661276
BPI 085605‡	*P. pseudodigitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1943	KF661262	n.d
DAOM 78074‡	*P. pseudodigitata*	*Endotropis crocea* subsp. *ilicifolia*	CA, USA	1943	KF661257	KF661272
BPI 085621‡	*P. pseudomesnieriana*	*Rhamnus saxatilis* subsp. *prunifolia*	Greece	1942	KF661263	KF661277
BPI 1111897	*P. pseudomesnieriana*	*Rhamnus saxatilis* subsp. *prunifolia*	Greece	1942	KF661264	KF661278
ISRt8870*	*P. pseudomesnieriana*	*Rhamnus lycioides* subsp. *graeca*	Israel	2006	KF661258	KF661273
ISRt8951*	*P. pseudomesnieriana*	*Rhamnus lycioides* subsp. *graeca*	Israel	2006	KF661259	KF661274
MA-Fungi 50171	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1971	KF661269	KF661282
MA-Fungi 50172	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1970	KF661270	KF661283
MA-Fungi 50174	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1970	KF661268	n.d
MA-Fungi 50176	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1975	KF661267	KF661281
MA-Fungi 50180	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1971	KF661266	KF661280
MA-Fungi 50182	*P. pseudomesnieriana*	*Rhamnus oleoides* subsp. *oleoides*	Spain	1975	KF661265	KF661279
DAOM 240982‡	*P. chunjii*	*Elymus* sp.	China	1996	HQ012446	HQ012473
(S)reg.nr. F34373	*P. coronata* var. *avenae* f.sp. *avenae*	*Avena sativa*	Sweden	2003	HM131257	KX944741
BPI 060333	*P. coronata* var. *avenae* f.sp. *avenae*	*Lolium perenne*	Afghanistan	1976	HM131271	HM147434
DAOM 240064	*P. coronata* var. *avenae* f.sp. *avenae*	*Avena* sp.	Canada	2006	HM057140	HM147405
K(M): 77013	*P. coronata* var. *avenae* f.sp. *avenae*	*Avena sterilis*	Greece	2000	HM131289	HM147422
BPI 871068	*P. coronata* var. *avenae* f.sp. *graminicola*	*Holcus lanatus*	USA	2005	HM131243	HM147432
K(M): 104797	*P. coronata* var. *avenae* f.sp. *graminicola*	*Glyceria maxima*	England	2002	HM131244	HM147423
PUR N1251	*P. coronata* var. *avenae* f.sp. *graminicola*	*Holcus lanatus*	USA	1992	HM131250	HM147403
(S)reg.nr. F46266	*P. coronata* var. *coronata*	*Frangula alnus*	Sweden	2005	HM131310	HM147420
B 70 0006597	*P. coronata* var. *coronata*	*Frangula alnus*	Germany	2003	HM131312	HM147427
BP 89076	*P. coronata* var. *coronata*	*Calamagrostis epigeios*	Hungary	1991	HM057141	HM147414
DAOM 220642	*P. coronati-agrostidis*	*Rhamnus* sp.	Canada	1995	HM131321	HM147397
PUR N114‡	*P. coronati-agrostidis*	*Agrostis stolonifera*	Finland	1977	HM131319	HM147393
PUR N1371	*P. coronati-brevispora*	*Bromus inermis*	USA	1997	HM131238	HM147387
PUR N652‡	*P. coronati-brevispora*	*Bromus inermis*	USA	1999	HM131235	KX944742
DAOM 107653	*P. coronati-calamagrostidis*	*Calamagrostis canadensis*	USA	1964	HM131304	HM147400
DAOM 204923	*P. coronati-calamagrostidis*	*Bromus ciliatus*	Canada	1918	HM131305	KX944748
DAOM 220889	*P. coronati-calamagrostidis*	*Elymus* sp.	Canada	1996	HM131350	HM147398
PUR N2268	*P. coronati-calamagrostidis*	*Calamagrostis canadensis*	USA	2000	HM131308	HM147385
DAOM 183691	*P. coronati-hordei*	*Elymus repens*	Canada	1982	HM057138	HM147399
PUR 89857‡	*P. coronati-hordei*	*Holcus vulgare*	USA	1992	HM131225	KX944744

Voucher no.	Rust species	Host species	Country	Year	GenBank ITSII28S	GenBank COI
PUR N1426	*P. coronati-hordei*	*Elymus repens*	USA	1995	HM131230	KX944743
PUR F16131‡	*P. coronati-japonica*	*Calamagrostis arundinacea*	Japan	1958	HM131317	HM147391
PUR N1055	*P. coronati-japonica*	*Berchemia pauciflora*	Japan	1990	HM131318	HM147384
PRC196‡	*P. coronati-longispora*	*Bromus erectus*	Czech Republic	2002	HM131232	KX944746
PRC247	*P. coronati-longispora*	*Rhamnus saxatilis*	Slovakia	2002	HM057142	KX944747
BR 150069-10	*P. graminis*	*Elymus repens*	Belgium	2001	HM131357	HQ012466
BR 68612-33	*P. hordei*	*Hordeum murinum*	Belgium	1997	HQ012448	HQ012479
K(M): 78624	*P. hordei*	*Hordeum murinum*	England	2000	HQ012449	HQ012480
DAOM 222447	*P. poae-nemoralis*	*Poa annua*	New Zealand	1991	HM057154	HM147443
DAOM 240189	*P. poae-nemoralis*	*Koeleria litvinowii*	China	1996	HM057155	HQ012476
DAOM 240188	*P. poarum*	*Tussilago farfara*	Canada	2006	HM057150	KX944740
BR 59352-85	*P. recondita*	*Elymus repens*	Belgium	1996	HM131361	HQ012487
PUR 89264	*P. recondita*	*Hordeum jubatum*	USA	1985	KX036373	KX036371
PUR N1240	*P. recondita*	*Elymus hystrix*	USA	1996	JX533588	KX944745
PUR N1253	*P. recondita*	*Elymus repens*	Canada	1994	HM057146	HQ012489
RS479A*	*P. striiformis*	*Triticum aestivum*	China	2006	HM057122	HQ012475

processor (Thermo Fisher Scientific Oy,Yantaa, Finland). Modifications to both protocols were as follows: prior to extraction, samples were homogenized using liquid nitrogen and sterile disposable micro-centrifuge tube pestles (PES-15-B-SI Axygen, Corning, NY USA), and DNA was suspended in 50 µl or 70 µl of elution buffer.

A fragment comprising the nrDNA internal transcribed spacer two (ITS2) and approximately 300 bp of the adjacent large subunit (28S) (hereafter referred to as ITSII28S) was amplified and sequenced using primers Rust2inv (Aime 2006) and ITS4Ru1 (Rioux et al. 2015). Exons one and two of the mitochondrial cytochrome oxidase subunit I (COI) gene were amplified and sequenced using primers P360f and P360r (http://www.boldsystems.org./index.php/Public_Primer_PrimerSearch). Attempts to amplify the β-tubulin (BT) and nuclear RNA Polymerase II subunit 2 (RPB2) genes with primers and methods used previously for rust fungi (Liu & Hambleton 2013) were unsuccessful.

The methods for PCR amplification and sequencing were as described in Liu & Hambleton (2013) with the following modifications. PCR thermocycler profiles included an initial denaturation at 95 °C for 3 min, followed by 45 cycles of 95 °C for 1 min, annealing for 45 s at 58 °C (ITSII28S) or 30 s at 51 °C (COI) and extension at 72 °C for 1 min 30 s, with a final extension at 72 °C for 8 min. Thermocycler profiles for the sequencing reactions of successfully amplified gene regions had an initial denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 30s, annealing for 15 s at 55 °C and extension for 60 °C for 2 min. An Applied Biosciences Prism® 3130xl Genetic Analyzer (Life Technologies™, Carlsbad, CA USA) was used to generate DNA sequences from the sequencing amplification reactions. Sequences were edited using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, MI USA) or Geneious 8.1.5 (Biomatters, Auckland, New Zealand).

Alignment and phylogenetic analysis

DNA sequences of ITSII28S for 55 taxa and COI for 50 taxa were submitted to MAFFT version 7 online service for multiple sequence alignment (Katoh et al. 2017; http://mafft.cbrc.jp/alignment/server/index.html, accessed on 3 Nov 2016). The iterative refinement methods E-INS-i (ITSII28S) and L-INS-i (COI) were used with default settings. DNA alignments were subjected to independent parsimony analyses using PAUP* 4.0b10 (Swofford 2002). Heuristic searches with 50 replicates of random stepwise addition and tree bisection-reconnection branch swapping were conducted with a limit of 1,000,000 re-arrangements set for each replicate. Bootstrapping analyses were set with 100 replicates with full heuristic search of random stepwise addition of 50 replicates and limit of 1,000,000 rearrangements per replicate. Clades with values (BS) ≥ 70 were recorded.

