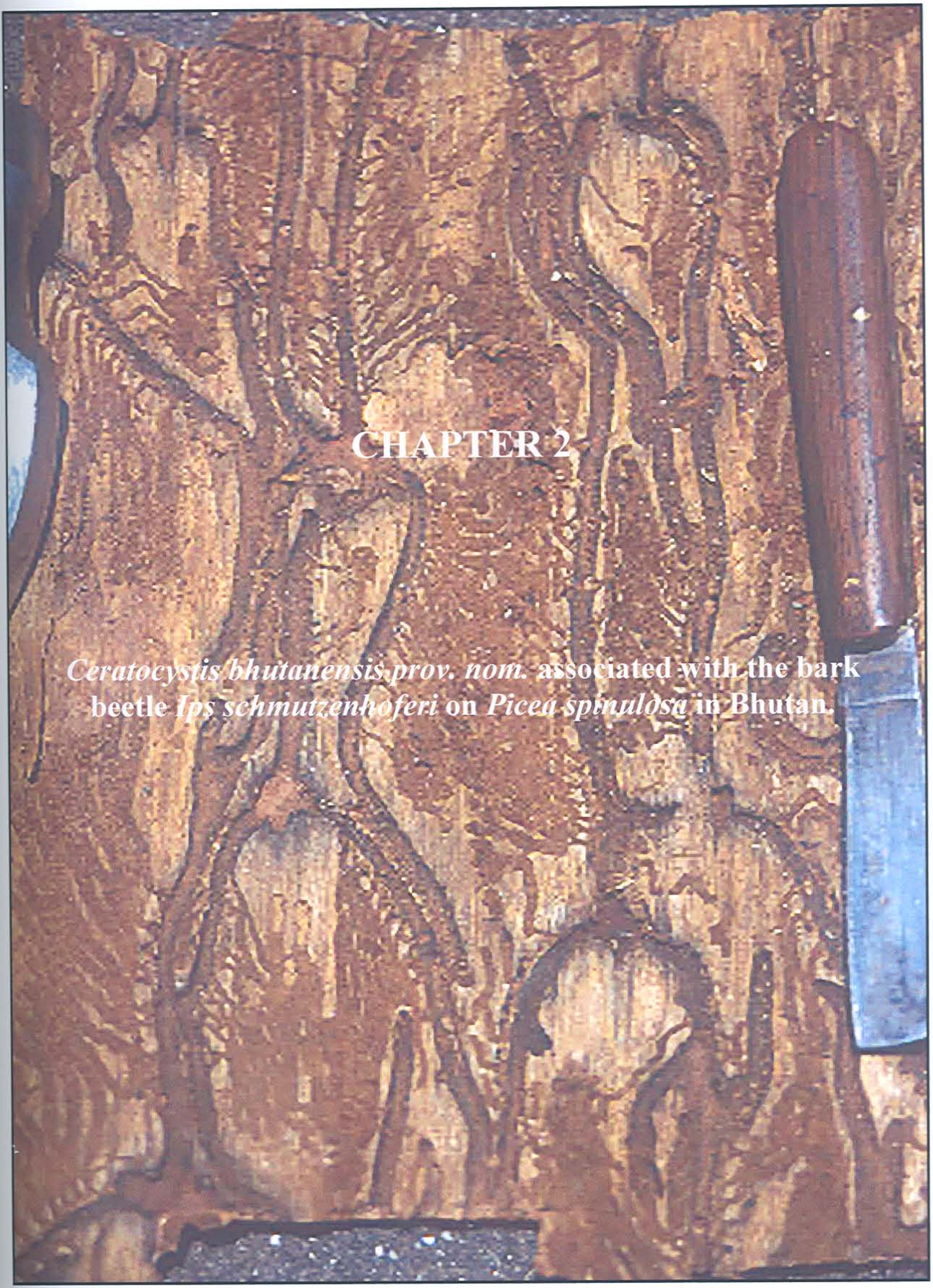

CHAPTER 2

Ceratocystis bhutanensis prov. nom. associated with the bark beetle *Ips schmutzenhoferi* on *Picea spinulosa* in Bhutan.

ABSTRACT

The Eastern Himalayan spruce bark beetle, *Ips schmutzenhoferi*, is a serious pest of *Picea spinulosa* and *Pinus wallichiana* in Bhutan. In 2001 a study was initiated that aimed to identify the ophiostomatoid fungi associated with this conifer bark beetle. During this survey, a *Ceratocystis* sp. was isolated from individuals of *I. schmutzenhoferi* collected from galleries on *P. spinulosa.* Morphological characteristics and comparisons of DNA sequence data were used to identify this fungus. Based on morphology, the *Ceratocystis* sp. from Bhutan resembled *C. moniliformis* and *C. moniliformopsis*, but was distinct from these species in colony morphology, micro-morphology, growth profiles at different temperatures, as well as the odour that it produces in culture. DNA sequence data of the ITS regions of the rDNA operon, β-tubulin and Elongation Factor 1-α genes, confirmed that this fungus represents a taxon distinct from *C. moniliformis*, *C. moniliformopsis* and all other species of *Ceratocystis*. Based on morphological characteristics, comparisons of DNA sequence data and its unique ecology, we, therefore, describe this fungus as new and provide the name *C. bhutanensis prov. nom.* Currently this fungus is only known from one locality in Western Bhutan and its geographical distribution, ecology, pathogenicity and vector relationships require further study.

INTRODUCTION

The Kingdom of Bhutan is renowned for its intact forest resources, which are of immense socio-economic and ecological importance for this Himalayan country. Sixty-four percent of Bhutan is covered by forests (FAO 1999, 2001). Conifer forests form the natural vegetation in most parts of the mountainous areas at elevations above 1800 m a.s.l. (FAO 1999). Eastern Himalayan spruce (*Picea spinulosa* (Griffith) A.) and Himalayan blue pine (*Pinus wallichiana* Jackson) are important tree species in these forests, forming either pure stands or mixed species stands together with other conifers.

Bark beetles (Coleoptera: Scolytidae) are amongst the most damaging agents affecting conifer forests, worldwide. Some of the most aggressive of these insects are species within the genus *Ips* de Geer (Postner 1974, Wood & Bright 1992). The best known of these is the eight-spined European spruce bark beetle, *I. typographus* L., that can cause extensive mortality of Norway spruce (*Picea abies* (L.) Karst.) in Europe (Postner 1974, Christiansen & Bakke 1988). In Bhutan, the Eastern Himalayan spruce bark beetle, *I. schmutzenhoferi* Holzschuh is a serious pest in conifer forests at elevations between 2500 and 3800 m a.s.l. (Schmutzenhofer 1988). This scolytid mainly attacks living trees and logs of Eastern Himalayan spruce and Himalayan blue pine, but logs of Himalayan larch (*Larix griffithiana* (Lindl. & Gord.) Carrière) are occasionally also infested (Schmutzenhofer 1988, Tshering & Chhetri 2000). During the 1980s, this insect caused a destructive outbreak in Western and Central Bhutan, during which 3000 ha of forest were affected and losses of approximately 2 million m^3 of timber occurred (Schmutzenhofer 1988).

Conifer infesting bark beetles are well known to carry blue-stain fungi belonging to the ascomycete genera *Ceratocystis* Ell. & Halst and *Ophiostoma* Von Syd. & Syd. and related anamorph genera (Francke-Grossman 1967, Upadhyay 1981, Whitney 1982, Webber & Gibbs 1989, Jacobs & Wingfield 2001). Members of these genera have also been referred to as the ophiostomatoid fungi (Wingfield, Seifert & Webber 1993). These fungi cause blue, grey or black discolouration in the sapwood of living trees, logs and lumber, mostly on conifers. This kind of damage results from the presence of pigmented fungal hyphae in the ray parenchyma cells and tracheids of the sapwood (Münch 1907, Liese & Schmid 1961, Seifert 1993). Damage due to sapstain is cosmetic rather than structural, and results in substantial financial losses, because markets prefer non-stained wood (Münch 1907, Seifert 1993, Uzunovic *et al.* 1999). Some bark beetle associated blue-stain fungi also cause vascular stain diseases on living conifer trees and are suspected to aid their insect vectors to exhaust the defence mechanisms of their host trees (Whitney 1982, Paine, Raffa & Harrington 1997).

Most fungal associates of bark beetles belong to the genus *Ophiostoma*. In contrast, *Ceratocystis* spp. usually have loose relationships with insects (Harrington 1987). However, there are three *Ceratocystis* spp. that are consistently associated with conifer bark beetles (Harrington & Wingfield 1998). The first of these, *C. polonica* (Siem.) Moreau, is associated with *I. typographus* and other species of *Ips* on *Picea abies* in Europe (Solheim 1986, 1992, Krokene & Solheim 1996, Kirisits, Grubelnik & Führer 2000), and with *I. typographus* f. *japonicus* Niijima on *Picea jezoensis* (Sieb. & Zucc.) Carr. in Japan (Yamaoka *et al.* 1997). The second, *C. laricicola* Redfern & Minter, is associated with the larch bark beetle *I. cembrae* Heer on *Larix* Miller spp. in Europe and Japan (Redfern *et al.* 1987, Yamaoka *et al.* 1998, Stauffer *et al.* 2001). The third, *C. rufipenni* Wingf., Harr. & Solh., is associated with the spruce bark beetle *Dendroctonus rufipennis* Kirby on *Picea engelmannii* Parry and *Picea glauca* (Moench) Voss in Western North America (Solheim 1995, Wingfield, Harrington & Solheim 1997).

The small number of *Ceratocystis* spp. that are associated with bark beetles display high levels of pathogenicity, when compared to *Ophiostoma* spp. from the same niche (Solheim 1988, Solheim & Safranyik 1997, Kirisits 1998, Krokene & Solheim 1998). *Ceratocystis polonica* is highly pathogenic to Norway spruce and contributes to tree death following attack by *I. typographus* (Christiansen 1985, Solheim 1988, 1992, Kirisits & Offenthaler 2002). Likewise, *C. laricicola* is considered to play an important role in the death of *Larix* spp. infested by *I. cembrae* (Redfern *et al.* 1987, Yamaoka *et al.* 1998, Kirisits 2001, Harrington *et al*. 2002) and *C. rufipenni* can kill Sitka spruce (*Picea sitchensis* (Bongard) Carrière) in mass inoculation experiments (Solheim & Safranyik 1997).

During a recent survey of ophiostomatoid fungi associated with *I. schmutzenhoferi* in Bhutan a *Ceratocystis* sp. resembling *C. moniliformis* Hedgc. and *C. moniliformopsis* Yuan & Mohammed was isolated. Despite its similarity with these *Ceratocystis* sp., the association of the *Ceratocystis* sp. from Bhutan with a conifer bark beetle aroused suspicion that it might be a separate, hitherto undescribed taxon. This study compares the *Ceratocystis* species from Bhutan with *C. moniliformis* and *C. moniliformopsis* and assesses their phylogenetic relationships based on the ITS region of the rDNA operon, β-tubulin and EF1-α gene sequences.

MATERIALS AND METHODS

Collection of material for fungal isolation

A survey of ophiostomatoid fungi associated with *I. schmutzenhoferi* in Bhutan was conducted in July 2001. Samples for fungal isolation were collected at several locations in Western and Central Bhutan (Fig. 1) where *I. schmutzenhoferi* attacked living trees or logs of *Picea spinulosa* and/or *Pinus wallichiana*. The collection sites included mixed conifer forests at Jelekha (3300 m a.s.l.), Changaphug (3600 m a.s.l.), Phobjikha valley (3100 m a.s.l.), and near the Renewable Natural Resources Research Center (RNR-RC) in Yusipang (2700 m a.s.l.) as well as wood depots at Gidakom (2200 m a.s.l.) and Ramtokto (2100 m a.s.l.) (Fig. 1). Logs and standing trees, infested by *I. schmutzenhoferi* were examined for suitable material to conduct fungal isolations. Galleries of the insects occurring in the bark or on the surface of the sapwood on logs and standing pine and spruce trees were inspected on site, with the aid of a 10x magnification hand lens, for the occurrence of sexual and asexual stages of ophiostomatoid fungi. At the research station in Yusipang adult beetles of *I. schmutzenhoferi* (2nd generation) were collected from a pheromone trap installed specifically for the purpose of insect specimen collection.

Adult and juvenile beetles, breeding galleries, stem discs and stem sections from beetle-infested *P. spinulosa* and *P. wallichiana* trees and logs were collected for further investigation. All samples were stored in plastic bags and transported to the laboratory at RNR-RC in Yusipang. Dry bark samples were sprayed with distilled water and the bags sealed for a few days to create a moist environment, conducive to sporulation of fungi within the beetle galleries. Reference specimens of *I. schmutzenhoferi* were stored in ethanol and are maintained at the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Universität für Bodenkultur Wien (BOKU), Vienna, Austria. In addition to material obtained from pine and spruce infested by *I. schmutzenhoferi*, wood samples were collected from broken *Cassia fistula* L. trees near Punakha (*ca* 1300 m a.s.l.) and Wangdi (*ca* 1100 m a.s.l.). The purpose of these collections was to search for *Ceratocystis moniliformis* on this subtropical hardwood species.

Fungal isolations

Fungi were isolated on 2 % Malt Extract Agar (MEA) (20 % w/v) (Biolab, Midrand, South Africa) or on 2 % Malt Agar (MA) (20 % w/v) (DiaMalt, Hefe Schweiz AG, Stettfurt, Switzerland) (W. Behrens & Co., Hamburg, Germany), both supplemented with 100 mg/L streptomycin sulphate (SIGMA, Steinheim, Germany, or VWR International GmbH, Vienna, Austria). In order to get a comprehensive view of the fungi associated with *I. schmutzenhoferi* various isolation methods were applied. Fungi were isolated directly from adult beetles (2nd generation) collected from two spruce logs at Jelekha, from young beetles (1st generation) obtained from a pine log at Ramtokto and from swarming beetles (2nd generation) collected from a pheromone trap at Yusipang. To obtain isolates directly from the insects, their body parts were dissected and spread onto 2 % MA.

Fungi were also isolated from the sapwood of one spruce tree from Jelekha. Six stem discs (*ca* 10-15 cm wide, with a diameter of 18-21 cm), were cut from the upper part of this tree. These were split vertically and isolations from the sapwood were done along radii underneath female galleries of *I. schmutzenhoferi*, following a similar procedure as that described by Solheim (1992). Three radii per disc were sampled, resulting in a total of 18 radii. Small pieces of sapwood were transferred onto 2 % MA plates. From each radius, samples were taken 2, 5 and 10 mm apart from the cambium into the sapwood.

Most isolations were made from ascospores and conidia taken directly from sexual and asexual fungal structures occurring in and around female and larval galleries and pupal chambers of the insects. Bark and sapwood samples from spruce and pine collected at the localities Jelekha, Gidakom, Ramtokto, Changaphug and Phobjikha valley were examined with a dissecting microscope at magnifications ranging from 10x to 40x. With a fine needle, ascospores and conidia accumulating at the apices of perithecia and on conidiophores, respectively, were carefully removed and transferred to 2 % MA or 2 % MEA plates. Isolation of *C. moniliformis* from *C. fistula* collected at Punakha and Wangdi was done in a similar manner, from ascospores obtained from perithecia occurring on the wood surface.

A selective method for the isolation of *Ceratocystis* spp. was also used (Moller & De Vay 1968). Fresh carrots were washed and lightly sprayed with 70 % ethanol. Carrot discs (5-10 mm thick) were cut and four to eight discs were placed in plastic Petri dishes (90 mm). Beetles, larvae and pupae of *I. schmutzenhoferi* were dissected and spread over the surface of the carrot discs. Larval frass, collected from the insect galleries was also put onto the carrots. The discs were examined for the incidence of perithecia after 5-10 days incubation at *ca* 20 °C.

Pure cultures were obtained by transferring ascospore or conidial masses as well as small pieces of mycelium from the primary isolation plates onto fresh 2 % MA or 2 % MEA plates. Fungal cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Universität für Bodenkultur Wien (BOKU), Vienna, Austria and the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Holotype material of the new *Ceratocystis* sp. from Bhutan, consisting of a dried culture of isolate CMW 8217 on 2 % MEA is kept at the National Fungal Herbarium (PREM), Pretoria, South Africa (Table 1).

