**Exobasidium darwinii**, a new Hawaiian species infecting endemic *Vaccinium reticulatum* in Haleakala National Park

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**Abstract** Hawaii is one of the most isolated archipelagos in the world, situated about 4,000 km from the nearest continent, and never connected with continental land masses. Two Hawaiian endemic blueberries, *Vaccinium calycinum* and *V. reticulatum*, are infected by *Exobasidium* species previously recognized as *Exobasidium vaccinii*. However, because of the high host-specificity of *Exobasidium*, it seems unlikely that the species infecting *Vaccinium calycinum* and *V. reticulatum* belongs to *Exobasidium vaccinii*, which in the current circumscription is restricted to *Vaccinium vitis-idaea*. We collected a fresh specimen of *Exobasidium* on *Vaccinium reticulatum* in Haleakala National Park, and analysed it by a combined morphological and molecular approach. The morphological and phylogenetic analyses (based on the LSU and the concatenated ITS+LSU sequence data) showed that *Exobasidium* on *Vaccinium reticulatum* belongs to an undescribed species, distinct from *Exobasidium vaccinii* and any other species. The name *Exobasidium darwinii* is proposed for this novel taxon. This species is characterized among others by the production of peculiar witches’ brooms with bright red leaves on the infected branches of *Vaccinium reticulatum*. Relevant characters of *Exobasidium darwinii* are described and illustrated, additionally phylogenetic relationships of the new species are discussed.

**Keywords** Exobasidiomycetes · ITS · LSU · Molecular phylogeny · Ustilaginomycotina

**Introduction**

*Exobasidium* Woronin is the largest genus within the Exobasidiales with 178 epithets included in the *Index Fungorum* (http://www.indexfungorum.org/Names/Names.asp). Some of these names are invalidly published, others are synonymous or represent species of other genera (e.g., *Arcticomyces* Savile, *Exobasidiellum* Donk, *Kordyana* Racib., *Laurobasidium* Jülich, *Muribasidiospora* Kamat & Rajendren). Yet the genus is poorly known and, except for Europe, there is no comprehensive study of *Exobasidium*. In the past, *Exobasidium* species were described based on supposed host specificity, but this concept was later abandoned by Burt (1915) and Savile (1959), who lumped together many species, especially within *Exobasidium vaccinii* (Fuckel) Woronin and *Exobasidium vaccini-uliginosi* Boud. The high level of host specialization was again postulated for European *Exobasidium* species by Nannfeldt (1981) on the basis of a detailed analysis of morphological characters correlated with host plant and physiological data (the latter elaborated by Sundström 1964). His ideas were basically supported by molecular analyses of Blanz and Oberwinkler (1983), Blanz and
Döring (1995) and Begerow et al. (2002), although in this latter study support for most branches was low and obtained resolution was not fully satisfactorily for conclusions about evolutionary relationships within both Exobasidium and the Exobasidiales.

Located in the middle of the Pacific Ocean, Hawaii is one of the most isolated archipelagos in the world. The islands are situated about 4,000 km from the nearest continent and have never been connected with continental land masses. The indigenous flowering plants of Hawaii include 1,029 species, of which ca. 89% are endemic to the islands (Sakai et al. 2002) likely harbouring many undescribed pathogenic fungi that are usually strictly the islands (Sakai et al. 2002) likely harbouring many undescribed pathogenic fungi that are usually strictly correlated with host plants. Two Hawaiian endemic blue-berries, Vaccinium calycinum Sm. and V. reticulatum Sm. (incl. V. peleanum Skottsb.), were reported as hosts for Exobasidium species producing peculiar witches’ brooms with bright red leaves on infected branches (Gardner 1985). Although the causal agent was first considered as a mycoplasma-like organism, it soon became evident that it is rather a species of Exobasidium, which has been referred to as Exobasidium vaccinii but with uncertainty whether it is native or alien on Hawaiian blueberries (Gardner 1985). However, in the light of the high specificity of Exobasidium, usually to the level of host species, rarely to a group of closely allied species (Nannfeldt 1981), it seems unlikely that the species infecting Vaccinium calycinum and V. reticulatum belongs to Exobasidium vaccinii. That latter species is quite a different fungus, producing localized, conspicuous and much thickened concavities on leaves of Vaccinium vitis-idaea L. Only occasionally it may cause deformations of all shoot-tips (Nannfeldt 1981), which however are never as strong as on the Hawaiian blueberries. Therefore, we hypothesize that Exobasidium on Vaccinium calycinum and V. reticulatum represents in fact one (or even two) distinct species. To test this hypothesis, we collected a fresh specimen of Exobasidium on Vaccinium reticulatum in Haleakala National Park, and analysed it by a combined morphological and molecular approach. Unfortunately, the corresponding specimen of Exobasidium on Vaccinium calycinum has not hitherto been re-collected.