The individual matrices and a combined ITSII28S-COI matrix were submitted to partitioned Bayesian analysis with MrBayes 3.2 (Ronquist et al. 2012). Evolutionary models for each locus were estimated with jModelTest 2.1.1 (Darriba et al. 2012) and ranked based on the Bayesian Information Criterion (BIC). Two independent runs were performed on multiple processors with models GTR+I+G (ITSII28S and ITSII28S-COI) or F81+G (COI). Clades supported by posterior probability values (PP) ≥ 80 were recorded. Each run was set four chains of 100,000,000 MCMC generations and sampling frequency was every 2000 generations. The Bayesian consensus tree was generated after 25 % burn-in.

Morphology and host associations

Telial morphology was examined with a Zeiss Discovery V12 stereomicroscope (Carl Zeiss MicroImaging GmbH, Jena, Germany), and photos were taken using an AxioCam HRc camera and AxioVision SE64 Rel.4.8.3 software (Carl Zeiss MicroImaging GmbH, Jena, Germany). Colours were recorded according to Kornerup & Wanscher (1978). Cross sections were cut with a MicroTome Cryostat, model HM500 OMs (MICROM Laborgeräte GmbH, Jena, Germany). To examine the spore morphology, small pieces of the dried specimens were temporarily softened in a moist chamber for at least 30 min. Clumps of spores were removed with tweezers and mounted on microscope slides in lactic acid with and without cotton blue stain. Slides were examined with an Olympus BX51 differential interference contrast light microscope (Olympus Canada Inc., Markham, ON Canada). Digital micrographs were taken with an Olympus DP 73 camera and analyzed by Olympus cellSens Life Science Imaging Software (Olympus Scientific Solutions Americas CORP., Waltham, MA USA) to obtain measurements. The length of spores was measured as the distance between the hilum to the apical wall including the wall but excluding the thickened part or the processes. In describing the processes, we followed the Dictionary of the Fungi (Kirk et al. 2008) definitions for tuberculate as having small wart-like processes and digitate as having deep radiating divisions or finger-like processes. Guyot ratio calculated as the length of the spores divided by the width of the lower cells (Guyot et al. 1948; résumé reprinted in Guyot 1951) has been effectively used to characterize the shape of leaf rusts on grasses (Marková & Urban 1998), therefore we calculated it for each lineage. Ranges of all the measurements were recorded using the upper and lower boundaries of 80 % of the values in the middle after sorting all the measurements for each criterion, and the minimum and maximum were recorded in parentheses as outliers.

The fungarium specimens of the *P. mesnieriana* complex examined in this study were collected between 1876 and 1975 and the identified associated *Rhamnus* hosts reflect the nomenclature in use at the time of collection. To clarify host associations of these rusts, on-line databases were searched for current names and recent systematic revisions of this large and diverse plant genus.

Results

DNA extraction, sequencing and phylogenetic analyses

The ITSII28S data matrix comprised 55 taxa and 673 characters, of which 105 were parsimony informative. Parsimony analysis resulted in 125787 most parsimony trees, with length = 329, CI = 0.584, RI = 0.849, RC = 0.495, HI = 0.416, G-fit = -80.783. The COI data matrix comprised 50 taxa and 348 characters but included only 14 parsimony informative characters and the parsimony analysis resulted in a generally unresolved tree. A holistic approach of combining individual loci with limited signals can be used to maximize the phylogenetic information (Smith 2000), therefore we combined the ITSII28S and COI data sets. The concatenated alignment of 55 taxa, with the five missing COI sequences coded as gaps, comprised 985 characters of which 135 were parsimony-informative. Parsimony analysis resulted in 126491 most parsimonious trees, length = 365, CI = 0.586, RI = 0.844, RC = 0.495, HI = 0.414, G-fit = -106.480. Bayesian analyses of each data matrix resulted in tree topologies similar to those from parsimony (data not shown). The Bayesian combined partitioned tree is shown in Fig. 1 with the support values for analyses of ITSII28S alone and ITSII28S-COI indicated (BS ITSII28S / PP ITSII28S / PP ITSII28S-COI). All specimens of *P. mesnieriana* and *P. digitata* sequenced in this study were placed within *P.* ser. *Coronata* (Clade A), confirming the hypothesized close relationship. Specimens from California grouped together with moderate support (Clade C; 71 / 88 / 96) in two strongly supported sub-clades, corresponding to *P. digitata* (100 / 100 / 100) and the new species *P. pseudodigitata* described here (99 / 100 / 100). Specimens from the Mediterranean region grouped together, here described as *P. pseudomesnieriana*, in a larger Clade D, but this group was unresolved

with *P. coronati-longispora* and *P. coronati-brevispora*, which were each in their own well- or moderately well-supported sub-clade (86 / 100 / 100 and – / 86 / 84, respectively). One single specimen from Portugal corresponding morphologically to *P. mesnieriana* appeared as a uniquely diverged orphan clade within *P.* ser. *Coronata*.

Morphology and host associations

Morphological examination revealed that the shapes of the telia on *Rhamnus* spp. were obviously different from those of *P.* ser. *Coronata* on oats and grasses. The former were circular pulvinate, or merged to large irregular patches and compact with hard (solid) surfaces. The latter were cylindrical or oval and relatively loose and soft. Therefore, considering the morphology of telia and the difference in pores are subapical (upper cell) and just below the transverse wall (lower cell). These germ pore positions are the same as those of *P. coronata* as illustrated in Arthur (1934: p. 152) and *P. coronata* on smooth brome grass (likely *P. coronati-brevispora* based on host, provenance and description) in Anikster et al. (2003).

On the basis of our molecular, morphological, host and distribution evidence, we designated each lineage as a distinct species, with the following descriptions and illustrations. All of the examined collections were dried specimens, therefore the recorded colours may not match those seen on fresh or living material. Current names for the *Rhamnus* hosts associated with each of the four lineages were sourced from the Global Biodiversity Information Facility (GBIF) portal (https://www.gbif.org/; accessed on 21 November 2018) and recent phylogenetic studies of the *Rhamnus* complex by Hauenschild et al. (2016a, b) (Tab. 3). In the taxonomy section, the original plant host identifications on specimen labels are retained in the typification and specimens examined sections, while the current names are listed in the Host field, in Tab. 1, in the Key to species, and on Fig. 1.

Tab. 2. Tabular key for four species in the *P. mesnieriana* complex. Numbers in bold indicate a differentiating measurement.

Characteristics	***P. digitata***	***P. pseudodigitata***	***P. mesnieriana***	***P. pseudomesnieriana***
Spore Dimension (µm)	(41)49–**74(83)** × (8)10–16(18)	(30)38–60(70) × (9)10–17(21)	(40)45–60(66) × (9)11–16(20)	(42)47–67(74) × (7)10–14(16)
Guyot Ratio	(2.5)3–**5.5(6.5)**	(1.5)2–4(5.5)	(2)2.5–4(5)	(2)3–5(7.5)
Length of Pedicel (µm)	(0)7–20(24)	0–17(25)	(7)10–**34(45)**	(2)7–**14**(26)
Shape of Apical Processes	Mainly digitate	Tuberculate, digitate	Mainly tuberculate	Mainly digitate
Number of Apical Digits/Tubercles	(1)2–7(9)	1–4(6)	1–3(4)	(1)3–5(7)
Host	*Endotropis crocea*	*Endotropis crocea*	*Rhamnus alaternus*	*Rhamnus lycioides, R. oleoides, R. saxatilis*
Distribution	California	California	Portugal	Greece, Spain, Israel

Taxonomy

Puccinia digitata Ellis & Harkn., Bull. Calf. Acad., 1: 27. 1884. – Figs. 2–13.

L e c t o t y p u s **(here designated).** – USA. California, Tamalpais, on leaves of *Rhamnus crocea*, n.d., *leg.* H. W. Harkness (NY 1840381). Type seen ML!

E p i t y p u s **(here designated).** – USA. California, Los Angeles Co., Palmer's Canyon, on *Rhamnus crocea* var. *ilicifolia,* 8 June 1935, *leg.* W. H. Wheeler (BPI 085602; ex-epitype sequences: ITS-28S KX036376, COI KX036372). Epitype seen ML!

hosts, it is easy to distinguish the *P. mesnieriana* complex from *P.* ser. *Coronata*. Among the four lineages within the complex that were revealed in our molecular analyses, variation in teliospore characters was more informative than those of telia even though this variation could be subtle and overlapping. In addition, we observed that the shape and the length of the apical processes were useful characters to differentiate the four species in the complex, therefore we made measurements for each one. Detailed morphological comparisons showed that by combining differences in the shape and size of teliospores, characteristics of the apical processes and length of pedicels, the four lineages could be differentiated (Tab. 2). Germ pores were not observed in our slide mounts. Teliospore germination of a *P. mesnieriana* collection from Israel (likely *P. pseudomesnieriana* based on provenance) was illustrated in Anikster & Wahl (1985: figs. 3–5), showing

Tab. 3. Original and current plant host names for the *P. mesnieriana* complex specimens examined or sequenced in this study.

