Histological description of *Exobasidium vexans* infection on tea leaves (*Camellia sinensis*)

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<th>Journal:</th>
<th><em>Songklanakarin Journal of Science and Technology</em></th>
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<td>Manuscript ID</td>
<td>SJST-2017-0350.R2</td>
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<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
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<td>Date Submitted by the Author:</td>
<td>26-May-2018</td>
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<td>Complete List of Authors:</td>
<td>MOHKTAR, NORSYUHADA; SCHOOL OF BIOLOGICAL SCIENCES; Nagao, Hideyuki; Universiti Sains Malaysia, School of Biological Sciences</td>
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<td>Keyword:</td>
<td>leaf gall, cell differentiation, mesophyll, phloem, disease severity</td>
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Original Article

Histological description of *Exobasidium vexans* infection on tea leaves

(*Camellia sinensis*)

Norsyuhada Mohktar\(^1\), Hideyuki Nagao\(^1\)

\(^1\)School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, 11800, Malaysia

*Corresponding author, Email address: norsyuhada.mohktar@gmail.com*
Abstract

Exobasidium vexans Massee is a parasitic fungus that causes tea blister blight. Tea blister blight is characterised by the swelling of the infected spots on the tea leaves. To date, there is limited information on the cellular alterations that occur during E. vexans infection. The aim of this study was to observe the histological changes on tea leaves infected by E. vexans. Diseased and healthy leaves were thin-sliced by cryostat microtome and examined by light microscopy. Observations on the thin sections revealed the mode of hymenium development in E. vexans in which the basidia protruded between the epidermal cells. The infection caused hypertrophy as the size of the cells at the infected spots became double the size of the healthy cells. The first description of venous infections of E. vexans were also described. The proliferation of the fungus on the vein resulted in the formation of hymenium on both lower and upper sides of the leaves and there was complete disruption of the vascular bundle in the leaves vein. Blister blight disease on the tea plantation in this study was found to be moderately severe. Morphological and molecular identification confirmed that the fungus isolated from the symptomatic leaves was E. vexans.

Keywords: Disease severity, leaf galls, cell differentiation, mesophyll, phloem, plant parasite

1. Introduction

Tea has been one of the most consumed drinks in the world. The drink originates from the leaves of tea plants (Camellia sinensis (L.) Kuntze) that are processed and dried for brewing. Exobasidium vexans Massee is a pathogenic plant fungus that infects tea plants and causes tea blister blight. The spread of this disease has brought tremendous loss to tea production industry and it has become a serious threat to all tea plantations across Asia including India, Sri Lanka,
Japan and Indonesia (Baby, 2002). As the number one tea producer to the global market, India has about 5 million hectares of land dedicated to tea plantation (Boriah, 2002). The country which had contributed up to 23% of total world tea production suffered a great loss due to tea blister blight as the pathogen attacks harvestable young shoots resulting in up to 40% yield losses (Gulati, Gulati, Ravindranath, & Chakrabarty, 1993; Basu, Bera, & Rajan, 2010). In addition, tea blister blight has inflicted a severe crop loss in Sri Lanka in which 33% of yield reduction was recorded in unprotected areas compared to fields which were sprayed with chemicals (de Silva, Murugiah, & Saravapavan, 1997). Transgenic tea plant that is resistant to *E. vexans* infection has been developed in which the clone expresses higher amount of chitinase enzyme to hydrolyse the cell wall of the invading fungus (Jeyaramraja, Pius, Manian, & Nithya Meenakshi, 2005; Jayaswall, Mahajan, Singh, Parmar, Seth *et al.*, 2016).

Symptoms of *E. vexans* infection include the swelling and formation of conspicuous white hymenium on the infected spots that will eventually necrotise. Several studies have been done on the histology of plants infected by other *Exobasidium* species. According to Mims and Richardson (2007) and Lee, Lee, Shin, Park, and Sieber (2015), the infection of *Exobasidium gracile* (Shirai) Syd, and P. Syd on *Camellia sasanqua* Thunb. was characterised by the formation of a distinct pseudoparenchymatous layer of intercellular hyphae which initially developed several layers above the lower epidermis. Eventually the lower epidermis layer became sloughed off to give rise to the white hymenium on the underside of the leaf (Mims & Richardson, 2007; Lee *et al.*, 2015). Meanwhile, Li and Guo (2008) described a different mode of hymenium development in the infection caused by *Exobasidium ovalifoliae* Z. Y. Li and L. Guo in which the basidia protruded between epidermal cells to form continuous white hymenium upon reaching the final stage of infection. Gadd and Loos (1949) reported that the formation of
hymenium in *E. vexans* progressed in the similar manner but they only provided an illustration to support their observation.