Culture characteristics and morphology

The growth of isolates CMW 8217, CMW 8241 and CMW 8244 representing the *Ceratocystis* sp. obtained from *I. schmutzenhoferi*, was determined on 2 % MEA (Fig. 2). Three isolates of *C. moniliformis* (CMW 9590, CMW 8238 & CMW 10134) and *C. moniliformopsis* (CMW 9986, CMW 10214 & CMW 10215) were used for comparisons in the growth study (Fig. 2). Prior to the growth assays, the isolates were grown in culture for two weeks at 25 °C (Fig. 3). Mycelial plugs were taken from actively growing cultures using a 5 mm cork borer and a single mycelial plug was transferred to the centre of a 90 mm Petri dish containing 2 % MEA. Five plates for each isolate were incubated at 4 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 35 °C, respectively. Colony diameter for each culture was assessed by taking two measurements at right angles to each other, every day, for four days or until the plates were almost completely covered by mycelium. Averages were computed separately for each isolate and each test temperature. The entire experiment was repeated once.

Morphological characteristics were described from 10-day-old cultures, on 2 % MEA supplemented with streptomycin sulphate (0.001 g vol^{-1}, SIGMA, Steinheim, Germany) and Thiamine (0.001 g vol^{-1}, SIGMA, Steinheim, Germany). Fungal structures were mounted in lactophenol containing cotton blue. Fifty measurements for each taxonomically relevant structure were made from isolate CMW 8217, and to correlate that these values are representative for the *Ceratocystis* sp. from Bhutan 10 further measurements were made for each of five other isolates (Table 1). Ranges, averages, and standard deviations of the corresponding measurements were calculated. The microscopic observations were made using a Carl Zeiss microscope and the photographic images were made with a Zeiss Axio Vision camera system. Colour descriptions were determined using the colour charts of Rayner (1970). The measurements and morphological characteristics of the *Ceratocystis* sp. from Bhutan were compared with descriptions of *C. moniliformis* (Hedgcock 1906, Upadhyay 1981) and *C. moniliformopsis* (Yuan & Mohammed 2002) (Table 2).

DNA extraction

Representative isolates of the *Ceratocystis* sp. from *I. schmutzenhoferi* in Bhutan as well as isolates of *C. moniliformis*, *C. moniliformopsis* and *C. virescens* (Davids.) Moreau (Table 1) were selected for DNA extraction and sequencing. From each culture a single ascospore mass was transferred, using a sterile needle, from actively growing and sporulating cultures, to 50 ml 3 % ME broth, in Erlenmeyer flasks, and incubated at 25 °C. After two weeks, the thick mycelial mats were filtered from the broth and lyophilised for two days. The freeze-dried mycelium was placed in liquid nitrogen and ground to a powder using a glass rod, and DNA was extracted using the method described by Barnes *et al.* (2001).

PCR amplification

The two ITS regions (ITS1 and ITS2) and the 5.8S gene of the rDNA operon were amplified using primers ITS1 and ITS4 (White *et al.* 1990) at an annealing temperature of 55 °C. A part of the β-tubulin gene was amplified using primers βt1a and βt1b at an annealing temperature of 55 °C (Glass & Donaldson 1995) and the EF1-α gene of the rDNA operon was amplified using primers EF1-728F and EF1-986R at an annealing temperature of 56 °C (Carbone & Kohn 1999).

Polymerase chain reaction (PCR) mixtures consisted of 200 nM of the forward and reverse primers, 200 μM of each dNTP, Expand High Fidelity PCR System enzyme mix (1.75 U) (Roche Diagnostics, Mannheim, Germany), 1 x Expand HF Buffer containing 1.5 mM $MgCl_2$ (supplied with the enzyme) and 2-10 ng DNA. Reaction volumes were adjusted to 25 μL with sterile Sabax water (Adcock Ingram Scientific, Johannesburg, South Africa). The PCR programme was set at 96 °C for 2 min, followed by 10 cycles at 94 °C for 20 s, x °C (x = the annealing temperature specified for each set of primers) for 40 s and 72 °C for

45 s. Further 30 cycles were included with the annealing time altered to 40 s and a 5 s extension after each cycle. A final step of 10 min at 72 ºC completed the programme. Amplification of the respective genes was confirmed on a 2 % agarose (Roche diagnostics, Mannheim, Germany) gel supplemented with ethidium bromide. PCR amplicons were purified using the Magic PCR Preps, Purification System (Promega, Madison, USA).

Sequencing and data analysis

PCR amplicons were sequenced in both directions using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California). The same primers as those in the PCR reactions were used for sequencing of the respective gene areas. Sequence reactions were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California, U.S.A) and sequences were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California).

The sequences obtained for the *Ceratocystis* sp. from *I. schmutzenhoferi* were compared with those of morphologically similar *Ceratocystis* spp. (Table 1). Sequences were aligned manually and analysed using PAUP version 4.0b10* [Phylogenetic Analysis Using Parsimony (and other methods)] (Swofford 2002). Gaps were treated as "newstate" and trees were obtained via stepwise addition of 1000 replicates with the Mulpar option in effect. The heuristic search based on parsimony with tree bisection reconnection was used to obtain the phylogram. Confidence intervals using 1000 bootstrap replicates were calculated. The out-group taxon *C. virescens* was rooted as a midpoint with respect to the in-group. All sequences derived from this study have been deposited in GenBank (Table 1). A partition homogeneity test (Swofford 2002) was used to determine whether the sequence data sets for the three different genome regions could be combined.

The Markov Chain Monte Carlo (MCMC) method (Larget & Simon 1999), with a Bayesian framework was used to estimate the posterior probability of nodes in the phylogenetic tree. One hundred thousand random trees were generated using the MCMC procedure, sampling every 100th tree and printing every 10th tree. To avoid including trees that might have been sampled before convergence of the Markov chain, 8600 trees were discarded. For the combined analysis of the three gene sequences, gamma rate heterogeneity was set, and no codon specific sites were included for the ITS gene. For β-tubulin and EF1-α sequences, codon specific sites were specified with a site-specific substitution rate and the site partition was treated as a by-codon.

RESULTS

Collection of material for fungal isolations

Conspicuous blue-stain was observed on the surface of the sapwood and in the bark around nuptial chambers and female and larval galleries of *I. schmutzenhoferi* on inspected spruce and pine trees and logs. However, intensive blue-stain, deeply penetrating into the sapwood was not seen on any of the inspected trees and logs, neither on spruce nor on pine. On the stem discs of the spruce tree from which isolations were made, a narrow zone of desiccation, extending 5 to 8 mm deep into the sapwood and recognizable by its white to yellowish colour, occurred.

Fungal isolations

From the isolations made, the *Ceratocystis* sp. was only isolated directly from beetles of the second generation collected from galleries on *P. spinulosa* at the locality Jelekha. In the sample of 20 beetles from this collection site which were used for fungal isolations, 16 (80 %) yielded growth of the *Ceratocystis* sp. and this fungus was thus among the dominant species recovered from this niche. Fourteen isolates of the *Ceratocystis* sp. representing isolations from separate beetles were initially maintained and 11 of these strains were used for phenotypic, morphological and molecular characterization of this fungus in the present study.

The *Ceratocystis* sp. mentioned above was neither isolated from beetles obtained from Ramtokto and Yusipang nor from desiccated sapwood of the spruce tree collected at Jelekha. Likewise, perithecia and conidiophores were never observed in galleries of *I. schmutzenhoferi* on spruce and pine. Subsequent attempts to isolate this fungus again from adult and juvenile beetles, larvae, and pupa or from larval frass on carrot discs, known to be selective for *Ceratocystis* spp., were unsuccessful.

Ascomata resembling those of *C. moniliformis* were common on the surface of the wood of broken *C. fistula* trees near Punakha and Wangdi. It was easy to isolate *C. moniliformis* by transferring ascospores from the perithecial tips to MA and MEA plates. Two isolates of this fungus from Bhutan were included for morphological and molecular comparisons with the *Ceratocystis* sp. from *I. schmutzenhoferi* (Table 1).

Cultural characteristics and morphology

All three *Ceratocystis* spp. tested grew very rapidly in culture, at least near their temperature optimum. *Ceratocystis bhutanensis prov. nom.*, *C. moniliformis* and *C. moniliformopsis* differed considerably in their growth profiles at different temperatures (Fig. 2). Only the results of the first study were used for the graphs (Fig. 2), the results of the second study were used to determine if the cultures had the same growth rate. The optimum temperature of the *Ceratocystis* sp. from *I. schmutzenhoferi* in Bhutan varied between 20 ˚C (Isolates CMW 8244 and CMW 8241) and 25 °C (Isolate CMW 8217), while for *C. moniliformis* and *C. moniliformopsis* it was 30 °C and 20 °C, respectively (Fig. 2). At 4 °C the *Ceratocystis* sp. from Bhutan grew, while no growth was observed for either *C. moniliformis* or *C. moniliformopsis*. There was diminished growth at 10 °C for all the isolates, while two of the *C. moniliformis* isolates (CMW 8238 and CMW 10134) had no growth at this temperature (Fig. 2). While *C. moniliformopsis* did not grow from 25 °C to 35 °C, *C. moniliformis* grew very fast at 25 °C and 30 °C and also showed no growth at 35 °C. The *Ceratocystis* sp. from Bhutan displayed fast growth at 25 °C, diminished growth at 30 °C for two of the isolates (CMW 8244 and CMW 8217) and no growth for one isolate (CMW 8241) and also did not grow at 35 °C (Fig. 2).

Within the species, considerable variation was observed (Fig. 2). For the three *C. moniliformis* isolates there was major differences observed at four critical temperatures (15 °C – 30 °C). The greatest variation was at 30 °C where one isolate (CMW 9590) had a growth rate of 90 mm in 3 days, while the other two isolates (CMW 8238 and CMW 10134) had a growth rate of 60 mm and 18 mm, respectively. For the isolates used to study *C. moniliformopsis* little variation was observed. One of the isolates for *C. moniliformopsis* (CMW 10214) seemed to have a better growth rate than the other two isolates (CMW 9986 and CMW 10215) at all temperatures studied. For the isolates used for *C. bhutanensis prov. nom.* variation was observed at two temperatures. One isolate (CMW 8241) had a very fast growth rate reaching 78 mm in just three days at 20 °C but the growth rate dropped dramatically at 25 °C and no growth was observed at 30 °C for this isolate. The other two isolates (CMW 8217 and CMW 8244) also had good growth rates at 20 °C but their growth tempo did not differ significantly at 25 °C but they still grew at 30 °C.

On MEA, cultures of the *Ceratocystis* sp. from *I. schmutzenhoferi* were light in colour when young, but turned grey and finally black as they became older. In young cultures, the submerged mycelium had a honey colour (19"b) and the aerial mycelium was cream-buff (19"d). In older cultures (> 14 days), the submerged mycelium was umber (15 m) with the aerial mycelium ecru-drab (13"""d) (Fig. 3). In even older cultures (> 28 days), the submerged mycelium tended to be black (7"""k) and the abundant aerial mycelium dark olive (21"m) (Rayner 1970). This is very different to *C. moniliformis* that produced white to cream-buff (19"d) mycelium (Fig. 3) and the *C. moniliformopsis* isolates that were described as having a colourless to white grey appearance with the centre becoming greenish brown due to sporulation of the ascomata (Yuan & Mohammed 2002) which was observed by us to be more brownish in colour (Fig. 3). Isolates of the unknown species of *Ceratocystis* from *I. schmutzenhoferi* produced an unpleasant rotten fruity odour. This was in contrast to the pleasant banana-oil odour typically produced by cultures of *C. moniliformis* (Davidson 1935) and little to no odour produced by *C. moniliformopsis* (Yuan & Mohammed 2002). The *Ceratocystis* sp. from Bhutan produces ascomata within a few (3-4) days, which are then overgrown by dense aerial mycelium and make the detection of ascomata difficult. *Ceratocystis moniliformis* and *C. moniliformopsis* also produce perithecia within a few days, but they can be clearly seen in older cultures.

The ascomatal bases of the *Ceratocystis* sp. isolated from *I. schmutzenhoferi* were black, globose and covered with short conical spines, resembling those of *C. moniliformis* (Fig. 4a-b). The bases of the necks also resembled *C. moniliformis* in being disc shaped and detaching from the bases of the ascomata when disturbed (Fig. 4b). The ostiolar hyphae were divergent (Fig. 4c), exuding sticky masses of hat-shaped ascospores (Fig. 4d). Two types of hyphae were present, one smooth-walled, and the other rigid and granular (Fig. 4e-f). The anamorph was typical of *Thielaviopsis* Went, with the phialidic conidiogenous cells producing both cylindrical and barrel-shaped conidia (Fig. 4g-i). No chlamydospores are present in the *Ceratocystis* sp. from Bhutan. When older cultures are examined with the dissecting microscope, accumulations of pigmented, thick-walled cells become obvious as black dots. These structures were originally suspected to represent chlamydospores, but were later identified as old conidia produced by the *Thielaviopsis* anamorph.

Care has to be taken when the cultures of the *Ceratocystis* sp. from Bhutan are to be preserved. There is a tendency of some cultures to degenerate on MEA plates. This phenomenon has also been observed in *C. moniliformis* and in *C. moniliformopsis*. The cultures that degenerate become white in colony morphology, they have a slower growth rate and no longer produce ascomata in culture. One alternative to preserve these cultures is to scrape the mycelium and fungal structures into an Eppendorf tube and freeze dry it. The culture can then be grown from there by placing a small amount of the freeze-dried material onto a MEA plate.

PCR amplification

Amplification of the ITS regions and the 5.8S gene of the rDNA resulted in amplification products of ~500 bp. Amplification of the β-tubulin gene resulted in amplification products of ~500 bp, while the amplification of the EF1-α gene resulted in amplification products of ~300 bp.

Sequencing and analyses

Partition homogeneity tests for the three sequence data sets gave a P-value of $P = 0.46$ for the ITS and β-tubulin, $P = 1.00$ for the ITS and EF1-α and $P = 0.14$ for the β-tubulin and EF1- α combinations. All datasets had a value greater than the minimum required value of $P = 0.05$ and they could thus be combined. The combined sequences of the three gene areas, resulted in a dataset that was 1491 bp long (Appendix), had a single most parsimous tree, with a consistency index (CI) of 0.9532, a homoplasy index (HI) of 0.0468, a retention index (RI) of 0.9291 and a rescaled consistency index (RC) of 0.8757. The posterior probability of the branch nodes of the combined tree, generated with the Bayesian inference programme supported the bootstrap values. The posterior probability for the branch nodes for the three clades representing *C. moniliformis*, *C. moniliformopsis* and the *Ceratocystis* sp. isolated from *I. schmutzenhoferi*, respectively, was 100 %.

A heuristic search resulted in a single well-resolved tree (Fig 5). Species of *Ceratocystis* included in this tree formed three distinct sub-clades (Fig. 5). One of these sub-clades included the *Ceratocystis* sp. isolated from *I. schmutzenhoferi* in Bhutan, supported by a bootstrap value of 100 %. The other sub-clades included isolates of *C. moniliformis* and *C. moniliformopsis*, respectively (Fig. 5).

TAXONOMY

Comparison of DNA sequence data confirmed morphological observations that the *Ceratocystis* sp. from *I. schmutzenhoferi* in Bhutan is related to *C. moniliformis* and *C. moniliformopsis*. The data, however, provided robust support for the view that this fungus represents a new and previously undescribed species of *Ceratocystis*. The fungus is, therefore, described as a new taxon.

Ceratocystis bhutanensis Van Wyk, Wingfield & Kirisits, *prov. nom.*

(Fig.3,4)

Etymology: Bhutanensis referring to the country where this species has been discovered, in Bhutan.