Rust species	Plant host names on fungarium specimens	Current names (GBIF)[a]
Puccinia digitata	*Rhamnus crocea*	*Endotropis crocea*
	R. crocea var. *pyrifolia*	*E. crocea* subsp. *pirifolia*
	R. ilicifolia	*E. crocea* subsp. *ilicifolia*
	R. crocea var. *ilicifolia*	*E. crocea* subsp. *ilicifolia*
P. pseudodigitata	*R. crocea* var. *ilicifolia*	*E. crocea* subsp. *ilicifolia*
P. mesnieriana	*R. alaternus*	*Rhamnus alaternus*
P. pseudomesnieriana	*R. lycioides* subsp. *oleoides*	*R. oleoides* subsp. *oleoides*
	R. palaestina	*R. lycioides* subsp. *graeca*
	R. prunifolia	*R. saxatilis* subsp. *prunifolia*

[a] Authorities for current names: *E. crocea* (Nutt.) Hauenschild; *E. crocea* subsp. *pirifolia* (Greene) Hauenschild; *E. crocea* subsp. *ilicifolia* (Kellogg) Hauenschild; *R. alaternus* L.; *R. oleoides* subsp. *oleoides* L.; *R. lycioides* subsp. *graeca* (Boiss. & Reuter) Tutin; *R. saxatilis* subsp. *prunifolia* (Sibth. & Sm.) Aldén

Description. – Spermogonia, aecia, uredinia not formed, basidia not documented/not seen. – Telia circular pulvinate, dark brown (6F3) to brownish black, hypophyllous, rarely epiphyllous or on twigs, compact, 0.5–2.5 mm diam., occasionally merged to large sori up to 4.5 mm, small sori covered with semi-transparent pale yellow (3A3), greyish orange (5B3) or brownish orange (5C5) epidermal layer, medium and large sori usually largely open with some remnants surrounding the edges; lesions on the opposite side of the leaf circular with clear or diffused edges, dull yellow (3B3) marked with reddish brown to dark-brown (6F3–6F8) veins. – Teliospores predominantly 2-, rarely 3-celled, oblong, oblong-clavate, clavate or cylindrical, (41)49–74(83) × (8)10–16(18) µm (n = 356), Guyot ratio (2.5)3–5.5(6.5), constricted or slightly constricted at the septum; wall of the upper cell dark orange to brownish yellow (5A8–5C8) or brownish orange (6C7–6C8) to light brown (6D7–6D8), light orange to greyish orange (5A4–5B4) at septum, greyish yellow to dark brown (4B4–6F8) at hilum, lower cell yellowish white to yellowish grey (4A2–4B2); apical wall thickened (2)3.5–8(13.5) µm, and tuberculate or digitate at the end forming (1)2–7(9) tubercles, most extended to finger-like processes, some branched, up to 11(16) µm long, (1.5)2–4.5(6.5) µm wide in the middle; pedicels similar colour with the lower cell of the spore or slightly darker, (0)7–20(24) µm long, hilum brownish orange (6C7–6C8), dark brown (4B4–6F8) or black, (4.5)6–10(12) µm wide.

Host. – *Endotropis crocea*, *E. crocea* subsp. *ilicifolia*, *E. crocea* subsp. *pirifolia*.

Distribution. – California, USA.

Other specimens examined. – USA. California, Gallagher's Canyon, Santa Catalina Island, upper-lower Sonoran Zone, on *Rhamnus crocea* var. *pyrifolia*, 16 July 1931, *leg.* F. R. Fosberg 7171 (BPI 085606); Mt. Wilson, on *Rhamnus ilicifolia*, 29 June 1920, *leg.* E. Bethel (BPI 085616 = North American Uredinales Elam Bartholomew 2455); Orange Co., Old Shaw Ranch, about 3 miles up Black Star Canyon, Santa Ana Mts., on *Rhamnus crocea* var. *ilicifolia*, 26 December 1938, *leg.* D. G. Nelson (DAOM 36609); on *Rhamnus crocea* s.d. *leg.* H. W. Harkness s.n. (DAOM 710570 = North American Fungi 1466).

Notes. – Morphologically, the specimens examined generally matched the short description by Ellis & Harkness (1884), but the range of spore length was wider. This species differs from *P. mesnieriana* in having fungal sori mainly located on the lower surface of the leaves, a majority of apical processes elongated to finger-like, up to 10(16) µm long, and shorter pedicels. In their original description, Ellis & Harkness (1884) did not designate a holotype. The Ellis collection at the Cryptogamic Herbarium, New York Botanical Garden (NY) holds six specimens collected by H. W. Harkness, including three N.A.F.1466 exsiccatae and three others, all from California on *Rhamnus crocea*, none with collection numbers or dates. Of these, NY 1840381 was selected for the lectotype because there is more precise location information, which matches the original description provenance of Tamalpais CA, although the handwriting is somewhat cryptic. The successfully sequenced specimen BPI 085602 was designated as epitype because the lectotype collection was not considered plentiful enough for DNA sampling.