**Materials and methods**

The Exobasidium specimen infecting Vaccinium reticulatum was collected in Haleakala National Park in Hawaii on 17 December 2007. This specimen was used in subsequent morphological and phylogenetic analyses. Information on the specimens of Exobasidium arescens Nannf. and E. rostrigii Nannf. sequenced for the present study is given in Table 1.

Morphological analyses

The macroscopic symptoms of host infection and microscopic features of Exobasidium on Vaccinium reticulatum were studied using dried herbarium material. The terminology used in the species description and in the text follows Nannfeldt (1981). Morphological observations and measurements of basidia, basidiospores and conidia were made by standard light and phase contrast microscopy (LM) of the material mounted in 3% KOH. A Nikon Eclipse 80i light microscope was used for LM studies. Thirty basidiospores and conidia were measured from the studied collection, using NIS-Elements BR 3.0 imaging software. LM micrographs were taken with a Nikon DS-Fil camera. The observations of basidia were supplemented by scanning electron microscopy (SEM). For this purpose, a fragment of infected leaf was glued to the carbon tab and fixed to an aluminium stub with double-sided transparent tape. The sample was sputter-coated with carbon using a Cressington sputter-coater and viewed with a Hitachi S-4700 scanning electron microscope, at 10 kV and 9,000 nA, with a working distance of ca. 12 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of the Jagiellonian University, Kraków (Poland).

Molecular analyses

Genomic DNA was isolated from the herbarium specimens. For methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data, see Lutz et al. (2004). We determined base sequences of the ITS1/2 region of the nuc-rDNA including the 5.8 S rDNA (ITS) and of the 5’-end of the nuc-LSU rDNA including the domains D1/D2 (LSU). The ITS was amplified using the primer pair ITS1f and ITS4 (Gardes and Bruns 1993). The LSU was amplified using the primer pair NL1 and NL4 (O’Donnell 1992, 1993). For amplification of both regions, we adjusted the annealing temperature to 45°C. DNA sequences prepared in the course of this study were deposited in GenBank; accession numbers are given in Table 1, and Figs. 1 and 2.

To obtain a hypothesis on the phylogenetic position of the specimen infecting Vaccinium reticulatum, we followed two strategies: (1) we analysed its LSU sequence together with sequences of most Exobasidium species available in GenBank, including sequences of all available species growing on Vaccinium L. and all available species producing witches’ brooms, respectively, and sequences of selected representatives of other genera of Exobasidiales (GenBank accession numbers are given in Fig. 1), and (2) we analysed the concatenated ITS+LSU sequences of the specimen from Hawaii within a dataset reduced to the
Table 1 List of species used in the molecular analyses with host plants, country of origin, voucher/strain information, GenBank accession numbers, and sources