Stat.conid.: *Thielaviopsis*

Coloniae juvenes cremeo-fulvidae, infra mellinae, seniores griseo-mustellinae, infra umbrinae, dein atro-olivaceae, infra nigrae. *Mycelium* plerumque in medio immersum; mycelium album aerium adest. *Crescit* optime ad 25 °C, nullo incremento supra 35 °C, deminuto ad 4 °C. *Hyphae* leves vel granulatae, in septis non constrictis, 1-3.5 μm latae. *Bases ascomatum* atrobrunneae vel nigrae, globosae, spinis hyphisque ornatae, spinis atrobrunneis vel nigris, (4.5-) 8-19 (-27) μm longis, bases (112-) 138-178 (-206) μm diametro. *Colla ascomatum* basin versus atrobrunnea vel nigra, apicem versus laetescentia, (450-) 453-519 μm longa, basi, 34-42 (-44) μm lata, apice (11-) 12-14 (-17) μ lata, apice discoideo. *Hyphae ostiolares* divergentes, hyalinae, (13-) 18-26 (-34) μm longae. *Asci* non visi. *Ascosporae* lateraliter visae cucullatae, aseptatae, hyalinae, in vagina investitae, cum vagina 4-6 x 2-5 μm, sine vagina 2-5 x 2-5 μm. Ascospora*e* in massis mucilagineis fulvo-luteis in apicibus collorum ascomatum cumulantes. *Anamorpha Thielaviopsis*: conidiophora in mycelio singula, hyalina, basi tumida, apicem versus angustata, (15-) 23-39 (-51) μm longa, basi (3-) 4-6 (-9) μm lata, apice 1–3 μm lata. Evolutio *conidii* phialidici per parietes annulares faciendas, *conidia* in catenis biformibus facta: conidia primaria hyalina, aseptata, cylindrica, (6-) 7-9 (-10) x 1-3 μm, conidia secondaria hyalina, aseptata, doliiformia, 3-5 x (1.5-) 2-3 (-3.5) μm.

Typus: **Bhutan**: *Thimphu dzongkhag*, Jelekha, isolated from *Ips schmutzenhoferi* collected from *Picea spinulosa*, 4 July 2001, *T. Kirisits and D. B. Chhetri*, (PREM 57804 - holotypus, living culture: CMW 8217).

Colonies that are young in culture had a honey colour for the submerged mycelium (19"b) the aerial mycelium being cream-buff (19"d). In older cultures (> 14 days), the submerged mycelium was umber (15 m) with the aerial mycelium ecru-drab (13""'d) (Fig. 3). In older cultures (> 28 days), the submerged mycelium was black (7""'k) and the abundant aerial mycelium dark olive (21"m). *Mycelium* submerged in medium, abundant white aerial mycelium present. *Optimal temperature* 20 °C, no growth at 35 °C, diminished growth at 4 °C and 30 °C. Isolates can grow up to 20 mm per day at the optimum temperature. *Hyphae* smooth or granulated, not constricted at septa, 1-3.5 µm wide. *Ascomatal bases* dark brown to black, globose, ornamented with spines and hyphae, spines dark brown to black, (4.5-) 8-19 (-27) µm long, bases (112-) 138-178 (-206) µm in diameter. *Ascomatal necks* dark brown to black at base, becoming light brown towards the apex, (450-) 453-519 µm long, 34-42 (-44) µm wide at the base, (11-) 12-14 (-17) µm wide at the apex, with a disc-like (disciform) base. *Ostiolar hyphae* divergent, hyaline, (13-) 18-26 (-34) µm long. *Asci* not observed. *Ascospores* cucullate in side view, aseptate, hyaline, invested in sheath, 4-6 x 2-5 µm with sheath, 2-5 x 2-5 µm without sheath. Ascospores accumulating in originally white and later buff-yellow (19d) mucilaginous masses on the apices of ascomatal necks. *Thielaviopsis anamorph*: conidiophores occurring singly on mycelium, hyaline swollen at the base, tapering towards the apex, (15-) 23-39 (-51) µm long, (3-) 4-6 (-9) µm wide at base, 1-3 µm wide at the apices. Phialidic *conidium* development through ring wall building, *conidia* formed in chains of two types: primary conidia hyaline, aseptate, cylindrical (6-) 7-9 (-10) x 1-3 µm, secondary conidia hyaline, aseptate, barrel-shaped 3-5 x (1.5-) 2-3 (-3.5) µm.

Additional specimens examined: **Bhutan**: *Thimphu dzongkhag*, Jelekha, isolated from *Ips schmutzenhoferi* collected from *Picea spinulosa*, 4 July 2001, T. Kirisits and D. B. Chhetri, (culture CMW 8241, PREM 57808; culture CMW 8242, PREM 57809; culture CMW 8108; culture CMW 8244, PREM 57811; culture CMW 8243, PREM 57810).

DISCUSSION

Very little is presently known about the occurrence of ophiostomatoid fungi in the Himalayas and their role as tree pathogens and agents of blue-stain in this part of the world. Prior to this study, only one ophiostomatoid fungus, *Ophiostoma himal-ulmi* Brasier & Mehrotra has been reported from the Western Himalayas, where it occurs on *Ulmus wallichiana* Planchon and is associated with elm bark beetles (Brasier & Mehrotra 1995). The discovery of *C. bhutanensis prov. nom.* and the detection of *C. moniliformis* in Bhutan represent the first reports of species of *Ceratocystis* from this country and the entire Himalayas. To determine wheter this fungus will have an impact on the forestry industry in Bhutan pathogenecity tests have to be conducted. *Ceratocystis bhutanensis prov. nom.*, described in this study is also the first fungus to be recorded as an associate of bark beetles, specifically of *I. schmutzenhoferi* in Bhutan. Many other ophiostomatoid fungi, including species of *Ophiostoma*, *Ceratocystiopsis* Upadhyay & Kendr., *Leptographium* Lagerb. & Melin and *Pesotum* Crane have been detected in the survey in Bhutan in 2001 (Kirisits, Wingfield & Chhetri 2002). Examination of these fungi is currently underway in order to unambiguously identify them and to provide formal names for the taxa that are considered as new to science. The survey in Bhutan was conducted in a very dry year and the dry weather conditions during spring and early summer 2001 may have accounted for the limited amount of stain observed on trees and logs infested by *I. schmutzenhoferi*. In a more humid year, more stain would be observed due to the more favorabile conditions for fungal growth.

Ceratocystis bhutanensis prov. nom. is morphologically very similar to *C. moniliformis* and *C. moniliformopsis*, but the occurrence of the fungus from Bhutan on a conifer tree in association with a bark beetle appeared to be untypical for the latter species and provided first evidence that the isolates obtained from *I. schmutzenhoferi* might represent a new species. This view was supported by the colony morphology and the distinct odour of the *Ceratocystis* sp. from Bhutan. Micro-morphological comparison of *C. bhutanensis prov. nom.* with *C. moniliformis* and *C. moniliformopsis* revealed small differences between these fungi and DNA sequence analyses of three nuclear genes finally provided unequivocal evidence that the isolates from Bhutan represent a new *Ceratocystis* sp. *Ceratocystis moniliformis*, *C. moniliformopsis* and *C. bhutanensis prov. nom.* are additional examples of morphologically almost indistinguishable, yet genetically and ecologically distinct species in the genus *Ceratocystis*. Other well-known examples are *C.*

polonica and *C. laricicola* that are morphologically identical, but show differences in their habitat and vectors, display host preference and can be differentiated by molecular markers (Harrington & Wingfield 1998, Kirisits 2001, Harrington *et al.* 2002).

Based on morphology, *C. bhutanensis prov. nom.* most closely resembles *C. moniliformis*. *Ceratocystis moniliformis* is typified by ascomatal bases covered with short conical spines, disc-shaped bases on its necks and hat-shaped ascospores (Davidson 1935, Nag Raj & Kendrick 1975, Upadhyay 1981). *Ceratocystis bhutanensis prov. nom.* shares all three of these characteristics. Morphologically, *C. bhutanensis prov. nom.* and *C. moniliformis* can be distinguished from each other by the much darker colour of the culture and the considerably more abundant production of aerial mycelium in the former species. *Ceratocystis bhutanensis prov. nom.* also produces an aroma very different to that of *C. moniliformis.* The two fungi have distinct types of hyphae; *C. bhutanensis prov. nom.* has smooth and granular hyphae, while *C. moniliformis* has only smooth hyphae. These morphological differences, as well as the different ecologies of the two fungi enable easy recognition of *C. bhutanensis prov. nom.* They also provide strong justification for the description of a new species.

Ceratocystis bhutanensis prov. nom. can be distinguished from *C. moniliformis* based on growth rate in culture. The former species grows considerably faster at temperatures below 15 °C than *C. moniliformis*, which hardly grows at temperatures below 15 °C. This ability to grow at low temperatures is consistent with the ecology of *C. bhutanensis prov. nom.* This growth at low temperatures of *C. bhutanensis prov. nom.* acts as a good distribution barrier. This fungus is associated with an insect that occurs at relatively high altitudes in the Himalayan mountain ranges, where it would be adapted to low temperatures. This is in contrast to *C. moniliformis,* which is common in the sub-tropics and tropics (Davidson 1935, Upadhyay 1981) where it is associated with temperatures much higher than those in the area where *C. bhutanensis prov. nom.* was found. Indeed, the two isolates of *C. moniliformis* from Bhutan examined in this study were collected near Punakha and Wangdi, at areas where sub-tropical climate and vegetation reach far north into the Himalayas. The growth-temperature relationships and the relatively low temperature optimum of *C. bhutanensis prov. nom.* resemble those of *O. himal-ulmi* that is also adapted to relatively low temperatures and has a temperature optimum between 22-25 °C (Brasier & Mehrotra 1995).

Another species very similar to *C. moniliformis* is *C. moniliformopsis* (Yuan & Mohammed 2002). *Ceratocystis bhutanensis prov. nom.* can easily be distinguished from *C. moniliformopsis*. The two fungi share the same characteristics with each other as they do with *C. moniliformis* (Davidson 1935, Yuan & Mohammed 2002). *Ceratocystis bhutanensis prov. nom.* can, however, be distinguished from *C. moniliformopsis* based on the culture morphology, as *C. bhutanensis prov. nom.* has a much darker mycelial growth than *C. moniliformopsis*. There is no description of aroma production of *C. moniliformopsis* but a similar, faint, fruity banana-oil odour that *C. moniliformis* produces was found, and not the fermenting fruit odour of *C. bhutanensis prov. nom.* The hyphae of *C. moniliformis* and *C. moniliformopsis* are the same and thus different to *C. bhutanensis prov. nom.* as described above. *Ceratocystis moniliformopsis* has convergent ostiolar hyphae in contrast to the divergent ostiolar hyphae of *C. moniliformis* and *C. bhutanensis prov. nom.* The ascomatal bases of *C. bhutanensis prov. nom.* and *C. moniliformis* are globose in shape while *C. moniliformopsis* has an ovoid ascomatal base shape. The ornamentation on the ascomatal base for *C. moniliformis* was described as conical spines (Hedgcock 1906) while *C. moniliformopsis* was described as having two types of ornamentation present on the ascomatal base. The first of these is the ampliform to conical, rostrate or obtuse at the apex spines. The second type is the hyphal hairs that are said to be distinct for this species, but hyphae have been observed on the bases of *C. bhutanensis prov. nom.* as well as on *C. moniliformis*. Yuan and Mohammed (2002) described two types of conidiogenous cells, both being phialidic only differing at the apices. In 1951, Bakshi also observed two types of conidiogenous cells for *C. moniliformis* only differing in width (Bakshi 1951). For *C. bhutanensis prov. nom.* only one type of conidiogenous cell was observed.

Other than *C. bhutanensis prov. nom.*, there are six *Ceratocystis* spp. known to have hat-shaped ascospores. These include *C. fimbriata* Ell. & Halst. (Upadhyay 1981), *C. moniliformis* (Davidson 1935), *C. albofundus* De Beer, Wingf. & Morr. (Wingfield *et al.* 1996), *C. moniliformopsis* (Yuan & Mohammed 2002), *C. pirilliformis* Barnes & Wingfield (Barnes *et al.* 2003b) and *C. acericola* Griffin (Griffin 1968). Of these species, only *C. moniliformis* and *C. moniliformopsis* have spines on their ascomatal bases and both have very characteristic disc-shaped bases on their ascomatal necks. *Ceratocystis bhutanensis prov. nom.* can be distinguished from both of these species based on host, biogeography, association with a conifer bark beetle, and odour in culture as well as various morphological characteristics noted previously.

A comparison of DNA sequences for three gene regions provided strong support for our view that *C. bhutanensis prov. nom.* represents a previously undescribed taxon. Sequence data for the ITS regions alone did not provide convincing separation between the new species, *C. moniliformis* and *C. moniliformopsis*. However, addition of β-tubulin and EF1-α sequences provided clear resolution to the clades in which these three species reside. Phylogenetically, *C. bhutanensis prov. nom.* grouped within the larger *C. coerulescens* clade (Witthuhn *et al.* 1998) together with *C. moniliformis* and *C. moniliformopsis* as its closest relatives. This clade is separate from the *C. fimbriata* clade, in which the other *Ceratocystis* spp. with hat-shaped ascospores reside (Witthuhn *et al.* 1999, Barnes *et al.* 2003a). This study also provides the first DNA sequence data for *C. moniliformopsis* and supports the view that this is a distinct species, even though it is morphologically very similar to *C. moniliformis* (Yuan & Mohammed 2002).

Besides *C. polonica*, *C. laricicola* and *C. rufipenni*, *C. bhutanensis prov. nom.* is the fourth *Ceratocystis* sp. known to be associated with a conifer bark beetle. The new *Ceratocystis* sp. from Bhutan is, however, very different to the other three species, morphologically, phenotypically and phylogenically. *Ceratocystis polonica*, *C. laricicola* and *C. rufipenni* are closely related to each other and form part of the *C. coerulescens* spp. complex on conifers (Harrington & Wingfield 1998, Witthuhn *et al.* 1998). In contrast, *C. bhutanensis prov. nom.* is more distantly related to species of the *C. coerulescens* complex on conifers, but phylogenetically groups closely with *C. moniliformis* and *C. moniliformopsis* that occur on hardwoods in tropical, sub-tropical and temperate areas of the world.

With its fast growth, its rapid degeneration under standard laboratory conditions and its intensive aroma *C. bhutanensis prov. nom.* phenotypically also resembles *C. moniliformis* and *C. moniliformopsis* and differs from other *Ceratocystis* spp. that are associated with conifer bark beetles. The intensive odour of *C. bhutanensis prov. nom.* is of special interest, since this is a general characteristic of *Ceratocystis* spp. that are not intimately and specifically associated with insects. The intensive aroma of these species is viewed as an adaptation to attract various insects that are unspecifically involved in dissemination of these fungi (Kile 1993, Harrington & Wingfield 1998). In contrast to *C. bhutanensis prov. nom.*, cultures of *C. polonica*, *C. laricicola* and *C. rufipenni* lack an intensive aroma, which is considered as a modification to the consistent association with bark beetles (Yamaoka *et al.* 1997, Harrington & Wingfield 1998).

Ips schmutzenhoferi is an insect that is biologically very similar to *I. typographus* and *I. cembrae* (Postner 1974, Christiansen & Bakke 1988, Schmutzenhofer 1988). Both of the latter insects carry a wide range of *Ophiostoma* spp. and their anamorphs, and they are particularly interesting in that they are also consistently associated with a pathogenic *Ceratocystis* sp. (Solheim 1986, Redfern *et al.* 1987, Solheim 1992, Yamaoka *et al.* 1997, Yamaoka *et al.* 1998, Kirisits 2001). In this respect, we might have expected to encounter a *Ceratocystis* sp. associated with *I. schmutzenhoferi* in Bhutan. However, the fact that *C. bhutanensis prov. nom.* was isolated only from adult insects at one locality and not from beetles obtained from other sites, from galleries or symptomatic sapwood tissue is intriguing and raises the question about the intimacy of the relationship between this fungus and *I. schmutzenhoferi*.