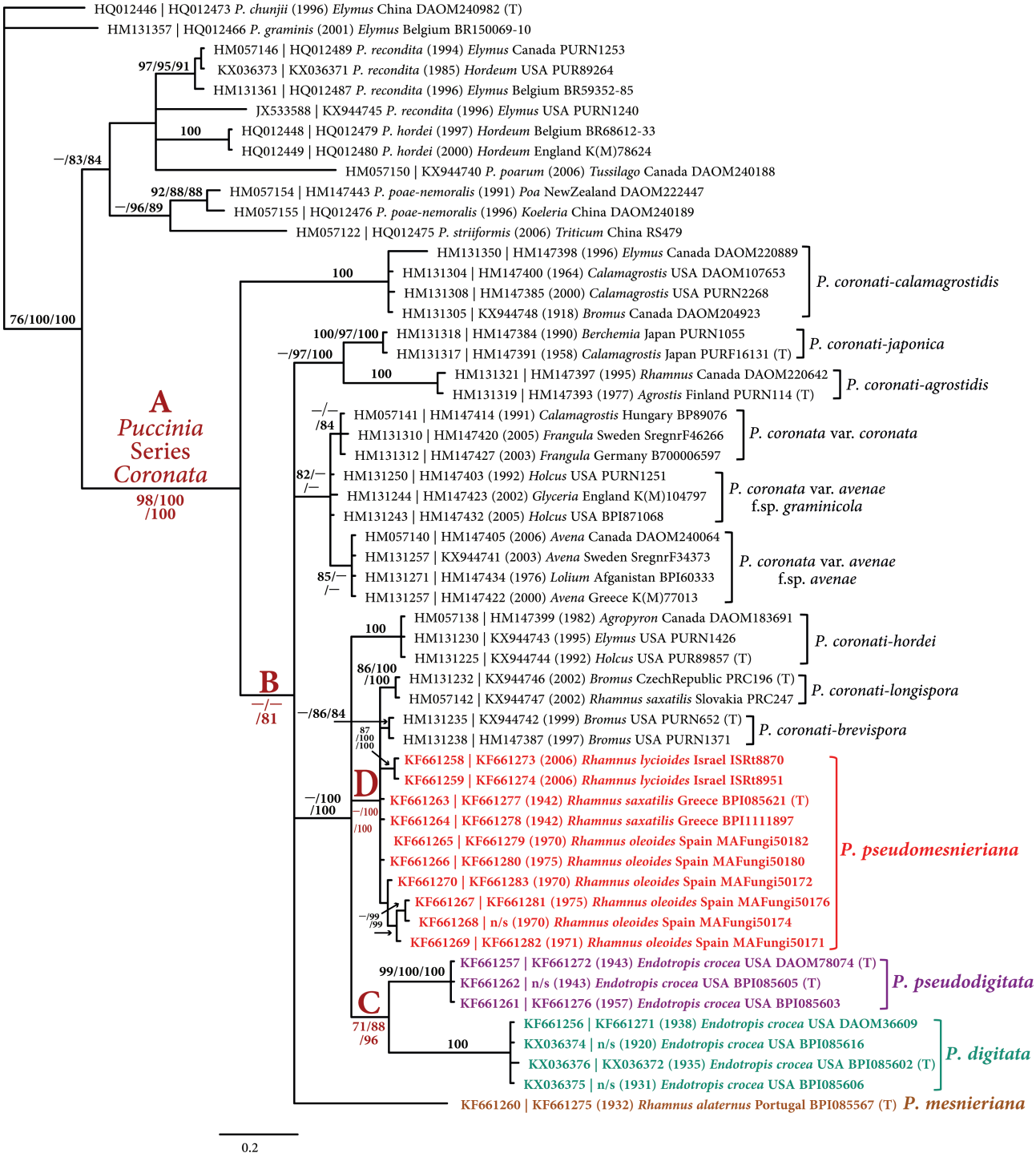
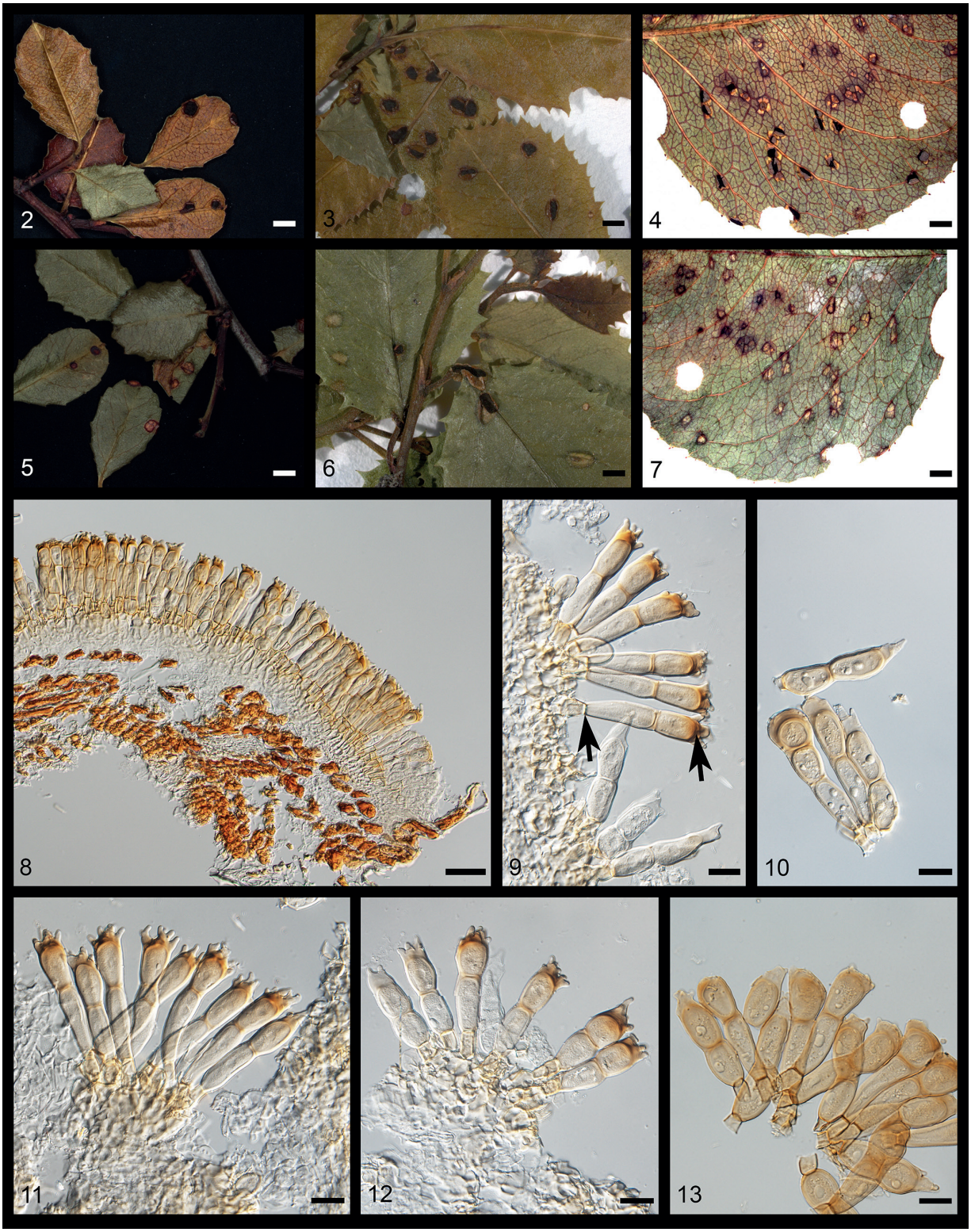
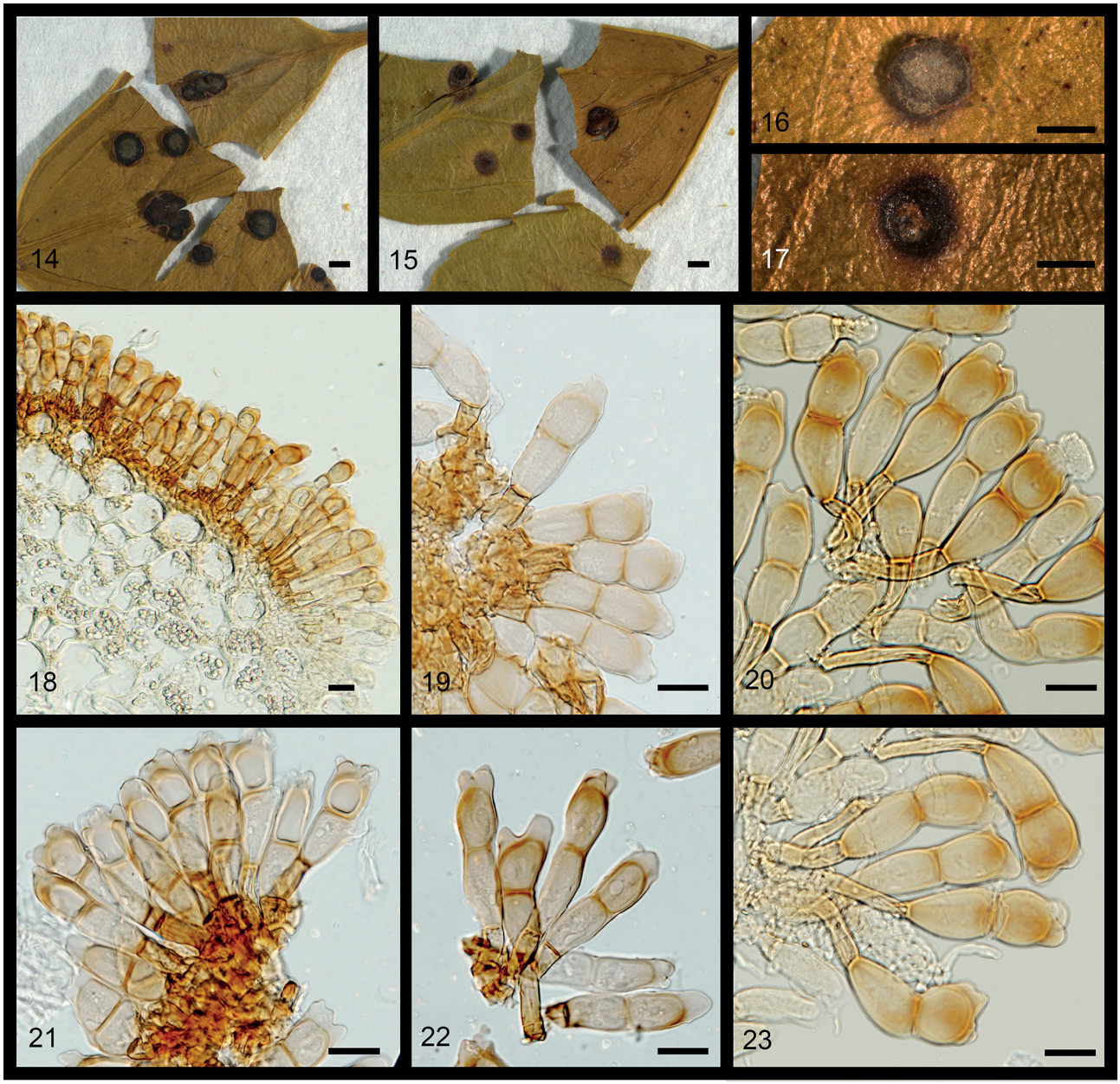


Fig. 1. Bayesian consensus tree inferred from a combined partitioned analysis of ITSII28S and COI sequences showing the phylogenetic relationships of four microcyclic species in the *Puccinia mesnieriana* species complex with members of *Puccinia* series *Coronata* (A). Three support values are shown above or beside nodes: BS ≥70 % ITSII28S / PP ≥80 % ITSII28S / PP ≥80 % ITSII28S-COI. (T) indicates a type specimen. See text for discussion of Clades B-D.



Figs. 2–13. *Puccinia digitata*. **2, 3, 4.** Telia on lower leaf surfaces; **5, 6, 7.** Upper surfaces of the infected leaves; **8.** Cross section of the telium; **9–13**. Teliospores of various shapes. **2, 5, 10, 13.** NY 1840381 (lectotype); **3, 6.** BPI 085602 (epitype); **4, 7, 9, 11, 12.** BPI 085616; **8.** NAF 1466 (DAOM 710570). Bars: **2–7** 2 mm; **8** 50 µm; **9–13** 20 µm. The length of the teliospores were measured as the distance between the two arrows (**9**), which excludes the thickened part of the apical walls.



Figs. 14–23. *Puccinia mesnieriana*. **14.** Telia on both sides of leaves; **15.** Opposite sides of the infected leaves; **16.** Telium on lower side of leaf; **17.** Upper side of the infected leaf; **18.** Cross section of a telium; **19–23.** Teliospores of various shapes and pedicels of various length. **14, 15, 18, 19, 21, 22.** BPI 085567 (epitype); **16, 17, 20, 23.** BPI 085572 (lectotype). Bars: **14–17** 1 mm; **18–23** 20 µm.

Puccinia mesnieriana Thüm., Mycoth. Univ., Cent. 9, no. 834, 1877. – Figs. 14–23.

Lectotypus **(here designated).** – PORTUGAL. Coimbra, on leaves of *Rhamnus alaternus*, July 1876, *leg.* Mesnier P. G. (BPI 085572, Mycotheca Universalis, Centurie 9 # 834). Type seen ML!