| Species                     | Host plant, country                  | Voucher/strain                                      | GenBank acc. no. (ITS/LSU) | Source                           |
|-----------------------------|--------------------------------------|-----------------------------------------------------|----------------------------|----------------------------------|
| Arcticomyces warmingii      | Saxifraga bryoides L., –             | R. Bauer 3081/–                                      | –/AF487380                 | Begerow et al. 2002             |
| (Rostr.) Savile             |                                      |                                                     |                            |                                  |
| Clinocladidium bullatum     | Phoebe neurophylla                   | M – Sydow Fungi Exot. ext. 553/–                    | –/AF487383                 | Begerow et al. 2002             |
| Syd.                        |                                      |                                                     |                            |                                  |
| Exobasidium arescens        | Vaccinium myrtillus L., Slovenia      | Slovenia, Bled, Pokljuka, Mocila, 28 Jul. 2006, leg. | FJ896135/FJ896136          | This study                       |
| Nannf.                      |                                      | M. Lutz, TUB 015031                                 |                            |                                  |
| Exobasidium bisporum        | Eubotryoides grayana (Maxim.) H. Har | IFO H-12038/IFO 30152                               | AB180368/AB177596          | GenBank, submitted by H. Nagao  |
| Sawada ex Ezuka             | a var. oblongifolia (Miq.) H. Har,   |                                                     |                            | in 2004                          |
|                            | Japan                                |                                                     |                            |                                  |
| Exobasidium bisporum        | Eubotryoides grayana (Maxim.) H. Har | IFO H-12021/IFO 9942                                | AB180364/AB177598          | GenBank, submitted by H. Nagao  |
| Sawada ex Ezuka             | a var. glabra (Komatsu) H. Har, Japan|                                                     |                            | in 2004                          |
| Exobasidium camelliae       | Camellia japonica L., Japan          | EC (TUK)/MAFF 238578                                | –/AB176712                 | GenBank, submitted by H. Nagao  |
| Shirai                      |                                      |                                                     |                            | in 2004                          |
| Exobasidium cylindrosporum  | Rhododendron macrosepalum Maxim.,   | TSH-B0127/MAFF 238177                               | –/AB176713                 | GenBank, submitted by H. Nagao  |
| Ezuka                       | Japan                                |                                                     |                            | in 2004                          |
| Exobasidium darwinii        | Vaccinium reticulatum Sm., Hawaii    | U.S.A., Hawaii, Maui, north facing slopes of Haleakala, near Hosmers Grove, 17 Dec. 2007, leg. P. Welton, holotype: KRAM F-47124, isotype: TUB 019166 | FJ896133/FJ896134          | This study                       |
| M. Piątek & M. Lutz         |                                      |                                                     |                            |                                  |
| Exobasidium gracile         | Camellia sasanqua Thunb., Japan      | –/TUK-E30                                           | –/AB177592                 | GenBank, submitted by H. Nagao  |
| (Shirai) Syd. & P. Syd.     |                                      |                                                     |                            | in 2004                          |
| Exobasidium inconspicuum    | Vaccinium hirtum Thub. var. pubescens | TSH-B0080/MAFF 238616                               | AB180347/AB177556          | GenBank, submitted by H. Nagao  |
| Nagao & Ezuka               | (Koidz.) T. Yamaz., Japan             |                                                     |                            | in 2004                          |
| Exobasidium japonicum      | Rhododendron indicum (L.) Sweet,     | TSH-B0011/MAFF 238176                               | –/AB177548                 | GenBank, submitted by H. Nagao  |
| Shirai                      | Japan                                |                                                     |                            | in 2004                          |
| Exobasidium karstenii      | Andromeda polifolia L., –           | R. Bauer 2052/–                                      | –/AB176713                 | GenBank, submitted by H. Nagao  |
| Sacc. & Trotter             |                                      |                                                     |                            | in 2004                          |
| Exobasidium kishianum       | Vaccinium hirtum Thub. var. pubescens| TSH-B0070/MAFF 238623                               | AB180353/AB177577          | GenBank, submitted by H. Nagao  |
| Nagao & Ezuka               | (Koidz.) T. Yamaz., Japan             |                                                     |                            | in 2004                          |
| Exobasidium kishianum       | Vaccinium hirtum Thub.               | NIAES 10519/MAFF 306199                             | –/AB178241                 | GenBank, submitted by H. Nagao  |
| Nagao & Ezuka               | var. pubescens (Koidz.) T. Yamaz.,   | (as Exobasidium sawadae, see text)                   |                            | in 2004                          |
|                            | Japan                                |                                                     |                            |                                  |
| Exobasidium miyabei        | Rhododendron dauricum L., Japan      | TSH-B0075/MAFF 38594                                | –/AB177561                 | GenBank, submitted by H. Nagao  |
| Nagao, Akimoto & Kishi      |                                      |                                                     |                            | in 2004                          |
| Exobasidium myrtilli       | Vaccinium myrtillus L., –           | R. Bauer 2055/–                                      | –/AF487390                 | Begerow et al. 2002             |
| Siegm.                      |                                      |                                                     |                            |                                  |
| Exobasidium nobeyamense    | Rhododendron wadanum Makino, Japan    | TSH-B0002/MAFF 238597                               | –/AB177582                 | GenBank, submitted by H. Nagao  |
| Nagao & Ezuka               |                                      |                                                     |                            | in 2004                          |
| Exobasidium notaniense     | Rhododendron reticulatum D. Don ex   | TSH-B0061/MAFF 238613                               | –/AB177593                 | GenBank, submitted by H. Nagao  |
| Ezuka                       | G. Don f. glabrescens (Nakai & H. Har) T. Yamaz., Japan |                                                     |                            | in 2004                          |
| Exobasidium oxyococi       | Vaccinium oxyococos L., –           | R. Bauer 2086/–                                      | –/AF487391                 | Begerow et al. 2002             |
| Rostr. ex Shear             |                                      |                                                     |                            |                                  |
| Exobasidium pachysporum     | Vaccinium uliginosum L., Japan       | –/MAFF 238621                                       | AB180352/AB177573          | GenBank, submitted by H. Nagao  |
| Nannf.                      |                                      |                                                     |                            | in 2004                          |

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closest relatives according to the results of the LSU analyses (GenBank accession numbers are given in Fig. 2). The species and sequences used in phylogenetic analyses are listed in Table 1.