At the cellular level, the enlargement of the infected spots caused by the infection of *Exobasidium* species is either due to hypertrophy or hyperplasia – an increase in the number of the cells. For example, Graafland (1960) reported that both hypertrophy and hyperplasia occurred in the infection of *Exobasidium japonicum* Shirai on the leaves of Azaleas. Meanwhile, the flowers of *Lyonia ferruginea* (Walter) Nutt. that were infected with *Exobasidium ferrugineae* Minnis, A.H. Kennedy & N.A. Goldberg only became hypertrophied but this observation was less common in the leaves of the plant (Kennedy, Goldberg, & Minnis, 2012).

Several methods have been developed to measure the severity of fungal diseases on tea plants. Webster and Park (1956) first evaluated the severity of *E. vexans* infection based on the disease incidence from a pool of sampled leaves. Meanwhile Balasuriya (2003) assessed blister blight infection according to the number of blistered lesion on tea leaves. Recently, Sinniah, Wasantha Kumara, Karunajeewa and Ranatunga (2016) developed an improvised assessment key to evaluate the severity of *E. vexans* infection on tea leaves that accounted for both host reactions and the sequential stages of symptom development.

To date, there have been no detailed descriptions on the histological aspects of *E. vexans* infection on *C. sinensis* except the illustration provided by Gadd and Loos (1949). Hence, the aim of this study is to provide a deeper understanding on the histological changes that occurred to *C. sinensis* leaf cells upon *E. vexans* infection. The mode of hymenium development and the effect of *E. vexans* infection on the architectural state of the leaf cells were examined. In addition, microscopic descriptions of venous infection were also presented. Disease severity of *E. vexans* infection on the tea plantation in this study was assessed and the morphology of
basidia and basidiospores of this fungus was described. Finally, a phylogenetic tree was constructed based on the internal transcribed spacer (ITS) gene to assess the position of *E. vexans* relative to other *Exobasidium* species.

2. Materials and Methods

2.1 Histology of *E. vexans* infection

Healthy and infected shoots of tea leaves were collected from MARDI Agro-technology Park, Cameron Highlands, Malaysia in August 2016 and preserved in formalin acetic acid solution. The vein and lamina of both healthy and infected spots were bounded to specimen blocks by Jung Tissue Freezing Medium® (Leica Microsystems, Nussloch, Germany). The samples were then transversely thin-sliced by cryostat microtome (-20°C) at 10µm thickness (Leica CM 1850 UV, Nussloch, Germany). The thin sections were viewed under light microscope (Olympus BX53, Tokyo, Japan) equipped with a camera (Olympus DP72, Tokyo, Japan). The cell sizes of healthy and infected leaves were measured and statistically analyzed by Mann Whitney-U Test.

2.2 Disease severity

Disease severity of *E. vexans* infection on tea plantation in MARDI Agro-technology Park, Cameron Highlands, Malaysia was assessed according to the assessment key developed by Sinniah *et al.* (2016). Thirty tea shoots showing symptoms of *E. vexans* infection were collected and the severity of infection on the third leaf of each shoot was rated according to the following assessment key. A 0 to 6 point scoring system was described according to the following conditions: (0) No translucent spots (TL) or no disease, (1) all TL show hypersensitive reaction, (2) TL cover <2% leaf area, (3) Blisters and/ or necrosis covering <5% of total leaf area, or TL...
covering 2-15% leaf area, (4) Blisters and/or necrosis covering 5-15% of total leaf area or TL covering >15% leaf area, (5) Blisters and/or necrosis covering 15-30% leaf area, and (6) Blisters and/or necrosis covering >30% leaf area or stem infections (Sinniah et al., 2016).

2.3 Morphological and molecular identification

Morphological observation of the basidia and basidiospores of *E. vexans* was done on fresh specimens according to Nagao, Akimoto, Kishi, Ezuka and Kakishima (2003a). Hymenia formed on blistered spots were scratched and mounted in Shear’s solution on a glass slide. The basidia and basidiospores were observed and measured under light microscope.

