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A new species of rust fungus on the New Zealand endemic plant, *Myosotidium*, from the isolated Chatham Islands

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Abstract

Pucciniastrum myosotidii sp. nov. is described from plants of the Chatham Island forget-me-not (*Myosotidium hortensium*), a host plant that has a conservation status of "nationally endangered". The rust has been found only on cultivated plants and not on wild plants. Although no teliospores were found, LSU and SSU sequence analysis showed that the new rust is closely related to some species of *Pucciniastrum* and *Thekopsora* forming a weakly supported clade together with *P. boehmeriae*, *P. epilobii*, *P. circaeae*, *P. goeppertianum*, *P. guttatum*, *P. pustulatum*, *T. minima* and *Melampsorella symphyti.* If this rust is endemic to Chatham Islands, then it must be accepted as a species of conservation value since the host plant is under threat from grazing animals and habitat loss.

Key words: Boraginaceae, endemic, megaherb, ornamental, phylogenetic analyses, *Pucciniastrum symphyti comb. nov.*

Introduction

Plants of the Chatham Island forget-me-not or giant forget-me-not (*Myosotidium hortensium* (Decne.) Baill.; Boraginaceae), growing in the Chatham Islands, were found to be infected by a rust fungus in January 2007 (Fig. 1 A–B). The rust was common in a garden on established plants and in a nearby nursery (Beever 2007). The rust has been since seen on cultivated plants, but never on wild plants. Although 63 species of rust fungi were recorded during earlier surveys of fungi in the Chatham Islands (McKenzie 1991, McKenzie & Johnston 1999), no rust was observed on *Myosotidium*. The host, which is a perennial and robust megaherb, is cultivated as an ornamental in both the North and South Islands of New Zealand although, in general, it struggles to survive outside of the Chathams. The Chatham Islands lie approximately 860 km east of Christchurch, New Zealand and have a total land area of less than 100,000 ha.

The genus *Myosotidium* is monotypic and endemic to the Chatham Islands where it grows naturally in coastal habitats on cliffs, rock outcrops and sandy and rocky beaches just above the strand zone (Heenan & Schönberger 2009). Although formerly abundant it has been significantly reduced to scattered remnants by farming, competition from marram grass, and trampling and browsing by animals. Consequently, it has been given a conservation status of "nationally endangered" (Hitchmough 2002).

Myosotidium has no obvious generic relatives in the Boraginaceae, but DNA analysis suggests that *Omphalodes nitida* Hoffmanns. & Link (1811) from Portugal and Spain is the closest relative of *Myosotidium*, which appears to represent a Chatham Island–Mediterranean disjunction (Heenan *et al.* 2010). It is also related to *Lappula squarrosa* and *Trichodesma scottii* (Heenan *et al.* 2010).

Based primarily on a phylogenetic analysis the rust on Chatham Island forget-me-not is herein described as a new species of *Pucciniastrum*. The question of whether or not this species is native or introduced to the Chatham Islands is also raised.