Epitypus **(here designated).** – PORTUGAL. Lisbon, Topada da Aejuda, Garden, Institute of Agronomy, on leaves of *Rhamnus alaternus*, May 1932, *leg.* Silva Teixeira (BPI 085567; ex-epitype sequences: ITS-28S KF661260, COI KF661275). Epitype seen ML!

Description. – Spermogonia, aecia, uredinia not formed, basidia not documented/not seen. – Telia circular pulvinate, dark brown (6F3) to brownish black at periphery, light grey (1C1–1D1) in the centre, amphigenous, compact, 0.5–2.5 mm diam., sori largely open with brown (4C7–5D7) epidermal remnants surrounding the edges; lesions on the opposite side of the leaf brown to greyish brown (6F3–6F8), circular or diffuse at

edge. – Teliospores 2-celled, oblong, oblong-clavate, clavate, (40)45–60(66) × (9)11–16(20) µm (n = 93), Guyot ratio (2)2.5–4(5), constricted or slightly constricted at the septum; apical wall thickened (2.5)4–9.5(11.5) µm, and tuberculate at the end forming (1)2–3(4) tubercles, up to 6(8) µm thick, (2)3.5–8(11) µm wide in the middle; pedicels similar colour with the lower cell of the spore or slightly darker, (7)10–34(45) µm long, hilum brownish orange (6C7–6C8), dark brown (4B4–6F8) or black, (5)6–10(12.5) µm wide.

Host. – *Rhamnus alaternus*.

Distribution. – Portugal.

Notes. – The 1932 specimen from Portugal (BPI 085567) formed an orphan clade in our analyses. Its geographic origin and morphology matched that of exsiccata specimen BPI 085572 (not sequenced), a potential type specimen collected by Mesnier P. G. in 1876, the same year and collector cited in Thümen's original description which was published with Mycotheca Universalis Centurie 9 (Thümen 1877) and again one year later in a journal (Thümen 1878). Thümen noted the pedicel was an elongated 35 µm which fits within the range of our measurements (7)10–34(45) µm. This species is clearly differentiated from *P. digitata* and the other two new species in having much longer pedicels. In addition, the apical processes often are more tuberculate than digitate, consistent with Thümen's description "saepe subcoronata" meaning "often not completely digitate". Although very few of the specimens accessioned under this name and sequenced for this study were confirmed as *P. mesnieriana* s. str., the species can be recognized by both molecular and morphological evidence. Data for more specimens are needed to assess its frequency of occurrence. Certainly in Portugal, rust teliospores are commonly found on *Rhamnus alaternus* plants every spring (pers. comm. Pedro Talhinhas). We selected BPI 085572 as the lectotype, because it is original material but Thümen (1877) did not specify a particular specimen as type. Isolectotypes may be found in other fungaria with holdings of Mycotheca Universalis Centurie 9 # 834. The successfully sequenced specimen BPI 085567 was designated as epitype because the lectotype collection was not considered plentiful enough for DNA sampling.

Puccinia pseudodigitata M. Liu & Hambl. **sp. nov.** – Figs. 24–36.

Mycobank no.: MB 816061

Diagnosis. – Similar to *Puccinia digitata,* apical wall of teliospores severely thickened and extended into 1–4(6) tuberculate or digitate processes, Guyot ratio (1.5)2–4(5.5), found only in California, USA.

Holotypus. – USA. California, Richardson Springs, Butte County, on leaves of *Rhamnus crocea* var. *ilicifolia,* 2 November 1943, *leg.* H. E. & S. T. Parks 6692 (BPI 085605 = California Fungi # 699; ex-holotype sequences: ITS-28S KF661262; isotype DAOM 78074; ex-isotype sequences: ITS-28S KF661257, COI KF661272). Type seen ML!

Description. – Spermogonia, aecia, uredinia not formed, basidia not documented/not seen. – Telia mainly hypophyllous, occasionally epiphyllous, compact, brownish black, 0.5–2.5 mm diam., sori largely open with some epidermal remnants surrounding the edges; lesions on the opposite side of the leaf surface circular or irregular with brown (5F8–6F8) to black edges or diffused, yellowish-grey (3B2–4B3) in the centre, sometimes depressed. – Teliospores predominantly 2-celled, but 1-, 3- even 4-celled spores are commonly found, ellipsoid, oblong, broad-clavate, or clavate, (30)38–60(70) × (9)10.5–17(21) µm (n = 345), Guyot ratio (1.5)2–4(5.5), slightly to strongly constricted at the septum; wall of the upper cell and the septum brownish orange (6C7–6C8) to light brown (6D7–6D8), lower cell light orange to greyish orange (5A4–5B4), greyish yellow to dark brown (4B4–6F8) at hilum; apical wall severely thickened (2)5–12.5(16) µm, some but not all tuberculate at the end forming up to 5 rarely 6 tubercles, some extended to finger-like processes, up to 8(10) µm long, (1.5)2.5–4.5(6) µm wide in the middle; pedicels similar colour with the lower cell of the spore or slightly darker, 0–17(25) µm long, hilum dark brown (4B4–6F8) or black, (5)6.5–10(13)µm. – Mesospores 28–30 × 18–20 µm, 3- and 4-celled spores 55–70 × 16–20 µm.

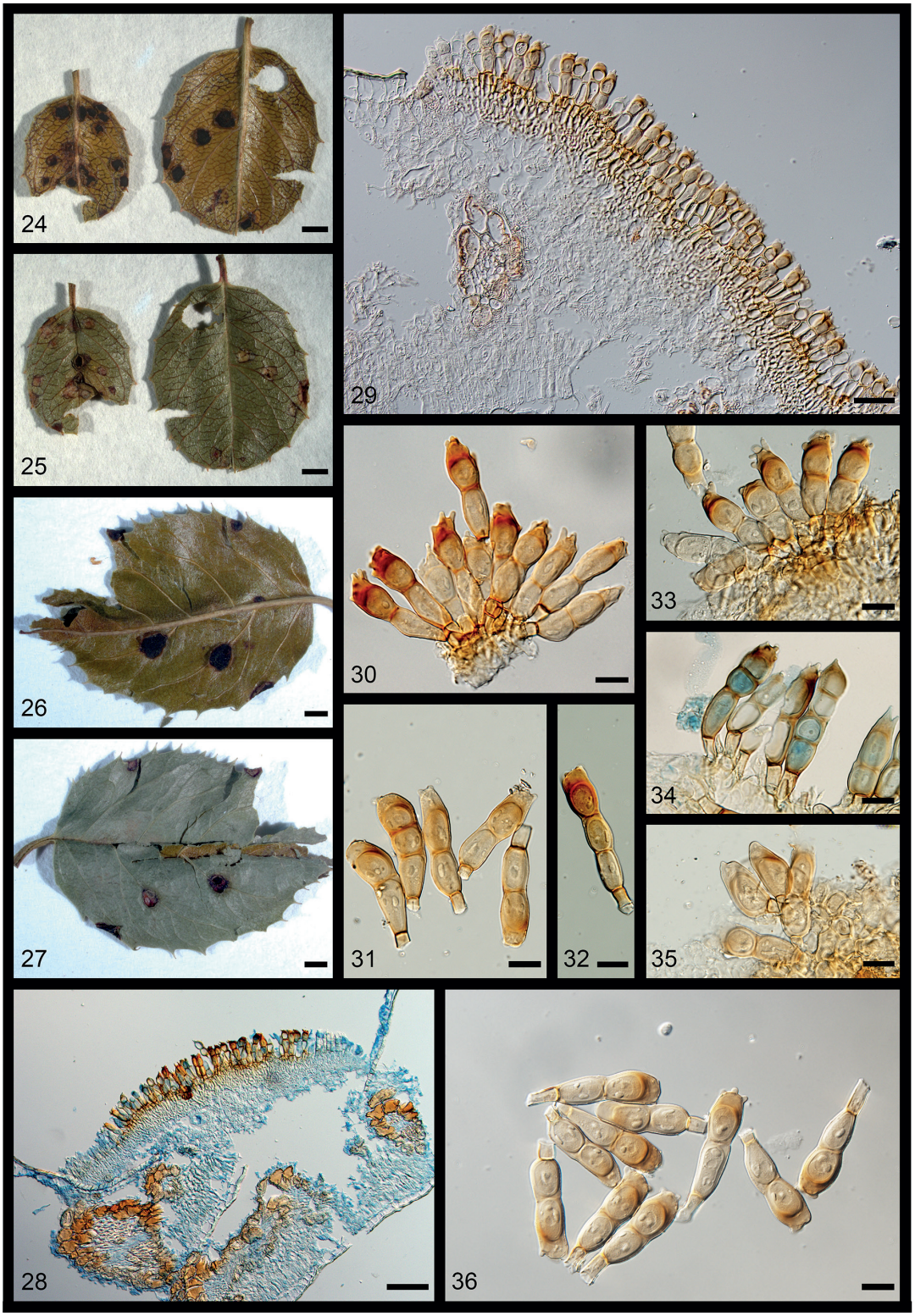
Etymology. – Refers to the close relationship with *Puccinia digitata*.