Molecular identification was done on *E. vexans* culture that was established according to the method described by Nagao *et al.* (2003a). To simplify, the infected spot on a tea leaf was cut into 2 mm² pieces and stuck onto 10 mm² agar block on the lid of a petri dish. The hymenium of the infected spot was set to face the potato dextrose agar (PDA) surface. The petri dish was incubated at 22°C in the dark, overnight to collect the released basidiospores. Then, the basidiospores were allowed to germinate and *E. vexans* colony formed after 1 month of culturing. DNA extraction was adapted from the method by Suyama *et al.* (1996). An extraction solution composed of 0.01% SDS, 50mM KCl, 1.5mM MgCl₂, 0.01% proteinase K, and 10mM Tris-HCL (pH 8.3 at 20 °C) was made. Small clumps of *E. vexans* colony were added to 20 µL of the extraction solution and incubated for 60 min at 37°C, followed by heating at 95°C for 10 min. After the incubation process, the extraction solution that contained the extracted DNA was used as the template for downstream polymerase chain reaction (PCR).

Two genes were amplified in this experiment. The large subunit (LSU) of the nuclear ribosomal RNA gene was amplified using the NL1 (5’- GCA TAT CAA TAA GCG GAG GAA
AAG -3’) and NL4 (5’- GGT CCG TGT TTC AAG ACG G-3’) primers (O’Donnell 1992, 1993). Amplification was done according to the following cycle: Initial denaturation at 94 °C for 2 min; 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 1 min and extension at 72 °C for 90 s; and a final extension at 72 °C for 10 min. Meanwhile, the ITS gene was amplified using the ITS1F (5’- CTT GGT CAT TTA GAG GTA A-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC -3’) primers according to the following cycle: Initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 1 min and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min (White, Bruns, Lee & Taylor, 1990; Gardes & Bruns, 1993; Virtudazo, Nakamura & Kakishima, 2001). The PCR mixture to amplify the two genes consisted of 20 mM of each dNTP, 1.5 mM MgCl₂, 4 µL of extracted DNA, and 0.2 µM of each ITS1F – ITS4 and NL1 – NL4 primers.

PCR products were sent to a service provider for sequencing.

Phylogenetic tree was constructed using the ITS sequence of *E. vexans* obtained from this study and other ITS sequences of *Exobasidium* species available on Genbank (Table 1). The sequences were aligned and Neighbour Joining tree was constructed using the software MEGA 7 (Kumar, Stecher, & Tamura 2016). The stability of clades was evaluated by generating 1000 bootstrap replicates and distance analyses were computed using the Maximum Composite Likelihood method. Gaps and missing data were completely deleted from the dataset.

3. Results

3.1 Histology of *E. vexans* infection on tea leaves

Leaves with mature *E. vexans* infection appeared swollen and the presence of white-velvety hymenium was observed in the lower epidermis. Meanwhile, if the infection occurred on the
For Review Only

veins, the hymenium could be observed on both lower and upper epidermis (Figure 1A and 1B). The leaf lamina that was close to the infected veins also had the presence of the hymenium in both lower and upper epidermis. Infection by *E. vexans* was normally localised and eventually caused the necrosis of the infected spots.

In the case of the hymenium developing on the underside of the leaf (Figure 1B), the structure of the lower epidermis was completely disrupted and filled with masses of slender hyphae and basidia (Figure 2B). The air cavities that were normally present in the healthy leaf were filled with networks of intercellular hyphae in the infected leaf (Figure 2B). As the infection reaching its final stage, intracellular hyphae could also be observed in the infected leaf cells. Mann-Whitney U test revealed that the size of the cells in the spongy mesophyll layer was significantly greater in the infected leaf (Mdn = 38.56 µm) than the healthy leaf (Mdn = 19.23 µm), *U* = 510, *p* <0.05. However, the structure of the upper epidermis and the palisade mesophyll of the infected leaf (Figure 2B) appeared similar to the ones in the healthy leaf (Figure 2A).

Infection on the vein of the leaf resulted in the proliferation of hymenium on both lower and upper side of the leaf (Figure 1A and 1B). In this case, both lower and upper epidermis of the leaf were completely absent and filled with exposed, slender hyphae which developed to become basidia that bore basidiospores (Figure 3A and 3B). The sclerenchyma layer in the infected vein (Figure 4B) was completely ruptured. Meanwhile, the normally organised and intact structure of the xylem and phloem became disrupted and the intercellular spaces were tightly packed with the proliferation of intercellular hyphae (Figure 4A and 4B).