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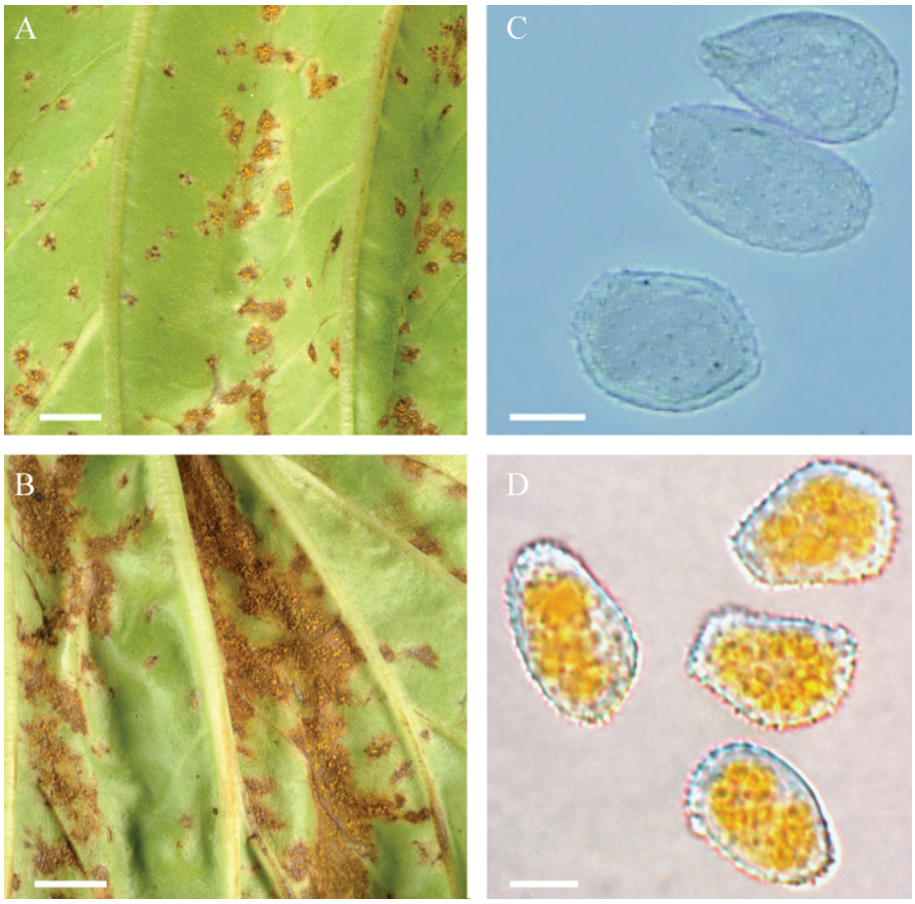

FIGURE 1. *Pucciniastrum myosotidii* on *Myosotidium hortensii*. A. Adaxial view. Scale bar = 5 mm B. Abaxial view. Scale bar = 5 mm C. Urediniospores. Composite image. Scale bar = 10 µm D. Urediniospores. Scale bar = 10 µm

Materials and methods

Isolates and morphology

For examination of morphological features the rust was mounted in lactophenol and examined with an Olympus BH-2 microscope. Voucher materials were deposited in Fungarium PDD.

Phylogenetic analyses

The rust fungus was also examined by molecular methods. Sori were excised and DNA was extracted using an X-tractor Gene System (Corbett Life Science, NSW, Australia). The nuclear ribosomal large subunit (LSU) locus was amplified with a rust-specific primer Rust2inv (Aime 2006) and LR6 (Vilgalys & Hester 1990), and sequenced with Rust2inv, LR6, LR3 (Vilgalys & Hester 1990), and LROR (Moncalvo *et al.* 1995). The nuclear ribosomal small subunit (SSU) was amplified with a rust-specific primer Rust 18S-R (Aime 2006) and NS1 (White *et al.* 1990), and sequenced with NS1, NS3, NS4, NS8 (White *et al.* 1990) and Rust 18S-R. The protocol for PCR conditions in Aime (2006) was followed. PCR products were diluted (1:5) before sequencing with BigDye Terminator sequencing enzyme v.3.1 (Applied Biosystems, Life Technologies New Zealand Limited, Auckland, NZ) in a 10 µl reaction. Sequencing reactions were cleaned by BigDye XTerminator® Purification Kit (Applied Biosystems), and sequenced on an Applied Biosystems 3100-Avant Genetic Analyzer.

Resulting LSU and SSU sequences were aligned with 33 sequences from this study and GenBank (Table 1) representing clades identified in Aime (2006) and Maier *et al.* (2003). *Eocronartium muscicola* (Pers.) Fitzpatrick (1918: 197) and *Tuberculina* Tode ex Saccardo (1880: 34) were used as outgroups. LSU and SSU datasets were analysed in PhyML 3.0 (Guindon & Gascuel 2003) using the GTR+Γ+I model of evolution and 1000 bootstrap support (MLBS) values were generated. Resulting phylogenies were examined to identify any regions of conflict before concatenating the datasets in Geneious Pro v7.0.5 (Biomatters, http://www.geneious.com/). Only taxa for which both

loci were present (except *Thekopsora areolata* (Fr.) Magnus (1875: 58) for which only LSU sequences were available) were included in the combined dataset. The concatenated dataset was analyzed as mentioned above.