Host. – *Endotropis crocea* subsp. *ilicifolia*.

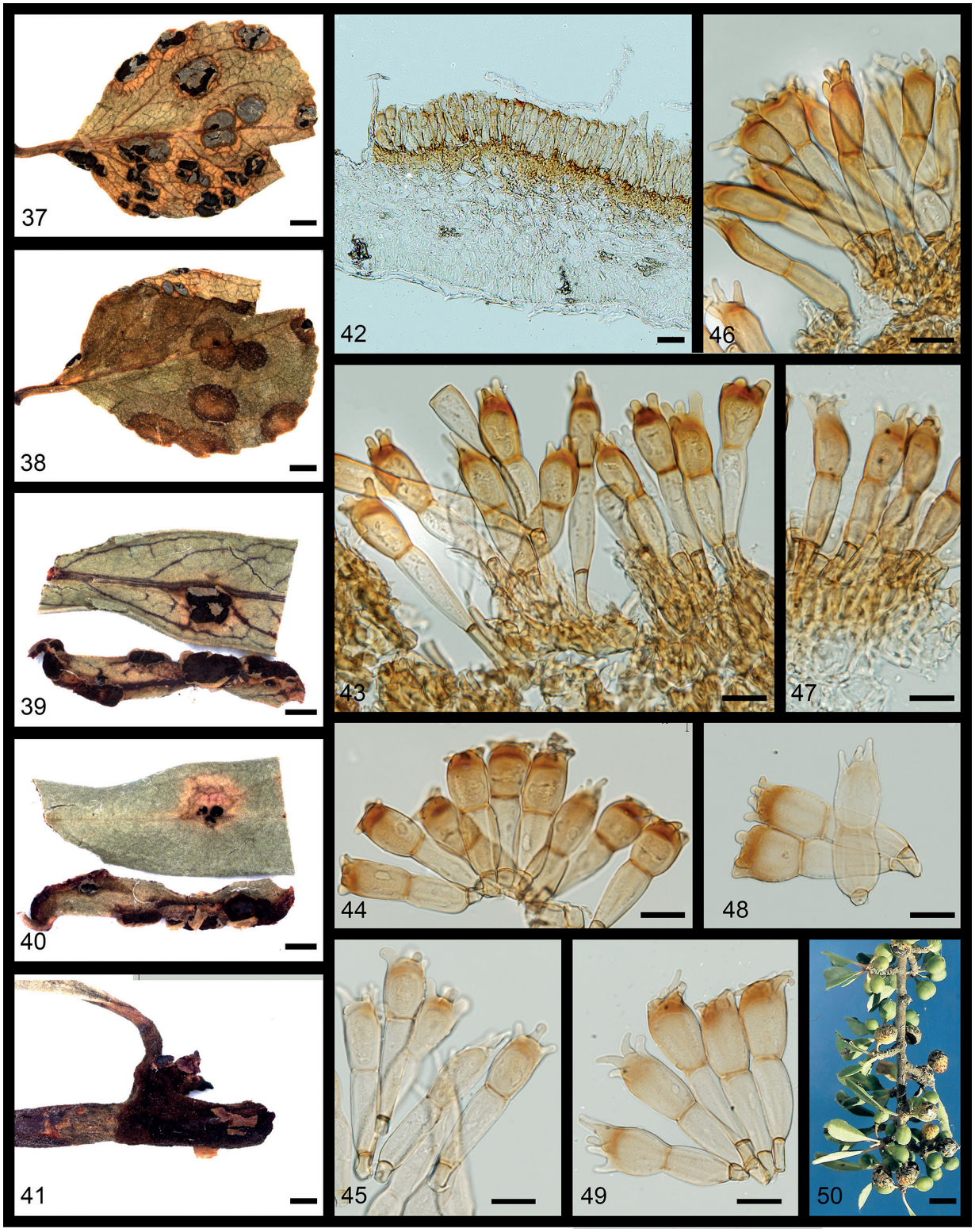
Distribution. – California, USA.

Other specimens examined. – USA. California, San Benito Co., the Pinnacles, on *Rhamnus crocea* var. *ilicifolia*, 1 June 1957, *leg.* H. J. Thomas (BPI 085603).

Notes. – Compared to *P. digitata*, this species produces generally shorter and wider teliospores, Guyot ratio (1.5)2–4(5.5) compared to (2.5)3–5.5(6.5). The apical wall is more severely thickened, produces fewer digits and is less tuberculate, though at times the tubercles extend to finger-like digits, and the pedicels are of similar length to *P. digitata*. One-, 3-, 4-celled spores are observed commonly. The holotype and isotype are exsiccatae specimens held in different fungaria (BPI and DAOM). Additional isotypes may be found in other fungaria with holdings of California Fungi # 699.



Figs. 24–36. *Puccinia pseudodigitata*. **24, 26.** Telia on lower leaf surfaces; **25, 27.** Upper surfaces of the infected leaves; **28.** Cross section of a telium on leaf surface mounted in cotton blue; **29.** Cross section of a telium; **30–36.** Teliospores of various shapes with **31**, **32** and **34** showing 3-, 4-celled spores, **35** showing mesospores. **24, 25**, **28–30, 33.** BPI 085603; **26, 27, 31, 32, 34–36.** BPI 085605 (holotype). Bars: **24–27** 2 mm; **28** 100 µm; **29** 50 µm; **30–36** 20 µm.



Figs. 37–50. *Puccinia pseudomesnieriana*: **37, 39.** Telia on lower sides of the leaves; **38**, **40.** Upper sides of the infected leaves; **41.** Telia on a twig; **42.** Cross section of a telium; **43–49**. Teliospores of various shapes. **50**. Freshly collected *Rhamnus palaestina* infected leaves and fruits from Israel. **37, 38, 42, 45, 48, 49.** BPI 085621 (holotype); **39, 40, 46, 47.** MA-Fungi 50182; **41, 43.** MA-Fungi 50174; **44**, BPI 1111897. Bars: **37–41** 1 mm; **42** 50 µm; **43–49** 20 µm; **50** 1 cm.

Puccinia pseudomesnieriana M. Liu & Hambl., sp. nov. Figs. 37–50.

Mycobank no.: MB 816062

Diagnosis. – Teliospores of variable shapes, oblong, oblong-clavate, clavate or cylindrical, Guyot ratio (2)3–5(7), apical processes mainly digitate, some tuberculate (1)3–5(7), pedicel up to 14(25) µm long, found in Greece, Israel and Spain.

Holotypus. – GREECE. Crete, Distr. Mylopotamous, Nida-Hochebene, on *Rhamnus prunifolia*, June 1942, *leg.* K. H. Rechinger (BPI 085621, Mycotheca Generalis Exsiccatae # 2033; ex-holotype sequences: ITS-28S KF661263, COI KF661277). Type seen ML!

Description. – Spermogonia, aecia, uredinia not formed, basidia not documented/not seen. – Telia amphigenous, and on twigs and fruits, brownish black, 0.5–2.5 mm diam. or merged up to 8 mm, sori with white to pale-grey (1B1–1B2) semi-transparent epidermal covering or open with some edge remnants; corresponding lesions on the opposite leaf surface circular with dark brown (5F8–6F8) to black edges or diffused. – Teliospores 2-celled with variable shapes, oblong, broad clavate, clavate or cylindrical, (41)47–67(74) × (6)10–14(17) µm (n = 148), Guyot ratio (2)3–5(7.5), slightly to strongly constricted at the septum; apical wall thickened (2.5)4.5–8(10) µm, forming (1)3–5(7) processes finger-like or branched horn-like, up to 12(17) µm long, (2)3–5(6.5) µm wide in the middle; pedicels similar colour with the lower cell of the spore or slightly darker, (2)6–14(25) µm long, hilum dark brown (4B4–6F8) or black, (4.5)5.5–8.5(11) µm wide.

Etymology. – Refers to the resemblance with *Puccinia mesnieriana*.

Host. – *Rhamnus saxatilis* subsp. *prunifolia, Rhamnus oleoides* subsp. *oleoides*.

Distribution. – Greece, Israel and Spain.

Other specimens examined. – GREECE. Kreta, Psiloriti-Gebirge (Idhi Oros), Mylopotamos, on *Rhamnus prunifolia,* 6 June 1942, *leg.* K. H. Rechinger (BPI 1111897); ISRAEL. Central Israel, on *Rhamnus lycioides*, June–December 1996, *leg.* Y. Anikster 8812, 8828; 2000, *leg.* Y. Anikster 8870; 2012, *leg.* Y. Anikster 8800; 2014, *leg.* Y. Anikster 88032; SPAIN. Malaga, Sierra de Molina, 20 km al NW de Antequera, 30SUG5015, 450 m, on *Rhamnus lycioides* subsp. *oleoides*, 19 June 1975, *leg.& det*. J. Mercé 3738 (MA-Fungi 50182); Granada, La Herradura, 35 km al W de Motril, 30SVF3465, 50 m, on *Rhamnus lycioides* subsp. *oleoides*, 7 June 1970, *leg. & det*. J. Mercé 1142 (MA-Fungi 50174).