Sequences were aligned for both datasets with MAFFT 6.611 (Katoh et al. 2002, 2005; Katoh and Toh 2008) using the L-INS-i option. Both alignments [length: 559 bp (LSU), 1,106 bp (ITS+LSU); variable sites: 185 (LSU), 163 (ITS+LSU)] were used throughout their length. We avoided both manipulation of the alignment by hand and manual exclusion of any positions as recommended by Giribet and Wheeler (1999) and Gatesy et al. (1993), respectively.

Table 1 (continued)

| Species             | Host plant, country | Voucher/strain | GenBank acc. no. (ITS/LSU) | Source                  |
|---------------------|---------------------|----------------|-----------------------------|-------------------------|
| Exobasidium pentasporium Shirai | Rhododendron obtusum (Lindl.) Planch. var. kaempferi (Planch.) E.H. Wilson, Japan | –/MAFF 238600 | –/AB177581 | GenBank, submitted by H. Nagao in 2004 |
| Exobasidium reticulatum S. Ito & Sawada | Thea sinensis L., – | –/DSM 4520 | –/AF487394 | Begerow et al. 2002 |
| Exobasidium rhododendri (Fuckel) C.E. Cramer | Rhododendron ferrugineum L., – Vaccinium oxyccocos L., Poland | Poland, Kotlina Sandomierska basin, Stary Jawornik, near Tarnów, 11 Jul. 2006, leg. J. Piątek & M. Piątek, KRAM F-47454, TUB 019165 | FJ896132// FJ896137 | This study |
| Exobasidium vaccinii (Fuckel) Woronin | Rhododendron degronianum Carrière, Japan | TSH-B0023/MAFF 238602 | –/AB177549 | GenBank, submitted by H. Nagao in 2004 |
| Exobasidium sundstroemii Nannf. | Andromeda polifolia L., – | R. Bauer 2051/– | –/AF487396 | Begerow et al. 2002 |
| Exobasidium symloci-japonicae | Symplocos lucida (Thunb.) Siebold & Zucc., Japan | NIAES 20521/MAFF 238811 | –/AB178255 | GenBank, submitted by H. Nagao in 2004 |
| Exobasidium vaccinii (Fuckel) Woronin | Vaccinium vitis-idea L., – | R. Bauer 945/– | –/AF009858 | Begerow et al. 1998 |
| Exobasidium vaccinii (Fuckel) Woronin | Vaccinium vitis-idea L., Japan | TSH-B0120/MAFF 238668 | AB180362/AB177560 | GenBank, submitted by H. Nagao in 2004 |
| Exobasidium woronichinii Nagao | Rhododendron brachycarpum D. Don ex G. Don, Japan | TSH-B0081/MAFF 238617 | –/AB177557 | GenBank, submitted by H. Nagao in 2004 |
| Graphiola phoenicis (Moug. ex Fr.) Poit. | Phoenix canariensis (Seub.) Franco, – Tradescantia L. sp., – | F. Oberwinkler 29350/– | –/AF009862 | Begerow et al. 1998 |
| Kordyana tradescantiae (Pat.) Racib. | Laurus azorica (Geyl.) Jülich, – | M. Piepenbring 2371/– | –/AF478403 | Begerow et al. 2002 |
| Laurobasidium lauri (Moug. ex Fr.) Port. | Rhus myosdensis (G. Don (published as Rhus lancea L.f.)), – | F. Oberwinkler 47397/– | –/AF352058 | Begerow et al. 2001 |
| Muribasidiospora indica Kram & Rajendren | Vaccinium L. sp., Japan | –/MAFF 410168 | –/AB178262 | GenBank, submitted by H. Nagao in 2004 |
| Tilletiopsis pallescens Gokhale | Rhododendron obtusum (Lindl.) Planch. var. kaempferi (Planch.) E.H. Wilson, Japan | –/MAFF 410169 | –/AB178263 | GenBank, submitted by H. Nagao in 2004 |
For phylogenetic analyses, we used both neighbour-joining analysis and a Bayesian approach. For neighbour-joining analysis, the data were first analysed with Modeltest 3.7 (Posada and Crandall 1998) to find the most appropriate model of DNA substitution. For the LSU dataset, the hierarchical likelihood ratio test proposed the SYM+I+G DNA substitution model, while for the ITS+LSU dataset, the GTR+I+G DNA substitution model was proposed. Bootstrap values were calculated for 1,000 replicates. For Bayesian analysis, we used for both datasets a Bayesian approach of phylogenetic inference using a Markov chain Monte Carlo (MCMC) technique as implemented in the computer program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). In each run, four incrementally heated simultaneous Markov chains were run over 2,000,000 generations using the general time reversible model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 70% majority-rule consensus tree computed from 18,000 trees that were sampled after the process had reached stationarity is shown. The topology was rooted with *Tilletiopsis pallescens* (AB178241, AB178262, AB178263). Numbers on branches before slashes are estimates for a posteriori probabilities, numbers on branches after slashes are percentage bootstrap values of 1,000 replicates. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. E. = *Exobasidium*, T. = *Tilletiopsis*