TABLE 1: GenBank accession numbers for taxa used in molecular analyses.

	Accession number	LSU	SSU
Chrysomyxa rhododendri	PDD 102088	KJ698630	KJ746824
Chrysomyxa sp.	PDD 94468	KJ716349	KJ746827
Coleosporium senecionis	PDD 98309	KJ716348	KJ746818
Cronartium ribicola		AF522166	
Eocronartium muscicola	MIN 796447	AF014825	AY123323
Gymnosporangium juniperi-virginianae	AFTOL 712	AY629316	DQ667158
Hyalopsora polypodii	PDD 71999	KJ698627	KJ746817
Kuehneola uredinis	AFTOL 987	AY745696	DQ92919
Melampsora hypericorum	PDD 97325	KJ716353	KJ746828
Melampsora ricini	PDD 98363	KJ716352	KJ746829
Melampsorella (Thekopsora) symphyti		AF426230	
Mikronegeria fuchsiae	PDD 101517	KJ716350	KJ746826
Naohidemyces vaccinii	BPI 871754	DQ354563	DQ354562
Naohidemyces vaccinii	MIN 928279	KJ698628	
Phragmidium violaceum	PDD 99246	KJ716351	KJ746822
Puccinia brachypodii	PDD 101651	KJ698624	KJ746820
Puccinia circaeae	TUB 14969	DQ917716	
Puccinia coronata	BPI 844300	DQ354526	DQ354525
Puccinia menthae	PDD 99229	KJ716344	KJ746825
Pucciniastrum boehmeriae	PDD 78509	KJ698626	
Pucciniastrum brachybotrydis	KUS-F 21087	KJ698625	KJ746819
Pucciniastrum circaeae	AFTOL 985	AY745697	
Pucciniastrum epilobii	ECS352	AF522179	AY123303
Pucciniastrum goeppertianum	TDB1497	AF522180	AY123305
Pucciniastrum guttatum	PDD 91889	KJ716345	KJ746830
Pucciniastrum myosotidii ined.	PDD 93251	KJ716347	KJ746815
Pucciniastrum myosotidii ined.	PDD 92567	KJ716346	
Pucciniastrum pustulatum	PDD 101572	KJ698631	KJ746816
Thekopsora areolata	PDD 102702	KJ698629	
Thekopsora areolata		AF426235	
Thekopsora minima	BRIP 57654	KC763340	
Thekopsora minima		GU355675	
Tuberculina sp.	PDD 101711	KJ698632	KJ746823
Uromyces appendiculatus	TDB	AF522182	AY123307
Uromyces viciae-fabae	PDD 101522	KJ716343	KJ746821

Results

The LSU sequence of PDD 93251 had a 95% sequence identity and 100% query coverage with *Thekopsora minima* (Arth.) Sydow (1915: 465) strain LD 1081 (HM439777), and 98% sequence identity and 69% query coverage with *T. minima* strain BRIP 57654 (KC763340). Two collections of the rust (PDD 93251 and PDD 92567) had identical LSU sequences. The SSU sequence had a 99% sequence identity and 100% query coverage with *Pucciniastrum epilobii* G.H. Otth (1861: 72) isolate ECS352 (AY123303). We were unable to amplify the SSU sequence for PDD 92567. In

the LSU phylogeny (Fig. 2), the rust on *Myosotidium* was recovered within a weakly supported clade (60.9% MLBS) of members of *Pucciniastrum* G.H. Otth (1861: 72), including the generic type *P. epilobii*, and *Thekopsora* Magnus (1875: 123). Phylogenetic analyses of the LSU and SSU datasets (Fig. 3), which did not include *Pucciniastrum guttatum* (J. Schröt.) Hylander, Jørstad & Nannfeldt (1953: 81), *Thekopsora minima*, and *Melampsorella symphyti* Bubák (1903: 356) sequences, recovered a highly supported clade (90% MLBS) that contained the rust on *Myosotidium* and three other *Pucciniastrum* species. In both analyses, *Pucciniastrum* and *Thekopsora* are polyphyletic. The generic type of *Thekopsora*, *T. areolata*, is sister to *Cronartium ribicola* J.C. Fischer (in Rabenhorst 1872: 182) in the LSU analyses (Fig. 2) and sister to *Chrysomyxa rhododendri* (DC.) de Bary (1879: 809) in the concatenated dataset (Fig. 3).