Ceratocystis bhutanensis prov. nom. may be a rare associate of *I. schmutzenhoferi* or may show a restricted geographical distribution. Variation in the assemblages of fungi associated with bark beetles between different study sites has also been well documented and this might explain the isolation results in the present study. For example, *C. polonica* has been reported as a frequent or even as the most dominate associate of *I. typographus* in some parts of Europe, while it was not recorded or occurred rarely in studies conducted in other parts of the continent (Solheim 1986, 1992, 1993, Kirisits *et al.* 2000, Kirisits 2001). It has also been suggested that the population dynamics of *I. typographus* has a strong influence on the incidence and frequency of *C. polonica*, the fungus occurring less frequently during endemic periods, but becoming more frequent during outbreaks of the insect (Solheim 1993). Similar phenomena may also occur in the *I. schmutzenhoferi – C. bhutanensis prov. nom.* system. Phoretic mites of bark beetles are also known to carry ascospores of *Ceratocystis* and *Ophiostoma* spp. (Moser, Perry & Solheim 1989, Moser, Perry & Furuta 1997) and it might be possible that *C. bhutanensis prov. nom.* was in fact isolated from mites attached to the beetles and not directly from *I. schmutzenhoferi*.

An alternative view is that the isolation of *C. bhutanensis prov. nom.* from *I. schmutzenhoferi* was only accidental and that this fungus is only casually associated with this conifer bark beetle. Its unusual features for a *Ceratocystis* sp. associated with conifer bark beetles, especially its intensive aroma, and its close phylogenetic relationship to two

Ceratocystis spp. from hardwoods might support this suggestion. At present the ecology of *C. bhutanensis prov. nom.* remains enigmatic. Further investigations, especially isolations from various niches at the type locality of the fungus and within the entire distribution range of *I. schmutzenhoferi* in Western and Central Bhutan are desirable, in order to understand the incidence and ecology of *C. bhutanensis prov. nom.* and its relationships with *I. schmutzenhoferi* more thoroughly.

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Table 1. Isolates of *Ceratocystis* used in this study.

Species	Isolate no.[d]	Alternative numbers[e]	GenBank accession nr.	Year of isolation	Host	Geographical origin	Associated insect	Collector(s)
C. moniliformis	CMW 8240[a]	None	AY528989[e] AY529000[f] AY529010[g]	2001	*Cassia fistula*	Punakha, Bhutan	None	M. J. Wingfield, T. Kirisits & D. B. Chhetri
"	CMW 8238[c]	"	N/A	"	"	"	"	"
"	CMW 9590[a, c]	"	AY528985[e] AY528996[f] AY529006[g]	2002	*Eucalyptus grandis*	Mpumalanga, South Africa	"	J. Roux
"	CMW 4114[a]	"	AY528986[e] AY528997[f] AY529007[g]	1997	*Schizolobium parahybum*	Ecuador, South America	"	M. J. Wingfield
"	CMW 10134[c]	"	N/A	2002	*Eucalyptus grandis*	Mpumalanga, South Africa	"	M. van Wyk
C. moniliformopsis	CMW 9986[a, c]	CBS 109441	AY528987[e] AY528998[f] AY529008[g]	1999	*Eucalyptus obliqua*	Tazmania, Australia	"	Z. Q. Yuan
"	CMW 10214[a, c]	None	AY528988[e] AY528999[f] AY529009[g]	1989	*Eucalyptus sieberi*	Victoria, Australia	"	M. J. Dudzinski
"	CMW 10215[c]	"	N/A	1990	"	"	"	"
C. bhutanensis prov. nom.	CMW 8215[a]	PREM 57805	AY528953[e] AY528958[f] AY528963[g]	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	M. J. Wingfield , T. Kirisits & D. B. Chhetri
"	CMW 8242[a, b]	CBS 112907 PREM 57809	AY528951[e] AY528956[f] AY528961[g]	"	"	"	"	"
"	CMW 8217[a, b, c]	PREM 57807	AY528952[e] AY528957[f] AY528962[g]	"	"	"	"	"

Table 1. (Continued) Isolates of *Ceratocystis* used in this study.

Species	Isolate no.[d]	Alternative numbers[e]	GenBank accession nr.	Year of isolation	Host	Geographical origin	Associated insect	Collector(s)
C. bhutanensis prov. nom.	CMW 8241[a, b, c]	PREM 57808	N/A	2001	*Picea spinulosa*	Jelekha, Bhutan	*Ips schmutzenhoferi*	M. J. Wingfield, T. Kirisits & D. B. Chhetri
"	CMW 8396[a]	BH 8/5 PREM 57812	N/A	"	"	"	"	"
"	CMW 8399[a]	BH 8/8	AY528954[e] AY528959[f] AY528964[g]	"	"	"	"	"
"	CMW 8243[a, b]	CBS 112908 PREM 57810	N/A	"	"	"	"	"
"	CMW 8108[a, b]	CBS 112905	N/A	"	"	"	"	"
"	CMW 8244[a, b, c]	PREM 57811	N/A	"	"	"	"	"
C. virescens	CMW 3276[a]	None	AY528984[e] AY528990[f] AY528991[g]	1963	*Quercus* sp.	Warrenber, N.Y., USA	None	T. Hinds

N/A refers to accession numbers not available at present.

[a, b, c, d, e, f, g] Isolates marked with [a] were sequenced, those marked with [b] were used for morphological descriptions and those marked with [c] were included in the growth studies, [d] CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. [e] CBS refers to the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, BH to the culture collection of the Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Universität für Bodenkultur Wien (BOKU), Vienna, Austria and PREM to the National Fungal Herbarium (PREM), Pretoria, South Africa. GenBank accession numbers that are marked with [e] represent the ITS sequences, those marked with an [f] represent the β-tubulin sequences and those marked with a [g] represent the elongation factor sequences.

Table 2. Comparison of *C. bhutanensis prov. nom.* with morphologically similar species, *C. moniliformis* and *C. moniliformopsis.*

Characteristic	***C. bhutanensis prov. nom.***	***C. moniliformis*** (Hedgcock 1906)	***C. moniliformopsis*** (Yuan & Mohammed 2002)
ASCOMATA			
Base			
Colour	Dark brown to black	Brown to black	Dark brown to black
Diameter	138 - 178 µm	90 - 180 µm	200 - 300 µm
Ornamentation	Conical spines and hyphal hairs	Conical spines (sparse)	Hyphal hairs & conical spines
Form	Globose	Globose	Ovoid
Neck			
Colour	Dark brown to black becoming light brown towards apex	Light brown becoming transparent at the apex [a]	Dark brown to black
Disc-form at base	Yes	Yes [a]	Yes
Length	453 - 519 µm	730 - 896 µm [a]	470 - 780 µm
Width (Tip)	12 - 14 µm	14 µm [a]	18 - 22 µm
Width (Base)	34 - 42 µm	39.2 - 51.8 µm [a]	40 - 50 µm
Ostiolar hyphae			
Shape	Divergent	Divergent	Convergent
Measurement	18 - 26 µm	12 - 18 x 2 µm	25 - 45 x 1.5 - 2 µm
Ascus	Not seen	Fugacious	Not Seen
Ascospores			
Colour	Hyaline	Hyaline	Hyaline
Shape (Side view)	Hat-shaped	Oval, one side flat	Hat-shaped
Measurements	4 - 6 x 2 - 5 µm	4 - 5 x 3 - 4 µm	4 - 5 x 2 - 2.5 µm
Texture	Mucilaginous	Slimy grey mass	Gelatinous sheath

[a] Description by Bakshi (1951).

Table 2 (continued). Comparison of *C. bhutanensis prov. nom.*with morphologically similar species, *C. moniliformis* and *C. moniliformopsis.*

Characteristic	***C. bhutanensis prov. nom.***	***C. moniliformis*** (Hedgcock 1906)	***C. moniliformopsis*** (Yuan & Mohammed 2002)
CONIDIOMATA			
Conidiophores			
Measurements	23 - 39 x 4 - 6 µm	(1) 3 - 13.7 x 3.5 - 8.9 µm [a] (2) 7.3 - 13.7 x 4.5 µm [a]	5 - 32.5 x 4 - 5.3 µm
Shape	Phialides	Phialides (2 types)	Phialides (2 types)
Conidia			
Shape	(1) Cylindrical (2) Barrel-shaped	(1) Oval or cylindrical [a] (2) Cylindrical [a]	(1) Cylindrical (2) Oblong or ellipsoidal
Measurements	(1) 7 - 9 x 1 - 3 µm (2) 3 - 5 x 2 - 3 µm	(1) 7.3 - 13.7 x 3.5 - 8.9 µm [a] (2) 4.3 - 15.5 x 1 - 2.5 µm [a]	(1) 13 - 21 x 2 - 3 µm (2) 12 - 17.5 x 5 - 7.5 µm
CULTURE			
Growth rate	20 mm per day at 25 °C in the dark	60 mm in 10 days at 22 °C in the dark [a]	6.3 - 7.5 mm per day at 22 °C in the dark
Colour	Cream-buff to dark olive to black	Hyaline to grey to black	Colourless to white grey, centre becoming greenish brown
Odour	Fermenting odour	Pear drops [a]	None
Mycelia	Smooth and granulated	Coarsely granular	Smooth

[a] Description by Bakshi (1951).

Figure 1. a) A map of Bhutan with all the administrative districts (dzongkhags) of the country and Bhutan´s capital Thimphu, b) A map of the dzongkhags Thimphu and Wangdue Phodrang (Wangdi) showing the localities where samples for fungal isolation were collected from *Picea spinulosa* and *Pinus wallichiana. Ceratocystis bhutanensis prov. nom.* was only isolated from individuals of *Ips schmutzenhoferi* obtained from Jelekha.

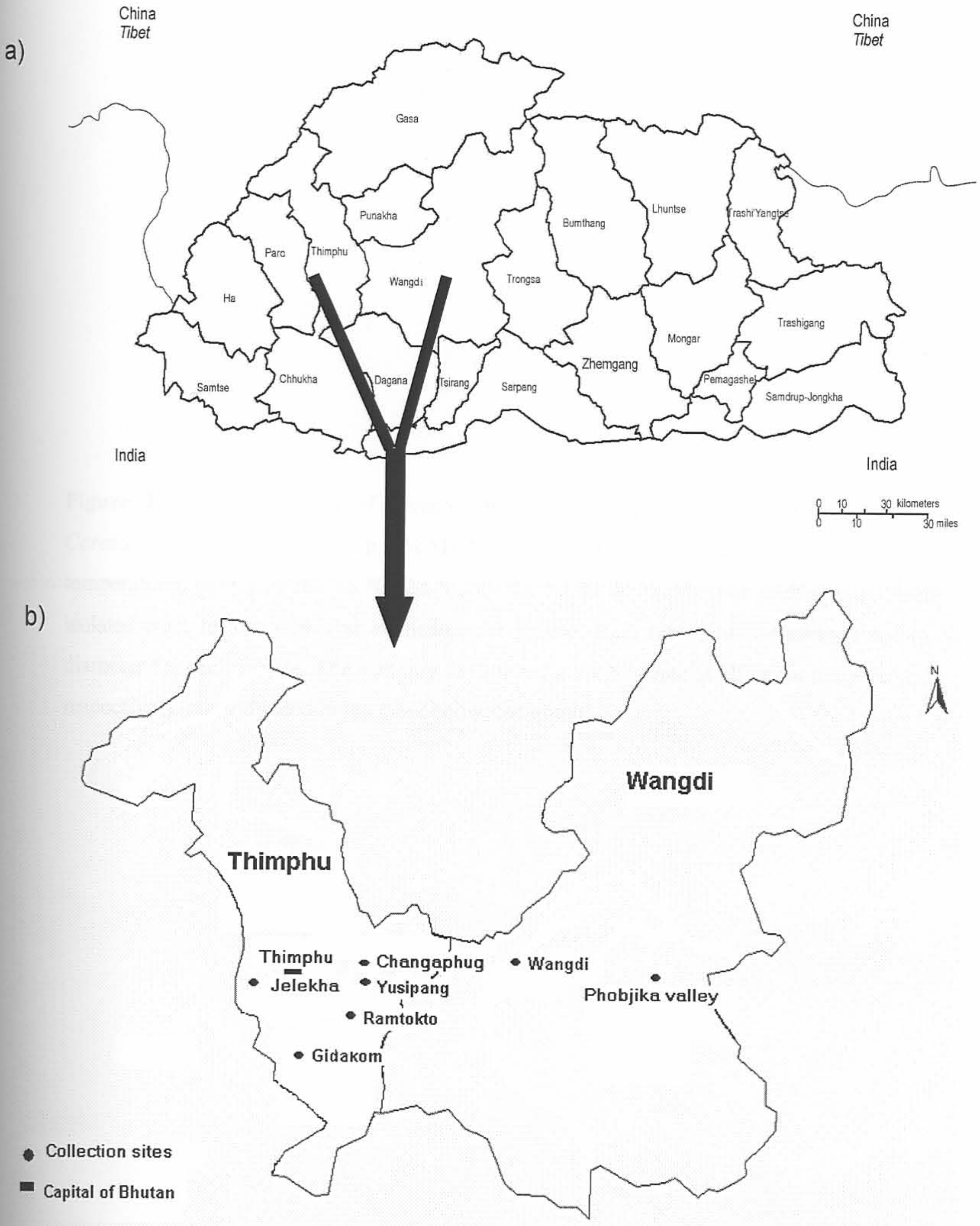
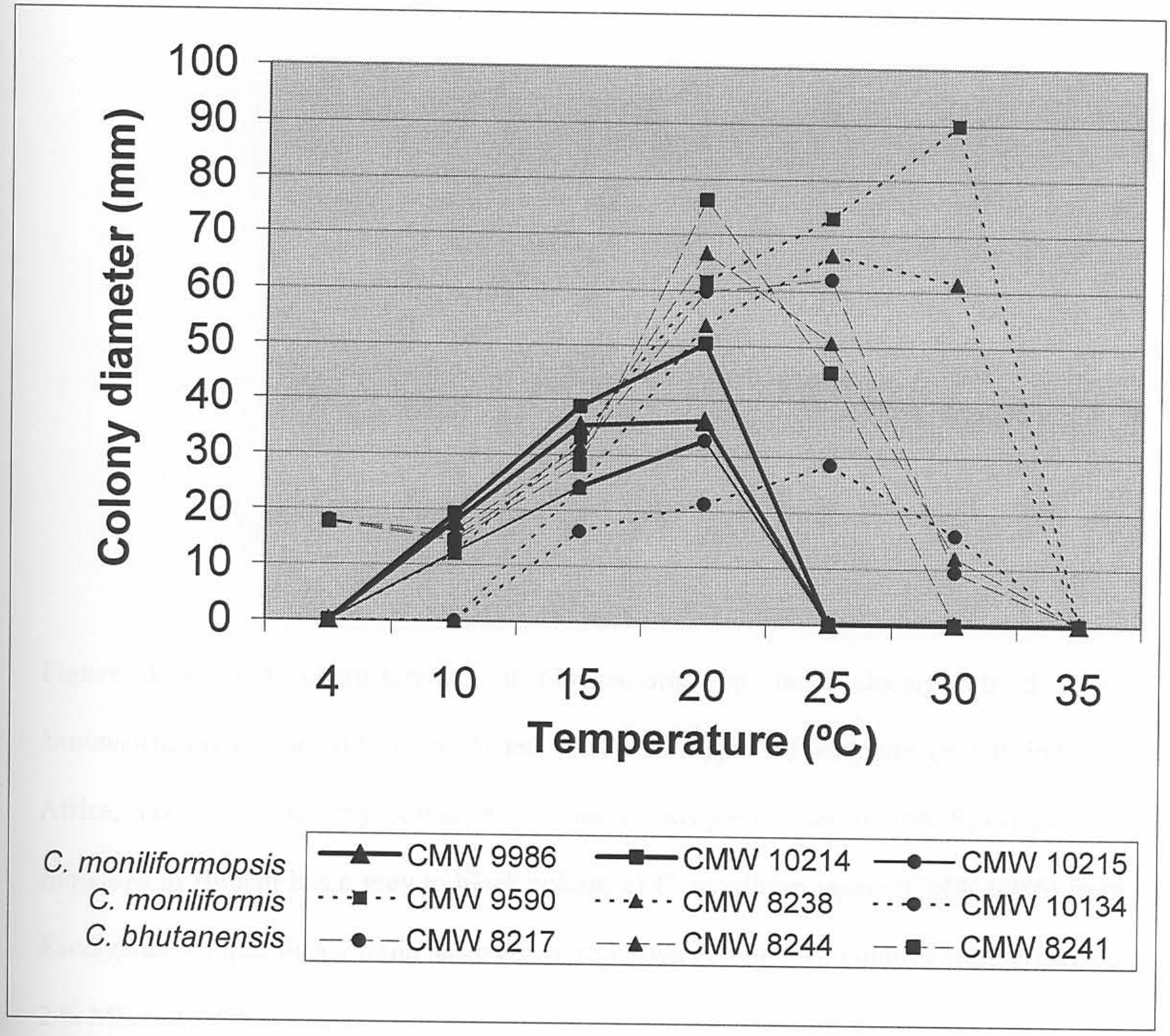

a)
China
Tibet
China
Tibet
Gasa
Punakha
Bumthang
Lhuntse
Trashi'Yangtse
Paro
Thimphu
Wangdi
Trongsa
Ha
Trashigang
Mongar
Zhemgang
Samtse
Chhukha
Dagana
Tsirang
Sarpang
Pemagashel
Samdrup-Jongkha
India
India
0 10 30 kilometers
0 10 30 miles
b)
N
Wangdi
Thimphu
Thimphu
Changaphug
Wangdi
Jelekha
Yusipang
Phobjika valley
Ramtokto
Gidakom
● Collection sites
▬ Capital of Bhutan

Figure 2. Colony diameter of *Ceratocystis moniliformopsis*, *C. moniliformis* and the *Ceratocystis* sp. from Bhutan on 2 % MEA after three days of incubation at eight different temperatures (4 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C & 35 °C). For each species three isolates were tested, with five replicates per isolate. Bars represent the average colony diameter for each isolate. The standard deviation for each isolate at all seven temperatures respectively, are indicated in the table below the graph.