### 3.2 Disease severity
Eighty percent of the sampled leaves in this study showed apparent symptoms of *E. vexans* infection which scored ≥ 3 points according to the assessment key by Sinniah *et al.* (2016). Meanwhile, 16.7% of the samples scored 6 points as the necrotic spots were observed to cover >30% of the leaf area (Table 2). The overall disease severity index (DSI) for tea plantation in this study was 2.07 according to the equation by Sinniah *et al.* (2016).

### 3.3 Morphological identification

The hymenium of *E. vexans* consisted of clavate to cylindrical basidia (46.98-86.42 µm × 4-5 µm) that generally had 2 sterigmata. Basidiospores (12.82-16.07 µm × 3.3-3.8 µm) were hyaline, aseptate and ellipsoid. Upon maturation, basidiospores can develop a single septation.

### 3.4 Molecular identification and phylogenetic tree

Amplification of the LSU and ITS genes of *E. vexans* yielded PCR products of size 556 bp and 586 bp respectively. The LSU and ITS sequences were deposited to Genbank database with accession number MG827275 and MG827276 respectively. BLAST search for the LSU sequence showed 99% similarity with several *E. vexans* sequences (AB180380.1, AB262789.1). Meanwhile the ITS sequence matched 100% (KC493154.1) and 99% (KY038487.1) with *Exobasidium reticulatum*. However, the ITS sequence obtained from this study did not match with an *E. vexans* ITS sequence that was readily available in the database (KC442794.1).

Neighbour Joining tree (NJ) in Figure 5 was constructed based on the ITS sequence analysis. The NJ tree of *Exobasidium* species was divided into four clades according to specific host groups. *E. vexans* isolated from this study belonged in the *Camellia* clade together with *E. reticulatum* S Ito & Sawada, *E. gracile* and *Exobasidium camelliae* Shirai. The separation of this clade from the other clades (*Rhododendron, Vaccinium* and *Symploci*) was supported by a high
NJ bootstrap value of 84%. Nonetheless, the ‘E. vexans KC442794.1’ sequence did not belong in the *Camellia* clade, instead it was found to be clustered with the outgroup of this NJ tree (*Tilletiopsis washingtonensis*).

### 4. Discussion

Observations on the mode of hymenium development during *E. vexans* infection on *C. sinensis* were coherent with the illustration provided by Gadd and Loos (1949) in which basidia protruded through the intercellular spaces of the lower epidermis. This finding is similar to the mode of hymenium development in the majority of other *Exobasidium* species including *E. ovalifoliae* and *Exobasidium tengchongense* Z.Y. Li and L. Guo (Li & Guo, 2008). Meanwhile, Wolf and Wolf (1952) reported that the hymenium of *E. gracile* developed differently than the rest of *Exobasidium* species including *E. vexans* in which the hymenium layer first formed beneath the lower epidermis and became exposed when the epidermal layer were sloughed off. This observation was further confirmed by Mims and Richardson (2007) and Lee *et al.* (2015).

In the case of hymenium formation only on the lower side of the leaf, the morphology of the palisade layer remained cylindrical indicating that the infection did not disrupt the cellular differentiation. Similarly, Li and Guo (2009) also reported the clear differentiation of palisade and mesophyll cells in the leaf of *C. sinensis* infected by *Exobasidium yunnanense* Z.Y. Li and L. Guo. This observation was different from the infection of *E. gracile* and *E. ovalifoliae* on *C. sasanqua* and *Lyonia ovalifolia* var. elliptica (Siebold & Zuccarini) Handel-Mazzetti respectively, in which the palisade layers in the diseased leaves were not able to differentiate properly (Mims & Richardson, 2007; Li & Guo, 2008).
In addition, the prominent hypertrophied condition primarily in the spongy mesophyll layer of *E. vexans* infected leaf was similar to the infection of several other *Exobasidium* species on their respective hosts (Gómez P & Kisimova-Horovitz, 1998; Chlebicki & Chlebická, 2007; Kennedy *et al.*, 2012). However, the effect of *Exobasidium* infection was not only limited to hypertrophy as Akai (1938) reported that the infection of *E. camelliae* on *Camellia japonica* L. resulted in both hypertrophy and hyperplasia of the leaf cells. Several researchers also reported the occurrence of both hypertrophy and hyperplasia in the infection of other *Exobasidium* species on the leaf (Graafland, 1960; Li & Guo, 2008; Lee *et al.*, 2015) and different plant organs such as flower (Kennedy *et al.*, 2012) and fruit (Nagao, Ogawa, Sato, & Kakishima, 2003b).