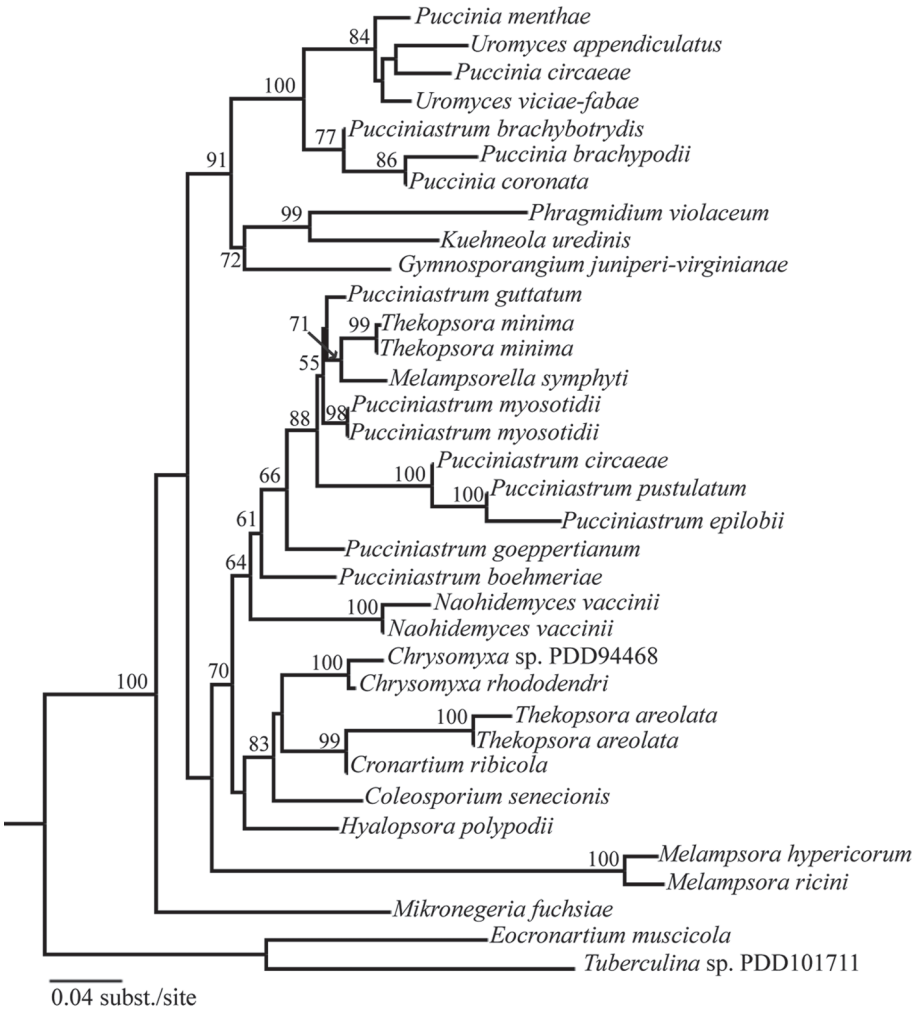

FIGURE 2. Phylogram obtained from maximum likelihood analysis of nuclear LSU rDNA. Bootstrap support values (> 50%) from a maximum likelihood search with 1000 replicates shown.

Discussion

Several rust fungi have been described or recorded on hosts within the family Boraginaceae. One of these, *Puccinia novozelandica* Bubák (1901: 5), was described from *Myosotis capitata* in the subantarctic Auckland Islands, New Zealand, but is known to produce only teliospores. The other two species are *Micropuccinia hydrophylli* (Peck & Clinton) Arthur & H.S. Jackson (1921: 42) that occurs on *Omphalodes luciliae* in Iran (Gjaerum 1986) and *Puccinia recondita* Roberge ex Desmazières (1857: 798) on *Lappula squarrosa* in Mongolia (Braun 1999).