Temperature/ Isolate	4 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
CMW 9986	0.0	0.2	2.1	1.3	0.0	0.0	0.0
CMW 10214	0.0	4.7	0.9	2.8	0.0	0.0	0.0
CMW 10215	0.0	1.0	2.0	5.2	0.0	0.0	0.0
CMW 9590	0.0	3.0	0.8	0.89	1.2	0.0	0.0
CMW 8238	0.0	0.0	2.6	0.3	1.5	1.0	0.0
CMW 10134	0.0	0.0	1.9	5.5	15.5	0.6	0.0
CMW 8217	1.6	1.5	1.0	1.2	2.7	0.4	0.0
CMW 8244	2.1	0.8	2.5	3.4	2.9	0.9	0.0
CMW 8241	1.2	0.5	2.7	5.9	3.6	0.0	0.0

Figure 3. Cultural characteristics of *Ceratocystis* spp. most closely related to *C. bhutanensis prov. nom.* a) *C. moniliformis* (CMW 9590) *from Eucalyptus grandis* in South Africa, has a white to grey colour, b) *C. bhutanensis prov. nom.* (CMW 8217) from *P. spinulosa* in Bhutan has a grey to black colour, c) *C. moniliformopsis* (CMW 9986) from *Eucalyptus obliqua* in Australia has a cream to brown colour. All cultures were grown on 2 % MEA at 20 °C for approximately 10 days.

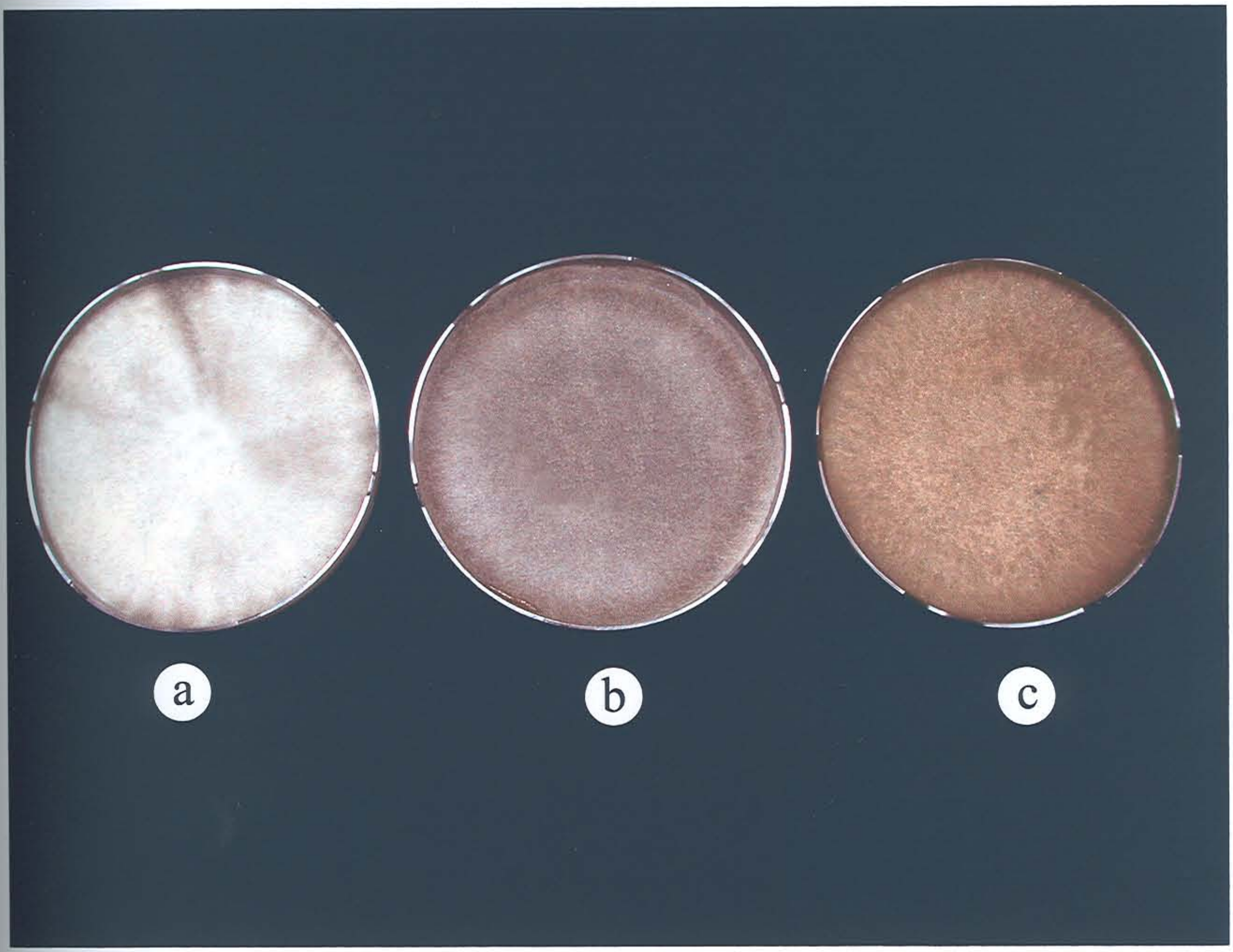

a
b
c

Figure 4. Morphological characteristics of *C. bhutanensis prov. nom.* (PREM 57807, CMW 8217), a) Globose ascomata with long neck (scalebar = 40 µm), b) Ascomatal base with short, conical spines and hyphal ornamentation (scalebar = 40 µm), c) Divergent ostiolar hyphae on the top of the ascomatal neck (scalebar = 10 µm), d) Hat-shaped ascospore in side view (scalebar = 5 µm), e) Hyphae with smooth edges (scalebar = 5 µm), f) Hyphae with rough edges (scalebar = 5 µm), g) Cylindrical conidia forming a chain (scalebar = 5 µm), h) Barrel-shaped conidia (scalebar = 5 µm), i) Phialidic conidiophore with emerging cylindrical conidium (scalebar = 10 µm)

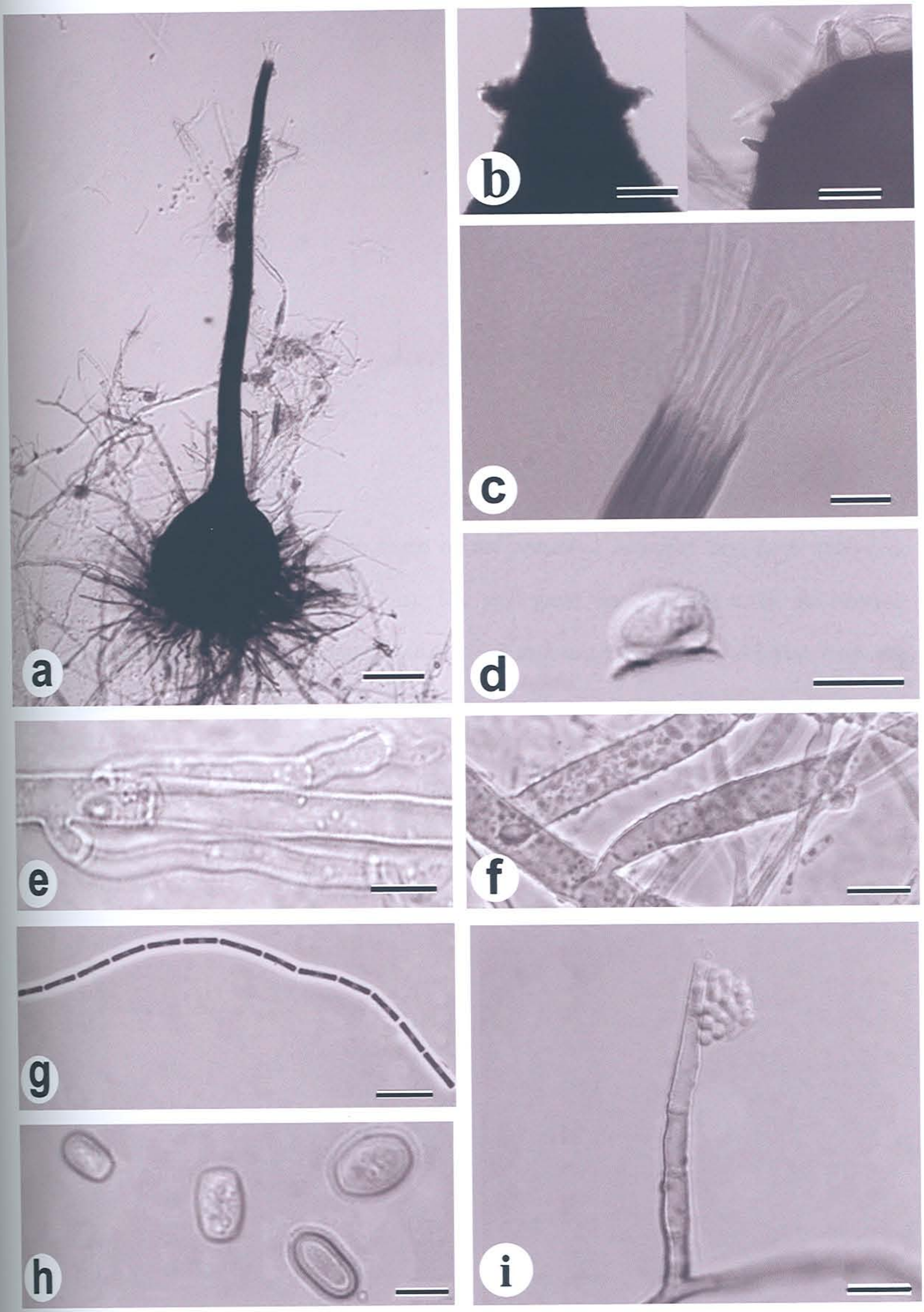
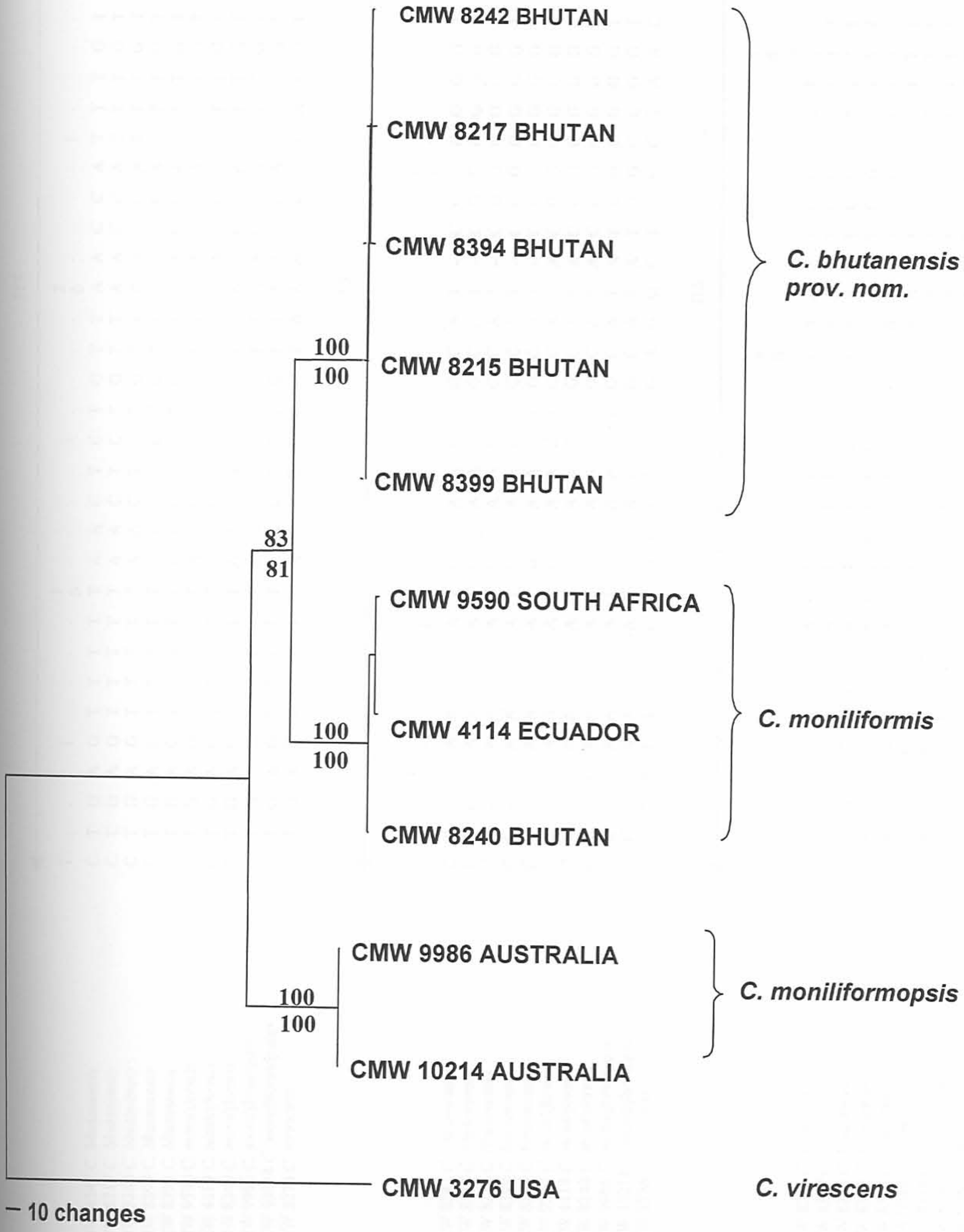

a
b
c
d
e
f
g
h
i

Figure 5. A phylogenetic tree based on the combined sequence data from three gene regions (ITS, β-tubulin and EF1-α). The phylogram was obtained using the heuristic search option based on parsimony and *C. virescens* was treated as the out-group. Bootstrap values are indicated above of the branches while Bayesian values are indicated below the branches.