Gadd and Loos (1949) described that *E. vexans* could invade *C. sinensis* by forming appressoria through either lower or upper sides of the leaf but eventually, the white hymenium still proliferated on the lower side of the leaf regardless of the entry side. In contrast, the observation in this study showed that the infection of *E. vexans* could result in the formation of hymenium on both the lower and upper sides of the leaf primarily on the leaf midrib and vein. Venous infection of *E. vexans* could relatively enhance the spread of the infection to other tissues. The sclerenchyma and phloem components of the vascular bundle in the infected vein were completely collapsed and filled with intracellular hyphae which were similar to the histological appearance of strawberry petiole infected with *Colletotrichum fragariae* (Milholland, 1982). Meanwhile, the cellular structure of the xylem became disorganised and the intercellular spaces were filled with *E. vexans* hyphae. Based on the descriptions of the different symptoms of *Exobasidium* infection provided by Nannfeldt (1981), *E. vexans* infection could be categorised as circumscribed and monocarpic in which the fungus usually shows localised infection spots and dies after sporulation.
Assessment of the disease severity on the tea plantation in this study showed that *E. vexans* infection almost consistently ends with the necrosis of the infected spots or stems as shown by the high percentage of scores ≥ 3. Some of the resistant tea strains infected by *E. vexans* would show hypersensitive reactions or smaller blisters with less sporulation (Sinniah et al., 2016). Histological observation in this study also supported this result as *E. vexans* infection on tea leaves led to the formation of hymenium to allow for sporulation and eventually, the symptomatic spots would necrotise. Susceptibility of the specific tea strain towards blister blight infection plays an important role in the scoring of the disease severity. The overall DSI score for tea plantation in this study was 2.07, in which it could be considered as moderately resistant as compared to a highly resistant tea strain that was reported to have a DSI score of 0.6 (Sinniah et al., 2016). However, it is crucial to note that the weather condition during the sampling period also plays a huge role in the disease development, thus the overall DSI score might increase as the weather becomes more favorable for *E. vexans* proliferation. For example, the monsoon season between October to March in Malaysia could worsen the disease as a combination of high relative humidity and less sunshine will result in severe blister blight infection (Homburg, 1955; Reitsma & Van Emden, 1950).

Extensive studies on the phylogenetics of *Exobasidium* species have been done by using different genes including ITS and LSU (Blanz & Doring, 1995; Begerow, Bauer & Oberwinkler, 2002; Piatek, Lutz & Welton, 2012). Nonetheless, *E. vexans* has never been included in any of the analyses involving ITS gene as its ITS sequence is not available in Genbank except for KC442794.1. Based on the ITS sequence obtained in this current study, *E. vexans* is clustered with other *Exobasidium* species that are parasitic to their respective hosts belonging in the *Camellia* clade. This result is coherent with the analysis by Blanz and Doring (1995) in which
Exobasidium species that are highly host specific co-speciate with their respective hosts. E. vexans was found to be in the same sub-clade with E. reticulatum as these fungi are the pathogens of tea plants. Nonetheless, E. reticulatum infection on tea plant causes tea net blight; a disease with distinctive symptoms than tea blister blight. The surface of the infected tea leaves appeared to be covered by networks of hymenium, but the infected spots are not swollen which is the prominent characteristics of E. vexans infection instead (Chen and Chen, 1982). In addition, the symptoms of tea net blight by E. reticulatum has never been observed in tea plantations in Malaysia. Only ITS gene was used to construct the Neighbour Joining tree in this study as the LSU sequence was found to not be able to resolve the phylogenetic relationship of Exobasidium species as reported by Piatek et al. (2012).

Interestingly, the E. vexans ITS sequence KC442794.1 that is available in the Genbank database was clustered with the outgroup of the Neighbour Joining tree (T. washingtonensis). Further analysis of this sequence revealed that it was 99% similar to Tilletiopsis pallascens with 100% query coverage. Hence, it is important to point out that the KC442794.1 sequence could be contaminated by co-occurring Tilletiopsis species that are common plant endophytes. The contamination of Tilletiopsis is common when a voucher specimen is used in the DNA extraction of Exobasidium species as reported by several other researchers (Begerow et al., 2002; Boekhout, 1995; Peatek et al., 2012). Thus, this current study eliminated the possibility of contamination by isolating the basidiospore and culturing E. vexans on artificial media though the culturing of Exobasidium species was known to be difficult (Begerow et al., 2002). In addition, the sequencing of two genes (LSU and ITS) and thorough description of infection histology further confirmed that the fungus isolated in this study was indeed E. vexans.
In conclusion, the findings from this study confirmed and expanded the observation provided by Gadd and Loos (1949). Several new observations were made including the occurrence of venous infection of *E. vexans* on *C. sinensis*. Phylogenetic analysis of the ITS gene revealed that *E. vexans* belonged in the same clade with other *Exobasidium* species whose host plants are in the genus *Camellia*. For future works, it would be interesting to study whether the infection on vascular bundles could affect the infection of *E. vexans* to other parts of the plant as the infection on the petioles and internodes of *C. sinensis* shoots were common.