**Fig. 1** Bayesian inference of phylogenetic relationships within the sampled Exobasidiales: Markov chain Monte Carlo analysis of an alignment of LSU base sequences using the GTR+I+G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 70% majority-rule consensus tree computed from 18,000 trees that were sampled after the process had reached stationarity is shown. The topology was rooted with *Tilletiopsis pallescens* (AB178241, AB178262, AB178263). Numbers on branches before slashes are estimates for a posteriori probabilities, numbers on branches after slashes are percentage bootstrap values of 1,000 replicates. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. E. = *Exobasidium*, T. = *Tilletiopsis*
the ITS+LSU analyses were rooted with *Exobasidium kishianum* Nagao & Ezuka (AB180353/AB177577) and *E. vaccinii* (AB180362/AB177560).

Clarification of species name in GenBank sequences attributed to *Exobasidium sawadae*

GenBank includes five sequences (AB180353, AB178241, AB177577, AB177555, AB180354) stored under the name “*Exobasidium sawadae* Nagao et Ezuka”. This is an apparently provisional name, which was never published by the authors because it would cause the creation of later homonym. The name *Exobasidium sawadae* G. Yamada 1919 is already available for a completely different species. Recently, the fungus included in GenBank as “*Exobasidium sawadae* Nagao et Ezuka” was described by Nagao et al. (2006) under the name *Exobasidium kishianum*. Thus, we used this latter name for these sequences, however, with one exception: our molecular analyses clearly showed that “*Exobasidium sawadae*” AB178241 in fact represents *Tilletiopsis pallescens* (see results and discussion).

**Results**

Molecular phylogenetic analyses

For both the LSU and the concatenated ITS+LSU dataset, all runs of Bayesian phylogenetic analyses that were performed yielded consistent topologies which were congruent to the results of the neighbour-joining analysis in respect to well-supported branchings (*a posteriori* probability greater than 60%, bootstrap support greater than 73%). For both datasets, we present the consensus tree of one run of Bayesian phylogenetic analyses to illustrate the results (Figs. 1, 2). Estimates for *a posteriori* probabilities are indicated on branches before slashes, bootstrap values from the neighbour-joining analysis are indicated after slashes.

The analyses of the LSU dataset placed the *Exobasidium* specimen infecting *Vaccinium reticulatum* from Hawaii as a sister taxon of *E. inconspicuum* Nagao & Ezuka within a well-supported group of four more *Exobasidium* species (*E. arescens*, *E. bisporum* Sawada ex Ezuka, *E. pachysporum* Nannf., *E. rostrupii*). Within that group, only the monophyly of *E. rostrupii* and *E. arescens* received considerable support values. The relationship between that group of species and most of the other *Exobasidium* species sampled remained unresolved using LSU sequences. Moreover, the relation between the sampled *Exobasidium* species and *Muribasidiospora indica* Kamat & Rajendren, *Arcticomyces warmingii* (Rostr.) Savile (both belonging to the *Exobasidiaceae*), *Kordyana tradescantiae* (Pat.) Racib. (Brachybasidiaceae), and *Graphiola phoenicis* (Moug. ex Fr.) Poit. (Graphiolaceae) was not resolved. However, the cryptobasidiaceous species *Clinoconidium bullatum* Syd. and *Laurobasidium lauri* (Geyl.) Jülich were revealed as sister taxon to all other Exobasidiales sampled. The sequence “*Exobasidium kishianum* AB178241” clustered together with the *Tilletiopsis pallescens* sequences AB178262 and AB178263 (Fig. 1). A blast search (Altschul et al. 1997) resulted in 100% identity of the sequence “*Exobasidium kishianum* AB178241” with the sequences of *Tilletiopsis pallescens* AB178263, AB178262, AJ235329, AJ235293, AJ235292, AJ235291, and of *T. albescens* Gokhale AJ235290.

In contrast to the analyses of the LSU dataset, those of the ITS+LSU dataset resolved the relationships within the cluster of six species mentioned above with good to moderate support. The *Exobasidium* specimen infecting...
Vaccinium reticulatum from Hawaii clustered as moderately supported sister taxon of E. bisporum in a well supported group together with E. pachysporum (Fig. 2).