CMW 8242 BHUTAN
CMW 8217 BHUTAN
CMW 8394 BHUTAN
CMW 8215 BHUTAN
CMW 8399 BHUTAN
100
100
C. bhutanensis prov. nom.
83
81
CMW 9590 SOUTH AFRICA
CMW 4114 ECUADOR
CMW 8240 BHUTAN
100
100
C. moniliformis
CMW 9986 AUSTRALIA
CMW 10214 AUSTRALIA
100
100
C. moniliformopsis
CMW 3276 USA
C. virescens
10 changes

ITS

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CMW 8242 *C. bhutanensis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 8217 *C. bhutanensis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 8215 *C. bhutanensis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 8399 *C. bhutanensis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 8394 *C. bhutanensis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 9590 *C. moniliformis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 4114 *C. moniliformis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 8240 *C. moniliformis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 9986 *C. moniliformopsis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 10214 *C. moniliformopsis*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	G	T	T	A	A	C	C	A	T	T	T	G	T	G	A	A	T	T	T	A
CMW 3276 *C. virescens*	C	T	G	A	G	T	T	T	T	T	A	A	C	T	C	T	-	T	A	A	A	C	C	A	T	A	T	G	T	G	A	A	C	A	T	A

ITS

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CMW 8242 *C. bhutanensis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	-	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 8217 *C. bhutanensis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	-	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 8215 *C. bhutanensis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	-	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 8399 *C. bhutanensis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	-	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 8394 *C. bhutanensis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	-	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 9590 *C. moniliformis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	**A**	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 4114 *C. moniliformis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	**A**	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 8240 *C. moniliformis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	**A**	**C**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 9986 *C. moniliformopsis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	**A**	**T**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 10214 *C. moniliformopsis*	C	C	-	-	A	A	A	C	A	T	G	-	A	A	C	T	G	C	A	T	**A**	**T**	G	G	C	G	G	G	T	C	T	C	C	C	G	C
CMW 3276 *C. virescens*	C	C	T	A	T	T	A	G	C	T	G	C	T	T	T	G	G	A	G	G	**C**	**T**	T	G	G	T	A	A	C	A	C	A	A	A	G	T

ITS

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CMW 8242 *C. bhutanensis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 8217 *C. bhutanensis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 8215 *C. bhutanensis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 8399 *C. bhutanensis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 8394 *C. bhutanensis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 9590 *C. moniliformis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 4114 *C. moniliformis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 8240 *C. moniliformis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 9986 *C. moniliformopsis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 10214 *C. moniliformopsis*	C	A	G	-	C	A	G	T	A	C	T	C	T	T	G	-	-	-	A	A	C	T	C	G	T	T	T	T	T	-	-	A	T	A	T	A
CMW 3276 *C. virescens*	C	T	G	C	C	G	G	T	A	G	T	A	T	T	T	T	A	A	A	A	C	T	C	T	T	T	T	T	T	T	T	A	T	T	C	T

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	.	110	.	.	.	.	|	.	.	.	.	120	.	.	.	.	|	.	.	.	.	130	.	.	.	.	|	.	.	.	.	140	.	.	.	.
CMW 8242 *C. bhutanensis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 8217 *C. bhutanensis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 8215 *C. bhutanensis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 8399 *C. bhutanensis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 8394 *C. bhutanensis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**T**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 9590 *C. moniliformis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 4114 *C. moniliformis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 8240 *C. moniliformis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 9986 *C. moniliformopsis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 10214 *C. moniliformopsis*	A	A	G	A	A	T	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	A	G	C	A	T	T	T	T	A	T	A	A	A	T
CMW 3276 *C. virescens*	A	A	A	G	A	A	T	T	A	T	T	C	A	T	T	G	C	T	G	A	**G**	T	G	G	C	A	T	T	A	A	C	A	T	A	A	T

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	|	.	.	.	.	150	.	.	.	.	|	.	.	.	.	160	.	.	.	.	|	.	.	.	.	170	.	.	.	.	|	.	.	.	.	180
CMW 8242 *C. bhutanensis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 8217 *C. bhutanensis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 8215 *C. bhutanensis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 8399 *C. bhutanensis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 8394 *C. bhutanensis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 9590 *C. moniliformis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 4114 *C. moniliformis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 8240 *C. moniliformis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 9986 *C. moniliformopsis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 10214 *C. moniliformopsis*	G	T	A	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A
CMW 3276 *C. virescens*	A	A	G	T	T	A	A	A	A	C	T	T	T	C	A	A	C	A	A	C	G	G	A	T	C	T	C	T	T	G	G	C	T	C	T	A

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CMW 8242 *C. bhutanensis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 8217 *C. bhutanensis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 8215 *C. bhutanensis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 8399 *C. bhutanensis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 8394 *C. bhutanensis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 9590 *C. moniliformis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 4114 *C. moniliformis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 8240 *C. moniliformis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 9986 *C. moniliformopsis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 10214 *C. moniliformopsis*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	A	G	T	A	A
CMW 3276 *C. virescens*	G	C	A	T	C	G	A	T	G	A	A	G	A	A	C	G	C	A	G	C	G	A	A	A	T	G	C	G	A	T	A	C	G	T	A	A

				220										230										240										250		
CMW 8242 *C. bhutanensis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 8217 *C. bhutanensis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 8215 *C. bhutanensis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 8399 *C. bhutanensis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 8394 *C. bhutanensis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 9590 *C. moniliformis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 4114 *C. moniliformis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 8240 *C. moniliformis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 9986 *C. moniliformopsis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 10214 *C. moniliformopsis*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T
CMW 3276 *C. virescens*	T	G	T	G	A	A	T	T	G	C	A	G	A	A	T	T	C	A	G	T	G	A	A	T	C	A	T	C	G	A	A	T	C	T	T	T

ITS

								260										270										280								
CMW 8242 *C. bhutanensis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 8217 *C. bhutanensis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 8215 *C. bhutanensis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 8399 *C. bhutanensis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 8394 *C. bhutanensis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 9590 *C. moniliformis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 4114 *C. moniliformis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 8240 *C. moniliformis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 9986 *C. moniliformopsis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 10214 *C. moniliformopsis*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	C	A	G	C	A	G	T	A	C	T	C	T	G	C	T	G	G	G	C	A
CMW 3276 *C. virescens*	G	A	A	C	G	C	A	C	A	T	T	G	C	G	C	C	T	G	G	C	A	G	T	A	T	T	C	A	G	C	C	A	G	G	C	A

ITS

		290										300										310										320				
CMW 8242 *C. bhutanensis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 8217 *C. bhutanensis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 8215 *C. bhutanensis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 8399 *C. bhutanensis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 8394 *C. bhutanensis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 9590 *C. moniliformis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 4114 *C. moniliformis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 8240 *C. moniliformis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	T	G
CMW 9986 *C. moniliformopsis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**A**	T	G
CMW 10214 *C. moniliformopsis*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**A**	T	G
CMW 3276 *C. virescens*	T	G	C	C	T	G	T	C	C	G	A	G	C	G	T	C	A	T	T	T	C	A	C	C	A	C	T	C	A	A	G	C	T	**C**	C	G

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	|	.	.	.	.	330	.	.	.	.	|	.	.	.	.	340	.	.	.	.	|	.	.	.	.	350	.	.	.	.	|	.	.	.	.	360
CMW 8242 *C. bhutanensis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 8217 *C. bhutanensis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 8215 *C. bhutanensis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 8399 *C. bhutanensis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 8394 *C. bhutanensis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 9590 *C. moniliformis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 4114 *C. moniliformis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 8240 *C. moniliformis*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**A**	**G**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 9986 *C. moniliformopsis*	C	T	T	G	**G**	T	G	T	T	G	G	A	G	**G**	**A**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 10214 *C. moniliformopsis*	C	T	T	G	**G**	T	G	T	T	G	G	A	G	**G**	**A**	C	C	T	G	T	G	C	T	A	T	G	C	G	C	G	G	G	C	C	T	C
CMW 3276 *C. virescens*	C	T	T	G	**T**	T	G	T	T	G	G	A	G	**G**	**A**	C	C	T	G	T	T	G	T	T	T	T	C	A	A	C	A	G	G	C	C	A

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CMW 8242 *C. bhutanensis*	T	-	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 8217 *C. bhutanensis*	T	-	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 8215 *C. bhutanensis*	T	-	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 8399 *C. bhutanensis*	T	-	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 8394 *C. bhutanensis*	T	-	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 9590 *C. moniliformis*	T	-	G	A	A	A	T	G	**T**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 4114 *C. moniliformis*	T	-	G	A	A	A	T	G	**T**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 8240 *C. moniliformis*	T	-	G	A	A	A	T	G	**T**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 9986 *C. moniliformopsis*	T	-	G	A	A	A	T	G	**T**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 10214 *C. moniliformopsis*	T	-	G	A	A	A	T	G	**T**	A	T	C	G	G	C	T	G	T	G	T	T	A	C	A	T	G	C	A	G	T	T	T	C	C	C	T
CMW 3276 *C. virescens*	C	C	G	A	A	A	T	G	**C**	A	T	C	G	G	C	T	G	T	T	A	T	A	C	T	T	G	C	A	G	C	T	T	C	C	C	T

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CMW 8242 *C. bhutanensis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 8217 *C. bhutanensis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 8215 *C. bhutanensis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 8399 *C. bhutanensis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 8394 *C. bhutanensis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 9590 *C. moniliformis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**T**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 4114 *C. moniliformis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**C**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 8240 *C. moniliformis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**C**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 9986 *C. moniliformopsis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**C**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 10214 *C. moniliformopsis*	G	T	G	T	A	G	T	A	A	A	A	C	T	T	T	A	**C**	T	G	T	T	G	C	A	C	T	T	T	G	A	A	A	T	T	C	T
CMW 3276 *C. virescens*	G	T	G	T	A	G	T	A	A	-	-	-	T	A	T	C	**T**	A	T	T	T	A	C	A	C	T	T	T	G	A	A	A	C	T	C	T

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CMW 8242 *C. bhutanensis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 8217 *C. bhutanensis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 8215 *C. bhutanensis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 8399 *C. bhutanensis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 8394 *C. bhutanensis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 9590 *C. moniliformis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**C**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 4114 *C. moniliformis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**C**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 8240 *C. moniliformis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**C**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	**T**
CMW 9986 *C. moniliformopsis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	-
CMW 10214 *C. moniliformopsis*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	T	A	A	A	C	A	A	C	C	C	-	-	A	A	T	T	T	T	T	T	-
CMW 3276 *C. virescens*	T	G	T	A	C	T	A	C	A	T	G	C	C	G	**T**	-	A	A	-	-	A	A	C	C	C	T	C	A	A	T	T	C	T	T	T	-

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CMW 8242 *C. bhutanensis*	**T**	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 8217 *C. bhutanensis*	**T**	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 8215 *C. bhutanensis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**T**	A	A	T	A	C	C	C	G	C	T	G
CMW 8399 *C. bhutanensis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 8394 *C. bhutanensis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**C**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 9590 *C. moniliformis*	**T**	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 4114 *C. moniliformis*	**T**	**T**	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 8240 *C. moniliformis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 9986 *C. moniliformopsis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 10214 *C. moniliformopsis*	-	-	A	G	G	T	T	G	A	C	C	T	C	**G**	G	A	T	C	A	G	G	T	A	G	**G**	A	A	T	A	C	C	C	G	C	T	G
CMW 3276 *C. virescens*	-	-	A	-	-	T	T	G	A	A	A	T	T	**T**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G

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CMW 8242 *C. bhutanensis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 8217 *C. bhutanensis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 8215 *C. bhutanensis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 8399 *C. bhutanensis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 8394 *C. bhutanensis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 9590 *C. moniliformis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 4114 *C. moniliformis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 8240 *C. moniliformis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**C**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 9986 *C. moniliformopsis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**T**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 10214 *C. moniliformopsis*	A	A	C	-	-	T	T	A	A	G	C	A	T	A	T	T	T	**T**	G	C	T	C	C	T	C	T	G	A	C	C	A	G	C	C	G	T
CMW 3276 *C. virescens*	A	A	A	G	G	T	T	G	A	-	-	-	-	-	-	T	T	**T**	G	C	T	C	C	C	C	T	G	A	C	C	A	G	T	C	G	C

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CMW 8242 *C. bhutanensis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 8217 *C. bhutanensis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 8215 *C. bhutanensis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 8399 *C. bhutanensis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 8394 *C. bhutanensis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 9590 *C. moniliformis*	G	G	C	G	C	**T**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 4114 *C. moniliformis*	G	G	C	G	C	**T**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 8240 *C. moniliformis*	G	G	C	G	C	**T**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 9986 *C. moniliformopsis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 10214 *C. moniliformopsis*	G	G	C	G	C	**C**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	T	G	T	C	A	G	C	G	T	T	C	C	T	G	A	G
CMW 3276 *C. virescens*	G	G	T	G	C	**T**	C	A	C	T	C	T	T	T	C	C	G	C	G	C	C	G	T	C	A	G	C	G	T	G	C	C	C	G	A	G

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CMW 8242 *C. bhutanensis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 8217 *C. bhutanensis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 8215 *C. bhutanensis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 8399 *C. bhutanensis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 8394 *C. bhutanensis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 9590 *C. moniliformis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**T**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 4114 *C. moniliformis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**T**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 8240 *C. moniliformis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**T**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 9986 *C. moniliformopsis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 10214 *C. moniliformopsis*	C	T	C	A	C	C	C	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G
CMW 3276 *C. virescens*	C	T	C	A	C	C	A	A	G	C	A	G	A	T	G	T	T	C	G	A	C	C	C	**C**	A	A	G	A	A	C	A	T	G	A	T	G

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CMW 8242 *C. bhutanensis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 8217 *C. bhutanensis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 8215 *C. bhutanensis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 8399 *C. bhutanensis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 8394 *C. bhutanensis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 9590 *C. moniliformis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 4114 *C. moniliformis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 8240 *C. moniliformis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 9986 *C. moniliformopsis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 10214 *C. moniliformopsis*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	C
CMW 3276 *C. virescens*	G	C	T	G	C	T	T	C	C	G	A	T	T	T	C	C	G	C	A	A	C	G	G	T	C	G	C	T	A	C	C	T	G	A	C	T

β-tubulin

```
                                6                   6                   6                   6
                              . 5 . . . . | . . . . 6 . . . . | . . . . 7 . . . . | . . . . 8 . . . .
                                0                   0                   0                   0
CMW 8242 C. bhutanensis       T G C T C T G C C A T T T T G T A A G T T - - - - - - - - - C C T T C T
CMW 8217 C. bhutanensis       T G C T C T G C C A T T T T G T A A G T T - - - - - - - - - C C T T C T
CMW 8215 C. bhutanensis       T G C T C T G C C A T T T T G T A A G T T - - - - - - - - - C C T T C T
CMW 8399 C. bhutanensis       T G C T C T G C C A T T T T G T A A G T T - - - - - - - - - C C T T C T
CMW 8394 C. bhutanensis       T G C T C T G C C A T T T T G T A A G T T - - - - - - - - - C C T T C T
CMW 9590 C. moniliformis      T G C T C T G C C A T C T T G T A A G T T A T - - - - - - - - - - - C T
CMW 4114 C. moniliformis      T G C T C T G C C A T C T T G T A A G T T A T - - - - - - - - - - - C T
CMW 8240 C. moniliformis      T G C T C T G C C A T C T T G T A A G T T A T - - - - - - - - - - - C T
CMW 9986 C. moniliformopsis   T G T T C T G C C A T C T T G T A A G T T A T T T T T G G A G G A A C T
CMW 10214 C. moniliformopsis  T G T T C T G C C A T C T T G T A A G T T A T T T T T G G A G G A A C T
CMW 3276 C. virescens         T G C T C T G C T A T C T T G T A T G T T - T T T - - - C A C C A A C T
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β-tubulin

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                                        6                   7                   7                   7
                              | . . . . 9 . . . . | . . . . 0 . . . . | . . . . 1 . . . . | . . . . 2
                                        0                   0                   0                   0
CMW 8242 C. bhutanensis       C C C C T G C A T C - A - T A C T T G G A G T A T C A T T C A C T A A C
CMW 8217 C. bhutanensis       C C C C T G C A T C - A - T A C T T G G A G T A T C A T T C A C T A A C
CMW 8215 C. bhutanensis       C C C C T G C A T C - A - T A C T T G G A G T A T C A T T C A C T A A C
CMW 8399 C. bhutanensis       C C C C T G C A T C - A - T A C T T G G A G T A T C A T T C A C T A A C
CMW 8394 C. bhutanensis       C C C C T G C A T C - A - T A C T T G G A G T A T C A T T C A C T A A C
CMW 9590 C. moniliformis      T T T T C   T T T G T A G T A T T T G G A G C A T G A T T C A C T A A C
CMW 4114 C. moniliformis      T T T T C - T T T G T A G T A T T T G G A G C A T G A T T C A C T A A C
CMW 8240 C. moniliformis      T T T T C - T T T G T A G T A T T T G G A G C A T G A T T C A C T A A C
CMW 9986 C. moniliformopsis   T T T T C G T T T C - A - T A T T T G G A G C A - C A T T G A C T A A C
CMW 10214 C. moniliformopsis  T T T T C G T T T C - A - T A T T T G G A G C A - C A T T G A C T A A C
CMW 3276 C. virescens         - - - - - - - - - - - A - T A T T T G - T C T A T C A T T - A G T - - -
```