Notes. – The specimens from Greece, Israel and Spain previously identified as *P. mesnieriana* grouped as a paraphyletic species (Fig. 1), which may represent an ancient taxon or several phylogenetic lineages. More specimens or data from additional DNA loci may bring more clarification. Based on the evidence available so far, it is prudent to recognize the group as one species to differentiate it from *P. mesnieriana* s. str. (see more in discussion). This species produces more variable teliospores with shorter pedicels and narrower, longer apical processes. The holotype is an exsiccata specimen and isotypes may be found in fungaria with holdings of Mycotheca Generalis # 2033.

Key to *P. mesnieriana* and related species

1. On *Endotropis crocea*, found in California, USA 2
1*. On *Rhamnus* spp., found in Mediterranean region 3
2. Teliospores 2-celled, rarely 3-celled, oblong, clavate or cylindrical, (41)49–74(83) × (8)10–16(18) µm, Guyot ratio (2.5)3–5.5(6.5), apical processes digitate .....*P. digitata*
2*. Teliospores 2-celled, but 1-, 3- even 4-celled are common, shorter and wider (30)38–60(70) × (9)10–17(21) µm, Guyot ratio (1.5)2–4(5.5), apical wall severely thickened with fewer digits, tuberculate .....*P. pseudodigitata*
3. Pedicels (2)7–14(26) µm .... *P. pseudomesnieriana*
3* Pedicels longer (7)10–34(45) µm... *P. mesnieriana*

Discussion

Recognition of four species

This study provides strong evidence for our recognition of *P. mesnieriana* and *P. digitata* as distinct, and the description of two new species, *P. pseudodigitata* and *P. pseudomesnieriana*. A combination of genetic, morphological, distributional and host association data differentiate these four species. Out of the nineteen specimens examined, only two were retained as *P. mesnieriana*. In the case of *P. pseudomesnieriana*, the molecular analyses do not confirm a monophyletic species. The sister species *P. digitata* and *P. pseudodigitata* are known only as yet from California USA, with *Rhamnus* hosts that are native to and restricted to North America, and now classified in the genus *Endotropis* (Hauenschild et al. 2016a, b). On the other hand, *P. mesnieriana* and *P. pseudomesnieriana* are restricted to the Mediterranean region and their hosts are classified in different sections of *Rhamnus*, sect. *Alaternus* which is also restricted to this region, and sect. *Rhamnus* with a broader distribution in Europe, Africa and Asia, respectively (Hauenschild et al. 2016a). Our DNA results validate the original hypothesis of Ellis & Harkness (1884) that *P. digitata* was a distinct species.

The Phylogenetic Species Concept sensu Mishler & Theriot (Mishler & Donoghue 1982; Donoghue 1985; Mishler 1985, 2000) and its widely-used derivatives in fungal systematics, i.e. using GCPSR, the Genealogical Concordance Phylogenetic Species Recognition method (Taylor et al. 2000), recognize species based on the evidence of monophyly. Species according to this concept must present on the multigene tree as one of the reciprocal monophyletic clades, which are recognized as a pair of sister species with the assumption that the ancestor species (stem species) has vanished in the evolutionary process (Taylor et al. 2000). According to topology-based monophyletic species concepts (Mishler & Donoghue 1982; Donoghue 1985; Mishler 1985, 2000; Taylor et al. 2000), the clade including *P. coronati-brevispora, P. coronati-longispora,* and *P. pseudomesnieriana* should be recognized as a single species, not three. Yet, lumping these three as one species is not appropriate unless we accept that rust fungi having dramatically different life-cycles can belong to the same species. In addition, *P. coronati-brevispora* and *P. coronati-longispora* did present as a pair of reciprocal monophyletic clades in our previous phylogenetic analyses based on multiple genes (BT, COI, ITS, RPB2) (Liu & Hambleton 2013), although samples of *P. pseudomesnieriana* were lacking at that time. We argue that in the case presented here, to recognize species merely based on the evidence of monophyly would be unstable.

The paraphyletic grouping of *P. pseudomesnieriana* could be a consequence of insufficient sampling or low resolution of the loci used. On the other hand, ample discussion has suggested that paraphyletic groups represent natural units in biological classification (Hörandl & Stuessy 2010). From an evolutionary point of view, diversification in a population can be symmetric and asymmetric. Additional evidence has shown that asymmetric speciation is common in nature (Rieseberg & Brouillet 1994), such as peripatric speciation, in which only small portion of the population diverges (Mayr & Bock 2002, De Queiroz 2005, Knapp et al. 2005, Harbaugh et al. 2009, Schaefer et al. 2009), and hybrid speciation, in which a previously diverged lineage backcrosses with its progenitor resulting in novel genotypes (retrogression, see Seehausen 2004, Rieseberg & Willis 2007, Soltis & Soltis 2009). Both cases result in the co-existence of progenitor and daughter species (Crawford 2010) as paraphyletic and monophyletic taxa in analyses. We hypothesize that the monophyletic *P. coronati-longispora* and *P. coronati-brevispora* are the progenies of the recently evolved paraphyletic species *P. pseudomesnieriana*, possibly as a result of asymmetric speciation. From a practical point of view, it is meaningful to recognize *P. pseudomesnieriana* as a species because many representatives in this study were identified as *P. mesnieriana,* which was shown in our analyses to be a separate lineage.

Relatedness of long-cycled and short-cycled rust species

The evolutionary relatedness of macrocyclic *P. coronata* and microcyclic *P. mesnieriana* was suggested by Dietel (1887) and, as noted in the introduction and in Scholler et al. (2019), there have been many hypotheses regarding such correlations put forward by taxonomists for other rust fungi. Tranzschel's Law refers to a pattern or rule, first recognized by Tranzschel in 1904 and further elaborated by others, which states that the telia of microcyclic rusts imitate the aecial habit of the related macrocyclic rust on its aecial host (Tranzschel 1904, Cummins & Hiratsuka 2003). In other words, microcyclic rusts are derived from macrocyclic rusts. Anikster & Wahl (1985) noted that Dietel's hypotheses about the direction of evolution for correlated species changed more than once between 1887 and 1918. Several conclusions can be inferred from our phylogenetic analyses, suggesting in this case both macro- to microcyclic and a micro- to macro evolutionary trends have occurred. Within *P.* ser. *coronata, P. coronati-calamagrostidis* is relatively ancestral. After its divergence, *P. mesnieriana* along with several macrocyclic lineages were diverged from a common ancestor (Node B, Fig. 1). Divergence of *P. pseudomesnieriana* and the two Californian species *P. digitata* and *P. pseudodigitata* were more recent than *P. mesnieriana*.

A previous study based on nrDNA (Zambino & Szabo 1993) showed that a strain of *P. mesnieriana* from Israel (likely belonging to *P. pseudomesnieriana* based on its provenance) is more closely related with *P. coronata* f. sp. *agropyri* (= *P. coronati-hordei*) than to *P. coronata* f. sp. *calamagrostis* (= *P. coronati-calamagrostis*) and a clade of *P. coronata* f. sp. *alopecuri*, f. sp. *avenae*, f. sp. *festucae* and f. sp. *lolii* (all members of *P. coronata* sensu Liu & Hambleton (2013). This result is consistent with ours. Additionally, both the ITSII28S (results not shown) and the ITSII28S-COI (Fig. 1) analyses showed a grouping of *P. coronati-brevispora*, *P. coronati-longispora* and *P. pseudomesnieriana* in which the two former species are more derived, i.e. macrocyclic species derived from microcyclic species. This evolutionary direction is in conflict with Scholler et al. (2019)

who argued against this trend, using the genus *Tranzschelia* as a case study. Although we acknowledge that our results are based on a limited sampling of DNA characters, we nevertheless speculate that the direction of the gain of function could be due to the retrogression or hybridization of *P. pseudomesnieriana* with a long-cycled species. These modes of speciation have been considered common in plants (Seehausen 2004, Soltis & Soltis 2009) and also played an important role in animal evolution (Mallet 2007, Mavarez & Linares 2008). Hybridization has proven common in cereal rust evolution resulting in novel biological specializations (Green 1971, Burdon et al. 1981), therefore it is reasonable to postulate that hybrid speciation has happened in this case.

As stated earlier, hypotheses regarding the relatedness of macro- and microcyclic species are common for rust fungi. Our study suggests that not only can microcyclic rusts be derived from macrocyclic species, macrocyclic rusts may also derive from microcyclic species. Whether or not our finding holds true with DNA analyses of more loci or for other pairs of correlated species remains to be tested in future studies.

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References

Aime M.C. (2006) Toward resolving family-level relationships in rust fungi (Uredinales). *Mycoscience* **47**: 112–122.

Anikster Y., Wahl I. (1985) Basidiospore formation and self-fertility in *Puccinia mesnieriana*. *Transactions of the British Mycological Society* **84**: 164–167.

Anikster Y., Eilam T., Manisterski J., Leonard K.J. (2003) Self-fertility and other distinguishing characteristics of a new morphotype of *Puccinia coronata* pathogenic on smooth brome grass. *Mycologia* **95**: 87–97.