Taxonomy

Exobasidium darwinii M. Piątek & M. Lutz, sp. nov. (Figs. 3 and 4)

MycoBank no. MB 518226

Fig. 3 Exobasidium darwinii sp. nov. on Vaccinium reticulatum (all from holotype: KRAM F-47124): a infected plants in natural habitat, b infected branches of the host plant with uniformly bright red, not hypertrophied and not enlarged leaves, c witches’ broom, d basidium emerging through stomatum, as seen by SEM, e basidium with four sterigmata, as seen by SEM, f–h basidia, as seen by LM, i–o variability of basidiospores, note the septa visible on j and m, as seen by LM, p–s bacilliform conidia, as seen by LM. Bars 10 μm (d, f–s), 5 μm (e)

Etymology Named after Charles Darwin (1809–1882), whose observations, among others on the oceanic Galapagos Islands, resulted in formulating the evolutionary theory.

Parasitus in Vaccinium reticulatum. Infectio systemica. Fungus in planta hospitali per proliferationem continuam parvorum novorum ramulorum conspicuas scopas strigae facit; rami infecti saepe modice deformati, contorti cum intumescentiis associatis. Folia infecta aequaliter pallide rubra ex utraque parte, non hypertrophica et non dilatata, prae foliis validis,
Parasitic on Vaccinium reticulatum hyaline, cylindrical, 20–30 μm longa, apice rotundata et 3–4 stergimatibus terminata. Sterigmata 2.2–5.5 μm longa et 1.4–1.9 μm in basi lata, ad apicem attenuata. Basidiospora hyalinae, cylindricae, subcylindricae, musaiformes, saepe in uno apice curvatae, plerumque versus hilum attenuatae 11.5–18.5 × 2.5–3.8 μm, 0–3-septatae, plerumque cum paucis guttulis oleosis, paries glaber. Conidia hyalina, bacilliformia, 5–10 × 1.5–2.5 μm, non-septata, paries glaber. Sequitiae acidi nucleici ITS/LSU typi in collectione sequentiarum acidi nucleici NCBI (GenBank) ut FJ896133/FJ896134 depositae sunt.

**Type** On Vaccinium reticulatum Sm., U.S.A., Hawaii, Maui, north facing slopes of Haleakala, near Hosmers Grove, elev. ca. 6,800 ft. (= ca. 2,072 m.a.s.l.), 17 Dec. 2007, leg. P. Welton (holotype: KRAM F-47124, isotype: TUB 019166).

Parasitic on Vaccinium reticulatum. Infection systemic. Fungus produces conspicuous witches’ brooms on the host plant through continuing proliferation of small young branches; infected twigs often somewhat deformed, contorted and accompanied with swellings. Infected leaves uniformly bright red on both sides, not hypertrophied and not enlarged in comparison with healthy leaves; hymenium hypophyllous, quite delicate, composed of basidia, basidiospores and conidia. Basidia emerging singly or in fascicles through stomata or directly from disintegrated epidermis, hyaline, cylindrical, 20–30 μm longa, 3.9–5.6 μm wide, apicis rotundata et 3–4 stergimatibus terminata. Sterigmata 2.2–5.5 μm longa et 1.4–1.9 μm in basi lata, ad apicem attenuatae. Basidiospora hyalina, cylindrica, subcylindrica, musaiforme, saepe in uno apice curvatae, plerumque versus hilum attenuatae 11.5–18.5 × 2.5–3.8 μm, 0–3-septatae, plerumque cum paucis guttulis oleosis, paries glaber. Conidia hyalina, bacilliformia, 5–10 × 1.5–2.5 μm, non-septata, paries glaber. The ITS/LSU hologenotype sequences are deposited in GenBank as FJ896133/FJ896134, respectively.

Distribution Vaccinium reticulatum is an endemic species of the Hawaiian Archipelago occurring most frequently on Maui and Hawai‘i, and rarely on Kaua‘i, O‘ahu and Moloka‘i at elevations from ca. 600–3,650 m.a.s.l. (Wagner et al. 1990). The type specimen of Exobasidium darwinii was collected near Hosmers Grove in Haleakala National Park on the Island of Maui. Gardner (1985) on the basis of aerial observations concluded that Exobasidium on Vaccinium reticulatum is common throughout extensive remote areas of the Park. However, it is not excluded that his observations may at least partly refer to the reddish coloration of juvenile leaves. It is desirable that future studies look for the exact distribution of Exobasidium darwinii on the Hawaiian Islands in order to establish an effective conservation management of this unique species.

**Discussion**

The morphological observations and phylogenetic analyses in the present study show that Exobasidium on Vaccinium reticulatum in the Hawaiian Archipelago belongs to an undescribed species, distinct from Exobasidium vaccinii and any other species. Thus, the name Exobasidium darwinii is proposed for this novel taxon.