β-tubulin

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                                                  7                   7                   7
                              . . . . | . . . . 3 . . . . | . . . . 4 . . . . | . . . . 5 . . . . | .
                                                  0                   0                   0
CMW 8242 C. bhutanensis       A T T C A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 8217 C. bhutanensis       A T T C A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 8215 C. bhutanensis       A T T C A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 8399 C. bhutanensis       A T T C A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 8394 C. bhutanensis       A T T C A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 9590 C. moniliformis      A T - - A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 4114 C. moniliformis      A T - - A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 8240 C. moniliformis      A T - - A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 9986 C. moniliformopsis   A T A T A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 10214 C. moniliformopsis  A T A T A T T T T C - - - - - - - - - - - - - - - - - - - - - - - T C T
CMW 3276 C. virescens         T T A T A T T T G C A T G G A T C T T T G C T A A C A C C T C T T T C T
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β-tubulin

	.	.	.	760	.	.	.	.	|	.	.	.	.	770	.	.	.	.	|	.	.	.	.	780	.	.	.	.	|	.	.	.	.	790	.	.
CMW 8242 *C. bhutanensis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 8217 *C. bhutanensis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 8215 *C. bhutanensis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 8399 *C. bhutanensis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 8394 *C. bhutanensis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 9590 *C. moniliformis*	-	-	-	-	-	**C**	**T**	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 4114 *C. moniliformis*	-	-	-	-	-	**C**	**T**	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 8240 *C. moniliformis*	-	-	-	-	-	**C**	**T**	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 9986 *C. moniliformopsis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 10214 *C. moniliformopsis*	-	-	-	-	-	-	-	-	-	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G
CMW 3276 *C. virescens*	T	T	C	G	T	**C**	**T**	T	T	A	T	A	G	C	C	G	T	G	G	T	A	A	G	G	T	C	G	C	C	A	T	G	A	A	G	G

β-tubulin

	.	.	|	.	.	.	.	800	.	.	.	.	|	.	.	.	.	810	.	.	.	.	|	.	.	.	.	820	.	.	.	.	|	.	.	.
CMW 8242 *C. bhutanensis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 8217 *C. bhutanensis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 8215 *C. bhutanensis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 8399 *C. bhutanensis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 8394 *C. bhutanensis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 9590 *C. moniliformis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 4114 *C. moniliformis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 8240 *C. moniliformis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 9986 *C. moniliformopsis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 10214 *C. moniliformopsis*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A
CMW 3276 *C. virescens*	A	G	G	T	T	G	A	G	G	A	C	C	A	G	A	T	G	C	G	C	A	A	C	G	T	C	C	A	G	A	A	C	A	A	G	A

β-tubulin

	.	830	.	.	.	.	|	.	.	.	.	840	.	.	.	.	|	.	.	.	.	850	.	.	.	.	|	.	.	.	.	860	.	.	.	.
CMW 8242 *C. bhutanensis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 8217 *C. bhutanensis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 8215 *C. bhutanensis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 8399 *C. bhutanensis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 8394 *C. bhutanensis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**T**	C	C	C	A	A	C	A	A	C	G
CMW 9590 *C. moniliformis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 4114 *C. moniliformis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**T**	C	C	C	A	A	C	A	A	C	G
CMW 8240 *C. moniliformis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**T**	C	C	C	A	A	C	A	A	C	G
CMW 9986 *C. moniliformopsis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 10214 *C. moniliformopsis*	A	C	T	C	C	T	C	T	T	A	C	T	T	C	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G
CMW 3276 *C. virescens*	A	C	T	C	C	T	C	T	T	A	C	T	T	T	G	T	T	G	A	G	T	G	G	A	T	**C**	C	C	C	A	A	C	A	A	C	G

β-tubulin

	|	.	.	.	.	870	.	.	.	.	|	.	.	.	.	880	.	.	.	.	|	.	.	.	.	890	.	.	.	.	|	.	.	.	.	900
CMW 8242 *C. bhutanensis*	T	C	C	A	G	A	C	C	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 8217 *C. bhutanensis*	T	C	C	A	G	A	C	C	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 8215 *C. bhutanensis*	T	C	C	A	G	A	C	C	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 8399 *C. bhutanensis*	T	C	C	A	G	A	C	C	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 8394 *C. bhutanensis*	T	C	C	A	G	A	C	C	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 9590 *C. moniliformis*	T	C	C	A	G	A	C	C	G	C	**T**	C	T	**T**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 4114 *C. moniliformis*	T	C	C	A	G	A	C	C	G	C	**T**	C	T	**T**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 8240 *C. moniliformis*	T	C	C	A	G	A	C	C	G	C	**T**	C	T	**T**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 9986 *C. moniliformopsis*	T	C	C	A	G	A	C	C	G	C	**T**	C	T	**C**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 10214 *C. moniliformopsis*	T	C	C	A	G	A	C	C	G	C	**T**	C	T	**C**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C
CMW 3276 *C. virescens*	T	C	C	A	G	A	C	T	G	C	**C**	C	T	**G**	T	G	C	T	C	C	A	T	T	C	C	T	C	C	C	C	G	T	G	G	C	C

β-tubulin

	.	.	.	.	|	.	.	.	.	910	.	.	.	.	|	.	.	.	.	920	.	.	.	.	|	.	.	.	.	930	.	.	.	.	|	.
CMW 8242 *C. bhutanensis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**T**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 8217 *C. bhutanensis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**A**	T	T	C	G	T	**T**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 8215 *C. bhutanensis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**T**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 8399 *C. bhutanensis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**T**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 8394 *C. bhutanensis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**T**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 9590 *C. moniliformis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 4114 *C. moniliformis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 8240 *C. moniliformis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**G**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 9986 *C. moniliformopsis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**C**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 10214 *C. moniliformopsis*	T	C	A	A	G	A	T	G	T	C	T	T	C	**C**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	T	A	C	T	G
CMW 3276 *C. virescens*	T	C	A	A	G	A	T	G	T	C	G	T	C	**T**	A	C	**C**	T	T	C	G	T	**C**	G	G	T	A	A	C	T	C	G	A	C	T	G

β-tubulin

	.	.	.	940	.	.	.	.	|	.	.	.	.	950	.	.	.	.	|	.	.	.	.	960	.	.	.	.	|	.	.	.	.	970	.	.
CMW 8242 *C. bhutanensis*	C	**T**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 8217 *C. bhutanensis*	C	**T**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 8215 *C. bhutanensis*	C	**T**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 8399 *C. bhutanensis*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 8394 *C. bhutanensis*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 9590 *C. moniliformis*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**C**	T	T	C	A	A	G	C	G	**T**	A	T	T	G	G	**C**	G	A	G	C	A	G	T
CMW 4114 *C. moniliformis*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**C**	T	T	C	A	A	G	C	G	**T**	A	T	T	G	G	**C**	G	A	G	C	A	G	T
CMW 8240 *C. moniliformis*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**C**	T	T	C	A	A	G	C	G	**T**	A	T	T	G	G	**C**	G	A	G	C	A	G	T
CMW 9986 *C. moniliformopsis*	C	**T**	A	T	C	C	A	G	G	A	G	C	T	**C**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 10214 *C. moniliformopsis*	C	**T**	A	T	C	C	A	G	G	A	G	C	T	**C**	T	T	C	A	A	G	C	G	**C**	A	T	T	G	G	**T**	G	A	G	C	A	G	T
CMW 3276 *C. virescens*	C	**C**	A	T	C	C	A	G	G	A	G	C	T	**G**	T	T	C	A	A	G	C	G	**T**	A	T	T	G	G	**C**	G	A	G	C	A	G	T

β-tubulin

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CMW 8242 *C. bhutanensis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 8217 *C. bhutanensis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 8215 *C. bhutanensis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 8399 *C. bhutanensis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 8394 *C. bhutanensis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 9590 *C. moniliformis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 4114 *C. moniliformis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 8240 *C. moniliformis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 9986 *C. moniliformopsis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 10214 *C. moniliformopsis*	T	C	A	C	T	G	C	C	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T
CMW 3276 *C. virescens*	T	C	A	C	T	G	C	T	A	T	G	T	T	C	C	G	T	C	G	C	A	A	G	G	C	T	T	T	C	T	T	G	C	A	T	T

β-tubulin

	.	1010	.	.	.	.	|	.	.	.	.	1020	.	.	.	.	|	.	.	.	.	1030	.	.	.	.	|	.	.	.	.	1040	.	.	.	.
CMW 8242 *C. bhutanensis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 8217 *C. bhutanensis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 8215 *C. bhutanensis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 8399 *C. bhutanensis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 8394 *C. bhutanensis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 9590 *C. moniliformis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 4114 *C. moniliformis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 8240 *C. moniliformis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 9986 *C. moniliformopsis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 10214 *C. moniliformopsis*	G	G	T	A	C	A	C	T	A	A	C	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A
CMW 3276 *C. virescens*	G	G	T	A	C	A	C	T	G	G	T	G	A	G	G	G	T	A	T	G	G	A	C	G	A	G	A	T	G	G	A	G	T	T	C	A

β-tubulin

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	|	.	.	.	.	1050	.	.	.	.	|	.	.	.	.	1060	.	.	.	.	|	.	.	.	.	1070	.	.	.	.	|	.	.	.	.	1080
CMW 8242 *C. bhutanensis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 8217 *C. bhutanensis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 8215 *C. bhutanensis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 8399 *C. bhutanensis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 8394 *C. bhutanensis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 9590 *C. moniliformis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	**A**	C	C	C	C	A	A	A
CMW 4114 *C. moniliformis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	**A**	C	C	C	C	A	A	A
CMW 8240 *C. moniliformis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	**A**	C	C	C	C	A	A	A
CMW 9986 *C. moniliformopsis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 10214 *C. moniliformopsis*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	-	C	C	C	C	A	A	A
CMW 3276 *C. virescens*	C	T	G	A	G	G	C	G	A	G	A	A	G	T	T	C	G	A	G	A	A	T	A	A	G	T	C	T	**C**	C	C	C	C	A	T	C

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									1090										1100										1110						
CMW 8242 *C. bhutanensis*	A	**C**	C	-	A	A	**T**	**C**	C	C	G	C	G	A	**C**	A	**T**	C	A	**A**	**T**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 8217 *C. bhutanensis*	A	**C**	C	-	A	A	**T**	**C**	C	C	G	C	G	A	**C**	A	**T**	C	A	**A**	**T**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 8215 *C. bhutanensis*	A	**C**	C	-	A	A	**T**	**C**	C	C	G	C	G	A	**C**	A	**T**	C	A	**A**	**T**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 8399 *C. bhutanensis*	A	**C**	C	-	A	A	**T**	**C**	C	C	G	C	G	A	**C**	A	**T**	C	A	**A**	**T**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 8394 *C. bhutanensis*	A	**C**	C	-	A	A	**T**	**C**	C	C	G	C	G	A	**C**	A	**T**	C	A	**A**	**T**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 9590 *C. moniliformis*	A	**C**	C	-	A	A	**A**	**C**	C	C	G	C	G	A	**T**	A	**C**	C	A	**G**	**C**	C	G	A	A	**C**	**T**	**G**	A	**A**	C	A	A	G	A	T
CMW 4114 *C. moniliformis*	A	**C**	C	-	A	A	**A**	**C**	C	C	G	C	G	A	**T**	A	**C**	C	A	**G**	**C**	C	G	A	A	**C**	**T**	**G**	A	**A**	C	A	A	G	A	T
CMW 8240 *C. moniliformis*	A	**C**	C	-	A	A	**A**	**C**	C	C	G	C	G	A	**T**	A	**C**	C	A	**G**	**C**	C	G	A	A	**C**	**C**	**G**	A	**A**	C	A	A	G	A	T
CMW 9986 *C. moniliformopsis*	A	**A**	C	-	A	A	**C**	**A**	C	C	G	C	G	A	**C**	A	**C**	C	A	**A**	**T**	C	G	A	A	**T**	**C**	**A**	A	**C**	C	A	A	G	A	T
CMW 10214 *C. moniliformopsis*	A	**A**	C	-	A	A	**C**	**A**	C	C	G	C	G	A	**C**	A	**C**	C	A	**A**	**T**	C	G	A	A	**T**	**C**	**A**	A	**C**	C	A	A	G	A	T
CMW 3276 *C. virescens*	C	**A**	G	T	C	T	**T**	**A**	C	A	T	A	T	T	**A**	T	**T**	G	A	**T**	**A**	T	C	A	T	**A**	**T**	**C**	C	**C**	T	C	C	G	T	T

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				1120										1130										1140										1150		
CMW 8242 *C. bhutanensis*	A	C	**C**	G	A	**T**	**A**	G	G	A	T	**G**	**T**	G	T	T	G	C	T	**A**	G	**T**	**G**	**A**	T	G	**C**	G	T	T	T	T	C	**T**	A	C
CMW 8217 *C. bhutanensis*	A	C	**C**	G	A	**T**	**A**	G	G	A	T	**G**	**T**	G	T	T	G	C	T	**A**	G	**T**	**G**	**A**	T	G	**C**	G	T	T	T	T	C	**T**	A	C
CMW 8215 *C. bhutanensis*	A	C	**C**	G	A	**T**	**A**	G	G	A	T	**G**	**T**	G	T	T	G	C	T	**A**	G	**T**	**G**	**A**	T	G	**C**	G	T	T	T	T	C	**T**	A	C
CMW 8399 *C. bhutanensis*	A	C	**C**	G	A	**T**	**A**	G	G	A	T	**G**	**T**	G	T	T	G	C	T	**A**	G	**T**	**G**	**A**	T	G	**C**	G	T	T	T	T	C	**T**	A	C
CMW 8394 *C. bhutanensis*	A	C	**C**	G	A	**T**	**A**	G	G	A	T	**G**	**T**	G	T	T	G	C	T	**A**	G	**T**	**G**	**A**	T	G	**C**	G	T	T	T	T	C	**T**	A	C
CMW 9590 *C. moniliformis*	A	C	**C**	G	A	**G**	**A**	G	G	A	T	**A**	**A**	G	T	T	G	C	T	**A**	G	**C**	**G**	**G**	T	G	**T**	G	T	T	T	T	C	-	A	C
CMW 4114 *C. moniliformis*	A	C	**C**	G	A	**G**	**A**	G	G	A	T	**A**	**A**	G	T	T	G	C	T	**A**	G	**C**	**G**	**G**	T	G	**T**	G	T	T	T	T	C	-	A	C
CMW 8240 *C. moniliformis*	A	C	**C**	G	A	**G**	**A**	G	G	A	T	**A**	**A**	G	T	T	G	C	T	**A**	G	**C**	**G**	**G**	T	G	**T**	G	T	T	T	T	C	-	A	C
CMW 9986 *C. moniliformopsis*	A	C	**A**	G	A	**T**	**C**	G	G	A	T	**G**	**A**	G	T	T	G	C	T	**G**	G	**T**	**C**	**A**	T	G	**C**	G	T	T	T	T	C	**C**	A	C
CMW 10214 *C. moniliformopsis*	A	C	**A**	G	A	**T**	**C**	G	G	A	T	**G**	**A**	G	T	T	G	C	T	**G**	G	**T**	**C**	**A**	T	G	**C**	G	T	T	T	T	C	**C**	A	C
CMW 3276 *C. virescens*	A	C	**G**	A	G	**C**	**T**	T	C	A	A	**T**	**C**	A	A	T	A	A	T	**G**	A	**A**	**A**	**A**	C	T	**T**	G	A	C	T	G	T	**T**	C	A