Thekopsora brachybotrydis Tranzschel (1907: 551) was described from China on *Brachybotrys paridiformis* (Boraginaceae), and it was later recombined as *Pucciniastrum brachybotrydis* (Tranzschel) Jørstad (1958). It has since been recorded from Austria, Japan, Korea and Russia on various species of *Brachybotrys*, *Myosotis*, *Omphalodes* and *Trigonotis* (Teppner *et al*. 1977, Hiratsuka *et al*. 1992, Cho & Shin 2004). Although attempts to amplify DNA from

available specimens of *P. brachybotrydis* on *Myosotis* were unsuccessful, DNA was amplified from specimens of *P. brachybotrydis* on *Trigonotis*. This species appears morphologically similar to the rust on *Myosotidium hortensium* but with smaller urediniospores. Teppner *et al*. (1977) summarised urediniospore measurements for *P. brachybotrydis* giving an extreme range of 16–23 × 11–18 µm. These spores are thus smaller than those of the rust on *M. hortensium*, which measure (19–)21–28(–30.5) × (12.5–)14–19(–21) µm with a mean size of 23.8 × 16.3 µm (Fig. 1 C–D). Additionally, *P. brachybotrydis* is molecularly distinct from the rust on *M. hortensium* and is recovered in a highly supported (99.7% MLBS) clade with *Puccinia coronata* Corda (1837: 6) and *P. brachypodii* G.H. Otth (1861: 81) (Fig. 2). *Pucciniastrum brachybotrydis* can possibly be recombined in *Puccinia* Persoon (1801: 225).

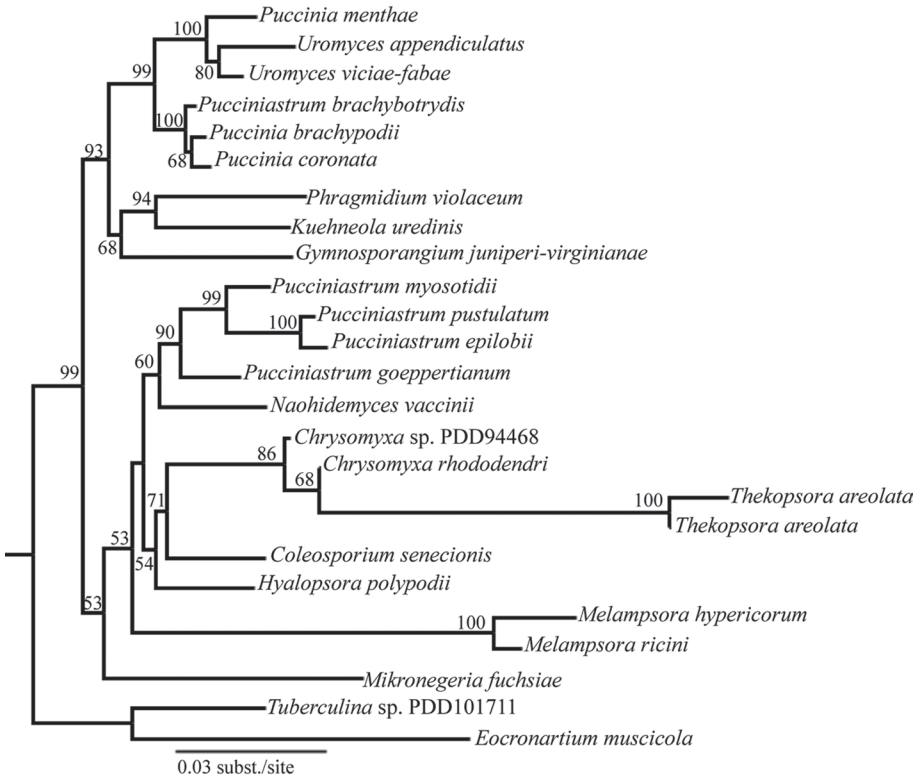

FIGURE 3. Phylogram obtained from maximum likelihood analysis of nuclear rDNA loci, LSU and SSU. Bootstrap support values (> 50%) from a maximum likelihood search with 1000 replicates shown.