Selection of Exobasidium sequences for molecular analyses

GenBank offers quite a lot of sequences of Exobasidium species generated by various authors, including a considerable number of sequences submitted by H. Nagao in 2004. Many Exobasidium sequences in GenBank were apparently taken from cultures linked with permanently preserved corresponding voucher specimens, others were taken directly from voucher specimens and still others from cultures not linked with herbarium specimens. When constructing the phylogenetic trees, we (1) primarily used sequences taken from Exobasidium species on their type hosts (with one exception—in the case of E. bisporum, the sequence from the type host was not available), (2) obtained from specimens in planta (preferably) or cultures linked with corresponding voucher specimens, and (3) avoided (with some exceptions, when other sequences were not available) using sequences taken from cultures not linked with any voucher specimen. The reason for the two latter choices lies in the fact that the macroscopic appearance of symptoms on the host plant is essential for delimiting the species in Exobasidium, while absence of voucher specimens prevents future verification of species identification. It is especially important because the species concept in Exobasidium was and still is not fully understood in many studies. We encourage to employ a similar approach in future molecular studies on the genus

![Fig. 4 Basidia and basidiospores of Exobasidium darwinii (from holotype: KRAM F-47124). Note the orientation of the basidiospores on the basidia. Bar 10 μm](image-url)
Exobasidium. This is in line with similar observation of Lutz et al. (2011) on urocystidalean species.

Not unimportantly, cultures taken from Exobasidium specimens can be contaminated by co-occurring Tilletiopsis Derx species, and, as Begerow et al. (2000) pointed out, it is difficult to obtain a pure culture of Exobasidium. In fact, Boekhout et al. (1995) found that the culture “Exobasidium vexans CBS 24752” (= sequence AJ235288) represents a Tilletiopsis species. Our analyses indicate that the sequence AB178241 of “Exobasidium kishianum MAFF 306199” was also taken from contaminated material and represents Tilletiopsis pallescens. Interestingly, according to GenBank data, the culture MAFF 306199 was obtained from voucher NIAES 10519, which is the holotype of Exobasidium kishianum (Nagao et al. 2006), included in GenBank under the provisional name “Exobasidium sawadae Nagao et Ezuka” (which is not E. sawadae G. Yamada 1919). Thus, the sequence “Exobasidium kishianum AB178241” should not be used in future phylogenetic studies on the genus Exobasidium, or considered as type sequence of Exobasidium kishianum.

The symptoms and infection caused by Exobasidium darwinii

On host plants, Exobasidium species can cause three different kinds of symptoms, depending on the manner of infection: circumscribed, surculicolous and systemic (Nannfeldt 1981). The infection may be monocarpic (annual, ephemeral) when the pathogen completely dies after sporulation, or polycarpic (perennial) when the pathogen survives in infected plants in the form of intercellular mycelium (Nannfeldt 1981). The circumscribed infection is a monocarpic, strictly localized infection forming leaf spots, leaf concavity galls or gall apples entirely surrounded by healthy tissues of the host plant. The surculicolous infection is also monocarpic, but in that case whole annual shoots are penetrated by the pathogen as in the case of the true systemic infection. In contrast to these two monocarpic symptom types, the systemic infection is polycarpic, and whole plants, single shoots or shoot complexes are invaded, depending on the Exobasidium species (Nannfeldt 1981). The main difference between systemic and surculicolous infections lies in the fact that in the systemic infection the mycelium of Exobasidium species survives in the host plant tissues during the winter season, while in the surculicolous infection it does not. This distinction, however, is often difficult to delineate, and even Nannfeldt (1981), who introduced these terms, pointed out that there may be transitional forms in some Exobasidium species. In the light of these data, the symptoms caused by Exobasidium darwinii could be assigned to the polycarpic, systemic infection because the pathogen surely penetrates whole shoot complexes of Vaccinium reticulatum leading to formation of witches’ brooms within which mycelium apparently survives through the years. The infection is, however, probably limited to certain areas of the host plant since witches’ brooms are formed only locally on certain branches while other branches are healthy.

Comparison of Exobasidium darwinii with other Exobasidium species on Vaccinium and other Exobasidium species causing witches’ brooms

Besides Rhododendron L., the members of the genus Vaccinium harbour the largest number of Exobasidium species. Together with Exobasidium darwinii, there are 22 species and one variety described on different Vaccinium species (Gómez and Kisimova-Horovitz 1998a, b; Hotson 1927; Nagao et al. 2006; Nannfeldt 1981; Nickerson 1984; Piepenbring et al. 2010; Raciborski 1909; Ramakrishnan and Ramakrishnan 1949; Savile 1959). Of them, 8 species and 1 variety are clearly circumscribed taxa, while the remaining 14 species are surculicolous or systemic. Exobasidium darwinii belongs to the latter group, having systemic and polycarpic infections. None of the hitherto described species is morphologically similar to E. darwinii because none of them produces distinct witches’ brooms. Exobasidium parvifolii Hotson on Vaccinium parvifolium Sm. and V. ovalifolium Sm. in North America forms perennial galls on the stems from which numerous cylindrical, clavarioid excrescences emerge. Nannfeldt (1981) interpreted these structures as highly disorganized shoots, which, however, cannot be confused with witches’ brooms of Exobasidium darwinii.