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								1160										1170										1180								
CMW 8242 *C. bhutanensis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**A**	A	G	G	T	**T**	G	A	G	T	T	G	**A**	T	T	T
CMW 8217 *C. bhutanensis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**A**	A	G	G	T	**T**	G	A	G	T	T	G	**A**	T	T	T
CMW 8215 *C. bhutanensis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**A**	A	G	G	T	**T**	G	A	G	T	T	G	**A**	T	T	T
CMW 8399 *C. bhutanensis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**A**	A	G	G	T	**T**	G	A	G	T	T	G	**A**	T	T	T
CMW 8394 *C. bhutanensis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**A**	A	G	G	T	**T**	G	A	G	T	T	G	**A**	T	T	T
CMW 9590 *C. moniliformis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**G**	**G**	A	G	G	T	**T**	G	A	G	T	T	G	**T**	T	T	T
CMW 4114 *C. moniliformis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**G**	**G**	A	G	G	T	**T**	G	A	G	T	T	G	**T**	T	T	T
CMW 8240 *C. moniliformis*	G	C	A	G	C	G	T	T	**C**	A	G	**T**	C	T	T	G	**T**	**A**	**T**	**A**	**G**	A	G	G	T	**T**	G	A	G	T	T	G	**T**	T	T	T
CMW 9986 *C. moniliformopsis*	G	C	A	G	C	G	T	T	**T**	A	G	**C**	C	T	T	G	**C**	**A**	**A**	**A**	**G**	A	G	G	T	**C**	G	A	G	T	T	G	**T**	T	T	T
CMW 10214 *C. moniliformopsis*	G	C	A	G	C	G	T	T	**T**	A	G	**C**	C	T	T	G	**C**	**A**	**A**	**A**	**G**	A	G	G	T	**C**	G	A	G	T	T	G	**T**	T	T	T
CMW 3276 *C. virescens*	C	C	G	G	T	T	T	C	**G**	A	A	**T**	T	C	G	T	**T**	**G**	**T**	**T**	**G**	G	A	T	C	**T**	G	A	G	T	C	T	**T**	G	T	G

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CMW 8242 *C. bhutanensis*	T	**C**	A	T	G	A	G	G	C	A	**G**	**C**	**C**	C	T	**A**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 8217 *C. bhutanensis*	T	**C**	A	T	G	A	G	G	C	A	**G**	**C**	**C**	C	T	**A**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 8215 *C. bhutanensis*	T	**C**	A	T	G	A	G	G	C	A	**G**	**C**	**C**	C	T	**A**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 8399 *C. bhutanensis*	T	**C**	A	T	G	A	G	G	C	A	**G**	**C**	**C**	C	T	**A**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 8394 *C. bhutanensis*	T	**C**	A	T	G	A	G	G	C	A	**G**	**C**	**C**	C	T	**A**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 9590 *C. moniliformis*	T	**G**	A	T	G	A	G	G	C	A	**A**	**T**	**A**	C	T	**A**	C	C	C	**A**	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 4114 *C. moniliformis*	T	**G**	A	T	G	A	G	G	C	A	**A**	**T**	**A**	C	T	**A**	C	C	C	**A**	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 8240 *C. moniliformis*	T	**G**	A	T	G	A	G	G	C	A	**A**	**T**	**A**	C	T	**A**	C	C	C	**A**	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 9986 *C. moniliformopsis*	T	**C**	A	T	G	A	G	G	C	A	**A**	**C**	**A**	C	T	**G**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 10214 *C. moniliformopsis*	T	**C**	A	T	G	A	G	G	C	A	**A**	**C**	**A**	C	T	**G**	C	C	C	-	C	G	C	C	-	T	G	G	C	G	G	G	G	T	A	G
CMW 3276 *C. virescens*	T	**C**	C	C	T	A	C	C	C	A	**C**	**C**	**T**	G	A	**A**	C	A	G	**C**	C	A	A	T	C	T	C	A	A	T	A	A	A	A	T	C

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	|	.	.	.	.	1230	.	.	.	.	|	.	.	.	.	1240	.	.	.	.	|	.	.	.	.	1250	.	.	.	.	|	.	.	.	.	1260
CMW 8242 *C. bhutanensis*	T	**G**	T	T	G	T	G	T	T	**G**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 8217 *C. bhutanensis*	T	**G**	T	T	G	T	G	T	T	**G**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 8215 *C. bhutanensis*	T	**G**	T	T	G	T	G	T	T	**G**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 8399 *C. bhutanensis*	T	**G**	T	T	G	T	G	T	T	**G**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 8394 *C. bhutanensis*	T	**G**	T	T	G	T	G	T	T	**G**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 9590 *C. moniliformis*	T	**T**	T	T	G	T	G	T	T	**A**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**A**
CMW 4114 *C. moniliformis*	T	**T**	T	T	G	T	G	T	T	**A**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**G**
CMW 8240 *C. moniliformis*	T	**T**	T	T	G	T	G	T	T	**A**	T	G	**A**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**G**
CMW 9986 *C. moniliformopsis*	T	**T**	T	T	G	T	G	T	T	**G**	T	G	**G**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**G**
CMW 10214 *C. moniliformopsis*	T	**T**	T	T	G	T	G	T	T	**G**	T	G	**G**	A	A	A	A	T	T	C	T	A	C	T	C	T	T	T	C	C	T	C	T	T	T	**G**
CMW 3276 *C. virescens*	C	**C**	A	T	G	C	T	T	G	**T**	T	C	**G**	A	A	C	C	C	C	A	C	T	A	G	C	C	T	A	G	G	A	T	T	G	A	**A**

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	.	.	.	.	|	.	.	.	.	1270	.	.	.	.	|	.	.	.	.	1280	.	.	.	.	|	.	.	.	.	1290	.	.	.	.	|	.
CMW 8242 *C. bhutanensis*	G	G	A	A	G	**A**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**T**	**A**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**C**	T	T
CMW 8217 *C. bhutanensis*	G	G	A	A	G	**A**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**T**	**A**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**C**	T	T
CMW 8215 *C. bhutanensis*	G	G	A	A	G	**A**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**T**	**A**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**T**	T	T
CMW 8399 *C. bhutanensis*	G	G	A	A	G	**A**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**T**	**A**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**T**	T	T
CMW 8394 *C. bhutanensis*	G	G	A	A	G	**A**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**T**	**A**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**C**	T	T
CMW 9590 *C. moniliformis*	G	G	A	A	G	**T**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**C**	**A**	A	T	T	A	C	C	C	C	G	C	T	**A**	T	**T**	T	T
CMW 4114 *C. moniliformis*	G	G	A	A	G	**T**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**C**	**A**	A	T	T	A	C	C	C	C	G	C	T	**A**	T	**T**	T	T
CMW 8240 *C. moniliformis*	G	G	A	A	G	**T**	**C**	A	T	A	C	A	A	G	G	G	T	**A**	**C**	**A**	A	T	T	A	C	C	C	C	G	C	T	**A**	T	**T**	T	T
CMW 9986 *C. moniliformopsis*	G	G	A	A	G	**C**	**T**	A	T	A	C	A	A	G	G	G	T	**G**	**T**	**T**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**T**	T	T
CMW 10214 *C. moniliformopsis*	G	G	A	A	G	**C**	**T**	A	T	A	C	A	A	G	G	G	T	**G**	**T**	**T**	A	T	T	A	C	C	C	C	G	C	T	**G**	T	**T**	T	T
CMW 3276 *C. virescens*	C	C	C	C	T	**G**	G	C	A	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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			1300										1310										1320										1330		
CMW 8242 *C. bhutanensis*	G	**T**	**A**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**G**	**G**	A	**A**	G	G	**T**	A	C
CMW 8217 *C. bhutanensis*	G	**T**	**A**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**G**	**G**	A	**A**	G	G	**T**	A	C
CMW 8215 *C. bhutanensis*	G	**T**	**A**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**G**	**G**	A	**A**	G	G	**T**	A	C
CMW 8399 *C. bhutanensis*	G	**T**	**A**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**G**	**G**	A	**A**	G	G	**T**	A	C
CMW 8394 *C. bhutanensis*	G	**T**	**A**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**G**	**G**	A	**A**	G	G	**T**	A	C
CMW 9590 *C. moniliformis*	G	**C**	**C**	C	C	C	T	G	T	A	C	A	A	**A**	G	A	C	C	C	G	**T**	T	**T**	T	G	T	G	**G**	**G**	A	**A**	G	G	**A**	A	C
CMW 4114 *C. moniliformis*	G	**C**	**C**	C	C	C	T	G	T	A	C	A	A	**A**	G	A	C	C	C	G	**T**	T	**T**	T	G	T	G	**G**	**G**	A	**A**	G	G	**A**	A	C
CMW 8240 *C. moniliformis*	G	**C**	**C**	C	C	C	T	G	T	A	C	A	A	**A**	G	A	C	C	C	G	**T**	T	**T**	T	G	T	G	**G**	**G**	A	**A**	G	G	**A**	A	C
CMW 9986 *C. moniliformopsis*	G	**T**	**C**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**A**	**A**	A	**G**	G	G	**C**	A	C
CMW 10214 *C. moniliformopsis*	G	**T**	**C**	C	C	C	T	G	T	A	C	A	A	**G**	G	A	C	C	C	G	**C**	T	**G**	T	G	T	G	**A**	**A**	A	**G**	G	G	**C**	A	C
CMW 3276 *C. virescens*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	T	C	C	**T**	C	**T**	T	A	C	A	**C**	**A**	A	**C**	A	A	**A**	C	C

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							1340										1350										1360								
CMW 8242 *C. bhutanensis*	C	C	T	G	G	T	**C**	A	**C**	**A**	**C**	**A**	C	**A**	T	C	**C**	**A**	**G**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 8217 *C. bhutanensis*	C	C	T	G	G	T	**C**	A	**C**	**A**	**C**	**A**	C	**A**	T	C	**C**	**A**	**G**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 8215 *C. bhutanensis*	C	C	T	G	G	T	**C**	A	**C**	**A**	**C**	**A**	C	**A**	T	C	**C**	**A**	**G**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 8399 *C. bhutanensis*	C	C	T	G	G	T	**C**	A	**C**	**A**	**C**	**A**	C	**A**	T	C	**C**	**A**	**G**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 8394 *C. bhutanensis*	C	C	T	G	G	T	**C**	A	**C**	**A**	**C**	**A**	C	**A**	T	C	**C**	**A**	**G**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 9590 *C. moniliformis*	C	C	T	G	G	T	**T**	A	**C**	**G**	**C**	**C**	C	**C**	T	C	**T**	**A**	**A**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 4114 *C. moniliformis*	C	C	T	G	G	T	**T**	A	**C**	**G**	**C**	**C**	C	**C**	T	C	**T**	**A**	**A**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 8240 *C. moniliformis*	C	C	T	G	G	T	**T**	A	**C**	**G**	**C**	**C**	C	**C**	T	C	**T**	**A**	**A**	T		-		C	A	T	G	C	A	**T**	G	**C**	T	C	T	**T**
CMW 9986 *C. moniliformopsis*	C	C	T	G	G	T	**C**	A	**T**	**C**	**T**	**C**	C	**A**	T	C	**C**	**C**	**A**	T	**C**	**T**	**A**	C	A	T	G	C	A	**A**	G	**G**	T	C	T	**C**
CMW 10214 *C. moniliformopsis*	C	C	T	G	G	T	**C**	A	**T**	**C**	**T**	**C**	C	**A**	T	C	**C**	**C**	**A**	T	**C**	**T**	**A**	C	A	T	G	C	A	**A**	G	**G**	T	C	T	**C**
CMW 3276 *C. virescens*	T	T	G	C	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	A	T	G	C	A	T	T	**G**	T	A	T	**C**

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		1370									1380										1390										1400				
CMW 8242 *C. bhutanensis*	-	**C**	G	**G**	**C**	**A**	**T**	**G**	**A**	**T**	**A**	**G**	**T**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**G**	T	T	G	G	T	T	**C**	G	C
CMW 8217 *C. bhutanensis*	-	**C**	G	**G**	**C**	**A**	**T**	**G**	**A**	**T**	**A**	**G**	**T**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**A**	T	T	G	G	T	T	**C**	G	C
CMW 8215 *C. bhutanensis*	-	**C**	G	**G**	**C**	**A**	**T**	**G**	**A**	**T**	**A**	**G**	**T**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**A**	T	T	G	G	T	T	**C**	G	C
CMW 8399 *C. bhutanensis*	-	**C**	G	**G**	**C**	**A**	**T**	**G**	**A**	**T**	**G**	**G**	**T**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**A**	T	T	G	G	T	T	**C**	G	C
CMW 8394 *C. bhutanensis*	-	**C**	G	**G**	**C**	**A**	**T**	**G**	**A**	**T**	**A**	**G**	**T**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**G**	T	T	G	G	T	T	**C**	G	C
CMW 9590 *C. moniliformis*	**A**	**C**	G	-	**T**	**A**	**T**	**G**	**G**	**T**	**G**	**G**	**G**	G	T	C	T	T	G	T	T	T	G	**G**	**G**	T	**A**	T	T	G	G	T	T	**A**	G	C
CMW 4114 *C. moniliformis*	**A**	**C**	G	-	**T**	**A**	**T**	**G**	**G**	**T**	**G**	**G**	**G**	G	T	C	T	T	G	T	T	T	G	**G**	**T**	T	**G**	T	T	G	G	T	T	**A**	G	C
CMW 8240 *C. moniliformis*	**A**	**C**	G	-	**T**	**A**	**T**	**G**	**G**	**T**	**G**	**G**	**G**	G	T	C	T	T	G	T	T	T	G	**G**	**T**	T	**G**	T	T	G	G	T	T	**A**	G	C
CMW 9986 *C. moniliformopsis*	**T**	**T**	G	**C**	**A**	**C**	**G**	**A**	**C**	-	-	-	-	G	T	C	T	T	G	T	T	T	G	**A**	**T**	T	**A**	T	T	G	G	T	T	**C**	G	C
CMW 10214 *C. moniliformopsis*	**T**	**T**	G	**C**	**A**	**C**	**G**	**A**	**C**	-	-	-	-	G	T	C	T	T	G	T	T	T	G	**A**	**T**	T	**A**	T	T	G	G	T	T	**C**	G	C
CMW 3276 *C. virescens*	-	-	-	-	**T**	A	T	G	**C**	-	-	-	-	A	T	G	C	C	A	T	T	T	A	**C**	**A**	A	**T**	A	A	-	-	-	-	-	-	-

						1410										1420
CMW 8242 *C. bhutanensis*	A	**C**	T	T	**C**	**A**	T	C	**T**	A	G	**C**	A	T	C	A
CMW 8217 *C. bhutanensis*	A	**C**	T	T	**C**	**A**	T	C	**T**	A	G	**C**	A	T	C	A
CMW 8215 *C. bhutanensis*	A	**C**	T	T	**C**	**A**	T	C	**T**	A	G	**C**	A	T	C	A
CMW 8399 *C. bhutanensis*	A	**C**	T	T	**C**	**A**	T	C	**T**	A	G	**C**	A	T	C	A
CMW 8394 *C. bhutanensis*	A	**C**	T	T	**C**	**A**	T	C	**T**	A	G	**C**	A	T	C	A
CMW 9590 *C. moniliformis*	A	**G**	T	T	**A**	**A**	T	C	**C**	A	G	**C**	A	T	C	A
CMW 4114 *C. moniliformis*	A	**G**	T	T	**A**	**A**	T	C	**C**	A	G	**C**	A	T	C	A
CMW 8240 *C. moniliformis*	A	**G**	T	T	**A**	**A**	T	C	**C**	A	G	**C**	A	T	C	A
CMW 9986 *C. moniliformopsis*	A	**C**	T	T	**A**	**G**	T	C	**T**	A	G	**G**	A	T	C	A
CMW 10214 *C. moniliformopsis*	A	**C**	T	T	**A**	**G**	T	C	**T**	A	G	**G**	A	T	C	A
CMW 3276 *C. virescens*	-	**C**	T	T	A	A	**A**	**C**	C	A	**A**	T	C	T	C	A