Although no teliospores were found in any of the Chatham Islands collections, despite collections being made at various times of the year, the new rust is closely related to some species of *Pucciniastrum* and *Thekopsora*. Molecular analysis showed that the new rust forms a weakly supported clade together with *P. boehmeriae* (Dietel) Sydow & P. Sydow (1903: 19), *P. epilobii*, *P. circaeae* (Schumach.) Spegazzini (1879: no 65), *P. goeppertianum* (Kuehn) Klebahn (1904: 391), *P. guttatum*, *P. pustulatum* Dietel (1897: 47), *T. minima* and *Melampsorella* [*Thekopsora*] *symphyti* (60.9% MLBS; Fig. 2). This clade was recognised by Maier *et al*. (2003) as "Group 3" within the Pucciniastreae. *Pucciniastrum epilobii* is the type species of *Pucciniastrum* (Cummins & Hiratsuka 2003). As mentioned by Maier *et al.* (2003), the genus *Thekopsora* is obviously polyphyletic and the type species, *T. areolata* (Cummins & Hiratsuka 2003) lies within a separate clade (Fig. 2). Traditionally, the new rust would be placed in the asexual genus *Uredo* Persoon (1801: 214). However, since 2011 all legitimate fungal names are treated equally for the purposes of establishing priority, regardless of the life history stage of the type (Article 59.1, Melbourne Code, McNeill *et al.* 2012). This implies that if the rust on *M. hortensium* was placed in *Uredo* now that it would eventually have to be recombined in another genus. The rust on *M. hortensium* is recovered in the combined analysis, within a well-supported clade (90% MLBS) consisting of three *Pucciniastrum* species (Fig. 3); thus we propose to place the rust within this genus instead of erecting another genus based on a single species. By doing so, we also propose that it is now necessary to recognise *Pucciniastrum minimum* Arthur (1905: 337) as the correct name for *Thekopsora minima* and to recombine *Melampsorella symphyti* in *Pucciniastrum*. When included in the molecular analyses, the generic type of *Melampsorella* J. Schröter (1874: 85), *M. caryophyllacearum* (DC.) J. Schröter (1874: 85) is sister to *Pucciniastrum goeppertianum* (87% MLBS; data not shown); however, support for the *Pucciniastrum* clade falls to 54% MLBS. Currently, we do not propose to recombine *M. caryophyllacearum* in *Pucciniastrum*, but acknowledge that this may be necessary in the future.

A question remains as to whether or not *P. myosotidii* is native to the Chatham Islands. The isolated position of the Chathams and the uniqueness of the host plant would suggest that the rust is indigenous, if not endemic. However, the failure to find this rust on wild plants suggests that the rust may be introduced. Either way, how did the rust get to the Chatham Islands? It is also of interest to note possible concerns over the conservation status of *P. myosotidii*. If this rust is endemic to Chatham Islands, then it must be accepted as a species of conservation value since the host plant is considered to be "nationally endangered" (Hitchmough 2002). A comparable situation exists with another endemic Chatham Islands rust, *Puccinia embergeriae* McKenzie & P.R. Johnston (2004: 657) (McKenzie & Johnston 2004), with a threat status listing of "nationally critical" since it occurs only on Chatham Islands sow thistle (*Embergeria grandifolia*), a plant that is under threat from grazing animals and is listed as "nationally endangered" (Hitchmough 2002).

Taxonomy

Pucciniastrum minimum Arthur, Résult. Sci. Congr. Bot. Wien 1905: 337. 1906.
≡ *Thekopsora minima* (Arth.) Syd. & P. Syd., Monogr. Uredin. (Lipsiae) 3(3): 465. 1915.
= *Uredo minima* Schwein., Schr. naturf. Ges. Leipzig 1: 70. 1822.
≡ *Uredo azaleae* Schwein., Schr. naturf. Ges. Leipzig 1: 70. 1822.
= *Caeoma azaleae* Schwein., Trans. Am. phil. Soc. Ser. 2, 4(2): 291. 1832.
= *Peridermium peckii* Thüm., Mitt. Ver. Österr. 2: 320. 1879.
≡ *Aecidium peckii* (Thüm.) Dietel, in Engler & Prantl, Nat. Pfanzenfam., Teil I (Leipzig) 1**: 78. 1897.