The unusual morphological aspect of Exobasidium darwinii is the production of witches’ brooms on the infected host plant (Fig. 3c). Witches’ brooms are produced by phytopathogenic fungi from different, unrelated systematic groups, but were rarely observed in Exobasidium species. To the best of our knowledge, the witches’ brooms were observed only in four species: Exobasidium uva-ursi (Maire) Juel on Arctostaphylos uva-ursi (L.) Spreng. in Europe and North America, E. dieterigimata L.D. Gómez & Kisim.-Hor. on Disterigma humboldtii (KL.) Nied. in Costa Rica, E. nobeyamense Nagao & Ezuka on Rhododendron wadanum Makino in Japan, and E. pentasporium Shirai on Rhododendron obtusum (Lindl.) Planch. var. kaempferi (Planch.) E.H. Wilson (type host) and R. macrosepalum Maxim. in Japan. Additionally, Nannfeldt (1981) mentioned another, presumingly undescribed, Exobasidium species on North American Rhododendron macrophyllum D. Don ex G. Don, but this species is not sufficiently known.

All these species are different from Exobasidium darwinii, not only because of other host plant genera but
also by metric characters of basidiospores or conidia. The basidiospores are 15–22×5–6 μm in *E. uvae-ursi* (Nannfeldt 1981), 12–21×2–5.5 μm in *E. nobeyamense* (Nagao et al. 2001) and ca. 14.4×4 μm in *E. pentasporium* (Nannfeldt 1981). Basidiospores were not observed in *E. disterigmacola*. However, its conidia (12–15.5×2.8–3.2 μm; Gómez and Kisimova-Horovitz 1998b) are much larger compared to those of *E. darwinii*. In many groups of *Exobasidium*, basidiospore size shows only a small variance between species. Therefore, these differences distinguish these species well. Additionally, in the phylogenetic analyses, *E. nobeyamense* and *E. pentasporium* are placed in the clade comprising species infecting members of *Rhododendron*, well separated from the clade including *E. darwinii*. The sequences of *E. uvae-ursi* and *E. disterigmacola* are still not available, and thus their phylogenetic position cannot be resolved. However, the morphological differences do not support their conspecificity with *E. darwinii*.

**Hypothesis on the origin of *Exobasidium darwinii***

Three Hawaiian *Vaccinium* species: *V. calycinum*, *V. dentatum* Sm., *V. reticulatum* and the Polynesian *V. crenatum* (L.f.) G. Forst. were included in *Vaccinium* sect. Macropelma (Klotzsch) Hook.f. (Vander Kloet 1993, 1996). Phylogenetic molecular analyses by Powell and Kron (2002) revealed that the Hawaiian *Vaccinium* species indeed form a monophyletic group that is nested within members of *Vaccinium* sect. *Myrtillus* Dumort. Powell and Kron (2002) suggested a boreal-arctic origin of the species instead of a southern Pacific origin and proposed that Hawaiian blueberries should be transferred to sect. *Myrtillus*. This view was recently accepted by Vander Kloet and Dickinson (2009).

Of the three Hawaiian *Vaccinium* species, no *Exobasidium* has so far been observed on *V. dentatum*, while the specimen on *V. calycinum* was not available for analysis. In the present study, *Exobasidium darwinii* on *V. reticulatum* is a member of a well-supported clade (LSU analysis, Fig. 1; ITS+LSU analysis, Fig. 2) containing *Exobasidium* species on different *Vaccinium* species as well as on *Eubotryoides grayana* (Maxim.) H. Hara var. *glabra* (Komatsu) H. Hara and var. *oblungifolia* (Miq.) H. Hara. The specimens used in the analyses come from Japan, Poland and Slovenia. What is interesting to note is that, except the evidently systemic *Exobasidium darwinii*, all the remaining taxa from this clade are circumscribed, monocarpic species, all producing merely leaf spots and each year renewing infection from apparently hibernated basidiospores or conidia. Thus, it is likely that the ancestral species of *E. darwinii* could have been a circumscribed species that changed its life-strategy during evolution. The geographical origin of this ancestral species is, however, difficult to indicate as the sequences of many species of *Exobasidium* on North American, South American and South-east Asian Ericaceae are still not available. It is evident that more intensive sampling and the analysis of *Exobasidium* species from these geographical regions are necessary to determine where and on which host plants the closest relatives of *Exobasidium darwinii* actually occur.

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