**Acknowledgement**

Authors would like to thank the USM 2017/2018 Fellowship Scheme for the support provided throughout the course of the study.

**References**


Figure 1. *vexans* infection on *C. sinensis* leaf. **A.** Upper side of the leaf with white and velvety hymenium (arrow) formed on the vein. **B.** Lower side of the same leaf in (A) with hymenium (arrow) formed on both of the vein and the leaf lamina. Bar = 5 mm.
Figure 2. Transverse section of healthy and infected leaf of *C. sinensis*. A. Healthy leaf with normally differentiated palisade (PM) and spongy mesophyll (SM) layer. Lower epidermis (LE) and air cavities are present and intact. Bar = 20 µm. B. Infected leaf with normally differentiated palisade mesophyll but hypertrophied spongy mesophyll cells that were double the size of healthy spongy mesophyll. Lower epidermis were absent and basidia were protruding between the cells (arrowhead). Bar = 50 µm.

Figure 3. Transverse section of healthy and infected vein of *C. sinensis*. A. Healthy vein with intact and normally organized sclerenchyma (S), xylem (X) and phloem (P). B. Infected vein with hymenium (arrowhead) proliferation on upper epidermis (UE) and lower
epidermis (LE). The structure of sclerenchyma, xylem and phloem were disrupted. Spongy mesophyll (SM), palisade mesophyll (PM). Bar = 100 µm.

Figure 4. Enlarged images of healthy and infected vascular bundle of *C. sinensis* in Figure 3. A. Intact and normally organized sclerenchyma (S), xylem (X) and phloem (P). B. Infected vascular bundle with collapsed sclerenchyma (S) and phloem (P) with intercellular hyphae (arrow) present in the xylem (X). Bar = 20 µm.
Figure 5. Neighbour Joining tree of *E. vexans* relative to other *Exobasidium* species constructed based on the ITS sequences with *Tilletiopsis washingtonensis* as the outgroup. Values shown at each node are obtained from 1000 bootstrap replicates, meanwhile the evolutionary distances were computed using the Maximum Composite Likelihood method. The sum of branch length is 1.0190 and a total of 420 nucleotides were used in the final dataset. The letters represent the clustering of *Exobasidium* species according to the host group. R = *Rhododendron*, V = *Vaccinium*, S = *Symploci*, C = *Camellia*
Table 1. List of *Exobasidium* species used to construct the phylogenetic tree with their respective hosts, isolate/strain, locations, Genbank accession numbers and sources

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<td><em>D. Don ex G. Don</em></td>
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<td>Tilletiopsis washingtonensis Nyland</td>
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<td>contaminant from an Exobasidium infection on Vaccinium uliginosum L.</td>
<td>Germany</td>
<td>Genbank, submitted by H. Doering in 2005</td>
<td>DQ025483.1</td>
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Table 2. Number of symptomatic host leaves as rated according to the blister blight severity assessment key by Sinniah et al. (2016). TL = Translucent spots

<table>
<thead>
<tr>
<th>Disease severity score</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (No TL or no disease)</td>
<td>1</td>
</tr>
<tr>
<td>1 (all TL show hypersensitive reaction)</td>
<td>2</td>
</tr>
<tr>
<td>2 (TL cover &lt;2% leaf area)</td>
<td>4</td>
</tr>
<tr>
<td>3 (Blisters and/ or necrosis covering &lt;5% of total leaf area, or TL covering 2-15% leaf area)</td>
<td>7</td>
</tr>
<tr>
<td>4 (Blisters and/ or necrosis covering 5-15% of total leaf area or TL covering &gt;15% leaf area)</td>
<td>4</td>
</tr>
<tr>
<td>5 (Blisters and/ or necrosis covering 15-30% leaf area)</td>
<td>7</td>
</tr>
<tr>
<td>6 (Blisters and/ or necrosis covering &gt;30% leaf area or stem infections)</td>
<td>5</td>
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