Pucciniastrum myosotidii McKenzie & Padamsee, *sp. nov.* (Fig. 3) MycoBank MB 808525

Type:—NEW ZEALAND. Chatham Islands: Rekohu, Te One, house garden next to Department of Conservation (DOC) base, on *Myosotidium hortensium*, 6 Jan 2007, *R.E. Beever & L. Guard* (PDD 94473).

Differs from *Pucciniastrum epilobii* by its larger urediniospores and its DNA phylogeny.

Telia and *teliospores* not observed. *Uredinia* hypophyllous, scattered but mainly grouped, sometimes concentric on spots up to 1.5 cm in diam., spots extend to upper surface of leaf, yellow-orange, orbicular, 0.25–0.5 mm diam., bullate, erumpent, opening initially through an apical pore that soon splits wider, becoming pulverulent. *Urediniospores* (19–)21–28(–30.5) × (12.5–)14.5–19(–21) µm (mean of 75 spores, 23.8 × 16.3 µm), subglobose, obovoid, broadly ellipsoidal or ellipsoid, sometimes somewhat angular, contents yellow; wall 1–1.5 µm thick, hyaline, echinulate, germ pores obscure.

Etymology:—Named after the host genus, *Myosotidium*.

Other specimens examined:—NEW ZEALAND. Chatham Islands: Rekohu, Te One, DOC house next to Ranger Station, on *M. hortensium*, 9 Jan 2007, *R.E. Beever* (PDD 94859); Rekohu, Te One, DOC house next to Ranger Station, on *M. hortensium*, 11 Jan 2007, *L. Guard* (PDD 94555); Rekohu, Henga, Admiral farm, on *M. hortensium*, 7 Jan 2007, *R.E. Beever* (PDD 94860); Rekohu, Te One, DOC Nursery and nearby house garden, on *M. hortensium*, 20 Jun 2007, *B. Gibb & L. Guard* (PDD 92565); Rekohu, Te One, front of DOC office (PDD 92566, 92567); Rekohu, Te One, on *M. hortensium*, 20 Sep 2007, *P.J. de Lange* (PDD 93251, **epitype, designated here**).

Note:—Only one other species of *Pucciniastrum*, *P. epilobii* (syn. *P. pustulatum*), has been recorded from New Zealand, on several genera within the Onagraceae. *Pucciniastrum epilobii* has smaller urediniospores (15–24 × 10–15 um; Cunningham 1931) than those of *P. myosotidii*, although in the current molecular analysis the two species lie within sister clades.

Pucciniastrum symphyti (DC.) McKenzie & Padamsee, *comb. nov.* MycoBank MB 808526
≡ *Uredo symphyti* DC., Encycl. Méth. Bot. 8: 232. 1808; basionym.
≡ *Melampsorella symphyti* (DC.) Bubák, Ber. dt. Bot. Ges. 21: 356. 1903.
≡ *Caeoma symphyti* (DC.) Schltdl., Fl. berol. (Berlin) 2: 122. 1824.
≡ *Erysibe symphyti* (DC.) Wallr., Fl. crypt. Germ. (Norimbergae) 2: 200. 1833.
≡ *Trichobasis symphyti* (DC.) Lév., Orbigny Dict. Univ. Hist. Nat.: 785 [19 extr.]. 1849.
≡ *Coleosporium symphyti* (DC.) Fuckel, in Kickx, Fl. Crypt. Flandres (Paris) 2: 88. 1867.

≡ *Thekopsora symphyti* (Bubák) Berndt, Untersuchungen zur Ultrastruktur und Anatomie der Melampsoraceen (Uredinales, Basidiomycetes): 198. 1993.

≡ *Thekopsora symphyti* (Bubák) J. Müll., Czech Mycol. 62: 97; 2010.

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