Arthur J.C. (1915) New species of Uredineae - IX. *Bulletin of the Torrey Botanical Club* **42**: 585–602.

Arthur J.C. (1934) *Manual of the rusts in United States and Canada*. Purdue Research Foundation, Layfayette, IN USA.

Arthur J.C., Jackson H.S. (1922) (Uredinales) Aecidiaceae Micropuccinia. *North American Flora* **7**: 541–542.

Burdon J.J., Marshall D.R., Luig N.H. (1981) Isozyme analysis indicates that a virulent cereal rust pathogen is a somatic hybrid. *Nature* **293**: 565.

Crawford D.J. (2010) Progenitor-derivative species pairs and plant speciation. *Taxon* **59**(5): 1413–1423.

Cummins G.B., Hiratsuka Y. (2003) *Illustrated genera of rust fungi*. 3rd edn. APS Press, St. Paul, MN USA.

Darriba D., Taboada G.L., Doallo R., Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

De Queiroz A. (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology & Evolution* **20**: 68–73.

Demers J.E., Liu M., Hambleton S., Castlebury L.A. (2017) Rust fungi on *Panicum*. *Mycologia* **109**: 1–17.

Dietel P. (1887) Beiträge zur Morphologie und Biologie der Uredineen. *Botanisches Centralblatt* **32**: 54, 84, 118, 152, 182, 217, 246, 1 Tafel.

Dietel P. (1918) Ueber die Wirtswechselnden Rostpilze. *Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten* **48**: 470–500.

Donoghue M.J. (1985) A critique of the biological species concept and recommendations for a phylogenetic alternative. *Bryologist* **88**: 172–181.

Ellis J.B., Harkness H.W. (1884) New californian fungi. *Bulletin of the California Academy of Sciences* **1**: 27.

Fischer E. (1898) *Entwicklungsgeschichtliche Untersuchungen über Rostpilze: Eine Vorarbeit zur monographischen Darstellung der schweizerischen Uredineen*. In: *Beiträge zur Kryptogamenflora der Schweiz* K.J. Wyss, Bern, Switzerland: 109–115 (Chapter Theoretisches).

Green G.J. (1971) Hybridization between *Puccinia graminis tritici* and *Puccinia graminis* secalis and its evolutionary implications. *Canadian Journal of Botany* **49**: 2089–2095.

Guyot A.L. (1951) *Revue Bibliographique Générale*. In: *Uredineana: Recueil d'études systématiques et biologiques sur les Urédinées du Globe* (ed. Guyot A.L.), Paul Lechevalier, Paris, France: 362–364.

Guyot A.L., Massenot M., Montégut J. (1948) Etudes experimentales sur les Uredinees heteroiques realisees au Laboratoire de Botanique de l'Ecole Nationale d'Agriculture de Grignon (Seine-et-Oise) au cours des annees 1947–1948. In: *Annales de l'ecole nationale d'agriculture de Grignon; ser. 3, t. VI* (ed. Lechevalier P.), Imprimerie National, Paris, France: 119–139.

Harbaugh D.T., Wagner W.L., Allan G.J., Zimmer E.A. (2009) The Hawaiian Archipelago is a stepping stone for dispersal in the Pacific: an example from the plant genus *Melicope* (Rutaceae). *Journal of Biogeography* **36**: 230–241.

Hauenschild F., Favre A., Salazar G.A., Muellner-Riehl A.N. (2016a) Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*. *Taxon* **65**: 65–78.

Hauenschild F., Favre A., Salazar G.A., Muellner-Riehl A.N. (2016b) Erratum. Corrigendum to Hauenschild, F. & al. 2016. *Taxon* **65**: 926–927.

Hörandl E., Stuessy T.F. (2010) Paraphyletic groups as natural units of biological classification. *Taxon*: 1641–1653.

Jackson H.S. (1931) Present evolutionary tendencies and the origin of life cycles in the Uredinales. *Memoirs of the Torrey Botanical Club* **18**: 27–40.

Katoh K., Rozewicki J., Yamada K.D. (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*: bbx108-bbx108.

Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. (2008) *Ainsworth & Bisby's Dictionary of the Fungi*. 10th edn. CABI Europe, UK, Egham, UK.

Knapp M., Stöckler K., Havell D., Delsuc F., Sebastiani F., Lockhart P.J. (2005) Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). *PLoS Biol* **3**: e14.

Kornerup A., Wanscher J.H. (1978) *Methuen Handbook of Colours*. 3rd edn. Eyre Methuen, London, UK.

Liu M., Hambleton S. (2010) Taxonomic study of stripe rust, *Puccinia striiformis sensu lato*, based on molecular and morphological evidence. *Fungal Biology* **114**: 881–899.

Liu M., Hambleton S. (2012) *Puccinia chunjii*, a close relative of the cereal stem rusts revealed by molecular phylogeny and morphological study. *Mycologia* **104**: 1056–1067.

Liu M., Hambleton S. (2013) Laying the foundation for a taxonomic review of *Puccinia coronata* s.l. in a phylogenetic context. *Mycological Progress* **12**: 63–89.

Liu M., Szabo L.J., Hambleton S., Anikster Y., Kolmer J. (2013) Molecular phylogenetic relationships of the brown leaf rust fungi on wheat, rye and other grasses. *Plant Disease* **97**: 1408–1417.

Mallet J. (2007) Hybrid speciation. *Nature* **446**: 279–283.

Marková J., Urban Z. (1998) The rust fungi of grasses in Europe. 6. *Puccinia persistens* Plow., *P. perplexans* Plow., and *P. elymi* Westend. *Acta Universitatis Carolinae Biologica* **41**: 329–402.

Mavarez J., Linares M. (2008) Homoploid hybrid speciation in animals. *Molecular Ecology* **17**: 4181–4185.

Mayr E., Bock W.J. (2002) Classifications and other ordering systems. *Journal of Zoological Systematics and Evolutionary Research* **40**: 169–194.

Mishler B.D. (1985) The morphological, developmental, and phylogenetic basis of species concepts in bryophytes. *Bryologist* **88**: 207–214.

Mishler B.D. (2000) The phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. In Wheeler Q.D., Meier M. (eds): *Species concepts and phylogenetic theory, a debate*: 119–132. Columbia University Press .

Mishler B.D., Donoghue M.J. (1982) Species concepts: a case for pluralism. *Systematic Zoology* **31**(4): 491–503.

Orton C.R. (1912) Correlation between certain species of *Puccinia* and *Uromyces*. *Mycologia* **4**: 194–204.

Rieseberg L.H., Brouillet L. (1994) Are many plant species paraphyletic? *Taxon*: 21–32.

Rieseberg L.H., Willis J.H. (2007) Plant speciation. *Science* **317**: 910–914.

Rioux S., Mimee B., Gagnon A.-È., Hambleton S. (2015) First report of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat in Quebec, Canada. *Phytoprotection* **95**: 7–9.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology* **61**: 539–542.

Schaefer H., Heibl C., Renner S.S. (2009) Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society of London B, Biological Sciences* **276**: 843–851.

Scholler M., Lutz M., Aime C.A. (2019) Repeated formation of correlated species in *Tranzschelia* (Pucciniales). *Mycological Progress* **18**: 295–303.

Seehausen O. (2004) Hybridization and adaptive radiation. *Trends in Ecology & Evolution* **19**: 198–207.

Smith J.F. (2000) Phylogenetic signal common to three data sets: combining data which initially appear heterogeneous. *Plant Systematics and Evolution* **221**: 179–198.

Soltis P.S., Soltis D.E. (2009) The role of hybridization in plant speciation. *Annual Review of Plant Biology* **60**: 561–588.

Swofford D.L. (2002) PAUP*. phylogenetic analysis using parsimony (*and other methods). version 4.0 b10. Sinauer Associates, Sunderland, MA.

Sydow P., Sydow H. (1904) *Monographia Uredinearum*. Fratres Borntraeger, Leipzig, Germany.

Taylor J.W., Jacobson D.J., Kroken S., Kasuga T., Geiser D.M., Hibbett D.S., Fisher M.C. (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.

Thümen F.v. (1877) Mycotheca Universalis, Centurie 9 (801–900), #834. *Bulletin de la Société Impériale des Naturalistes de Moscou*.

Thümen F.v. (1878) Contributiones ad floram mycologicam lusitanicam. *Jornal de Sciencias Mathematicas, Physicas e Naturaes Lisboa,* ser. 6 **(no. 21–24)**: 238.

Tranzschel W. (1904) Über die Möglichkeit, die Biologie wirtswechselnder Rostpilze auf Grund morphologisher Merkmale vorauszusehen. *Arbeiten der Kaiserlichen St. Petersburger Naturforschenden Gesellschaft* **35**: 311–312.

Travelbee H.C. (1914) Correlation of certain long-cycled and short-cycled rusts. *Proceedings of the Indian Academy of Sciences (Plant Sciences)* **1914**: 231–234.

Zambino P.J., Szabo L.J. (1993) Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. *Mycologia* **85**: 401–414.

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