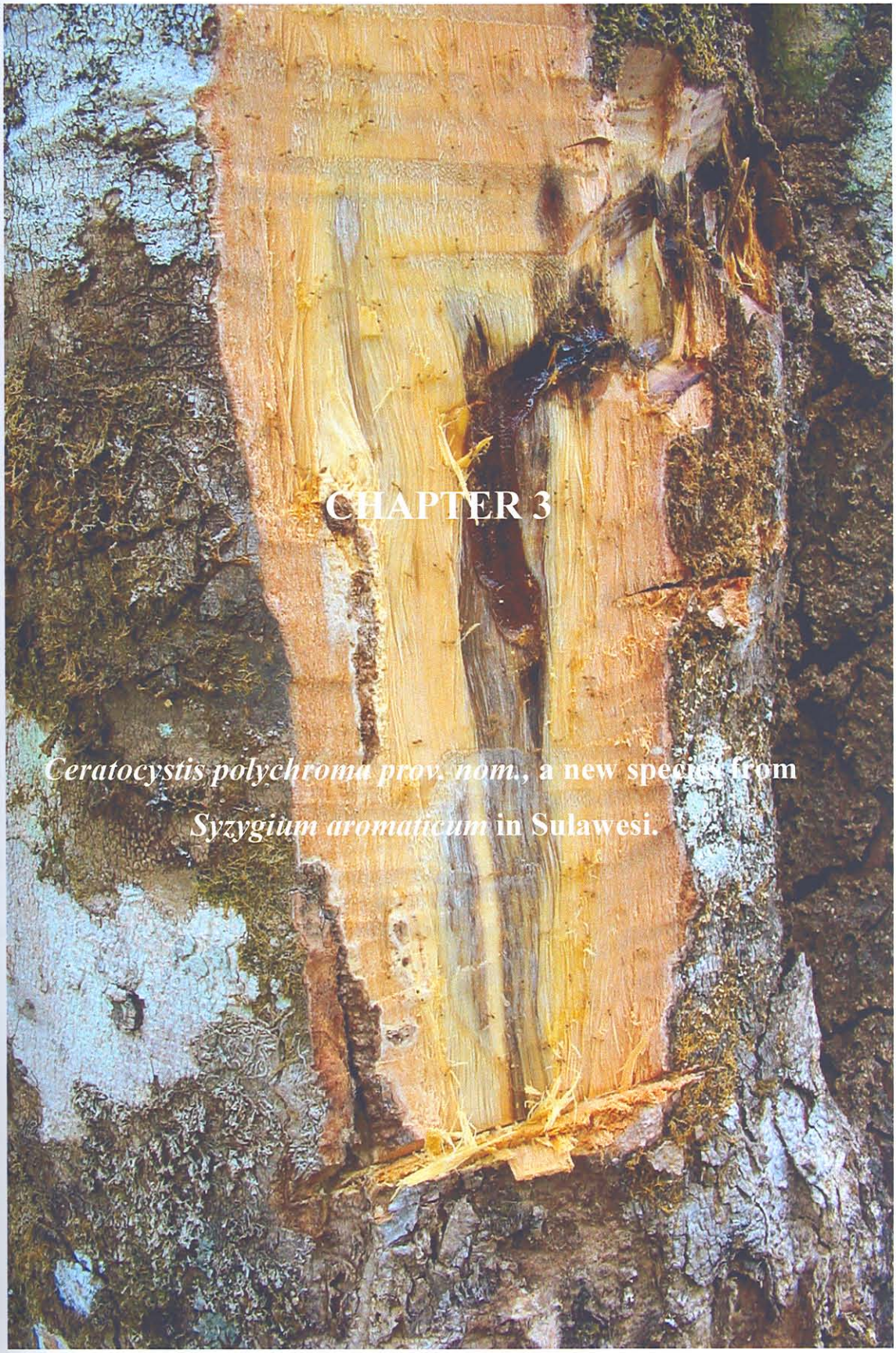

CHAPTER 3

Ceratocystis polychroma prov. nom., a new species from *Syzygium aromaticum* in Sulawesi.

ABSTRACT

Clove decline is the most serious disease affecting *Syzygium aromaticum* in Northern Sulawesi, Indonesia. The aetiology of this disease has never been established. Diseased *S. aromaticum* trees show symptoms of wilt and defoliation, and die in large numbers. Clove decline was found to affect 20-80 % of trees at eighteen sites investigated during a recent survey of the disease. Dying trees are typically infested with the woodborer *Hexamitodera semivelutina*. Larval tunnels are associated with extensive discolouration of the xylem vessels, which has a streaked appearance. Isolations from discoloured wood and larval galleries consistently yielded a *Ceratocystis* spp. very similar to *C. fimbriata.* Comparisons of DNA sequence data for the Internal Transcribed Spacer (ITS) regions, β-tubulin and the Transcription Elongation Factor 1-α (EF1- α) region showed clearly that this *Ceratocystis* sp. resides in a clade distinct from *C. fimbriata* or any of the other known *Ceratocystis* spp. It can also be distinguished from other *Ceratocystis* spp. based on colony morphology and a distinct ecology. We, therefore, describe it as a new taxon to be known as *C. polychroma prov. nom.*

INTRODUCTION

Clove represents a commonly used spice worldwide. It is produced from the unopened flower buds of the evergreen tree, *Syzygium aromaticum* L. Merr. & Perry (Myrtaceae) (Nutman & Roberts 1971, Purseglove *et al.* 1981). The tree is indigenous to the Molucca islands, but has been spread to many countries where it is now commercially cultivated. Most clove plantations are in developing countries, providing an important source of income to small-scale farmers. The trees flourish in tropical environments that are hot and humid with high, annual rainfall (Nair 2000).

Syzygium aromaticum is affected by a number of pests and pathogens (Purseglove *et al.* 1981, Nair 2000). The best-known disease is Sumatra Disease, which is caused by the bacterium *Pseudomonas syzygii* Roberts, Eden-Green, Jones and Ambler (Roberts *et al.* 1990). The woodborer, *Hexamitodera semivelutina* Hell. (Coleoptera: Cerambycidae) that infests living trees is the most serious insect pest of clove (Purseglove *et al.* 1981). This borer is particularly serious in Sulawesi where it is commonly associated with extensive dieback of clove trees.

Although *H. semivelutina* is closely associated with clove dieback in Sulawesi, it has been hypothesised that this dramatic disease could be associated with other factors, including pathogens. A preliminary survey was conducted during September 2001 and December 2002, and isolations were made from symptomatic tissue, especially that associated with woodborer damage. A *Ceratocystis* sp. was commonly found in the tunnels of *H. semivelutina* and consistently isolated from discoloured wood associated with the borer. This fungus was tentatively identified as *Ceratocystis fimbriata* Ell. & Halst. (Liew *et al.* 2003) based only on morphological characteristics. The aim of the present study was to identify the *Ceratocystis* sp. from dying clove trees more comprehensively, based on morphological and DNA sequence comparisons.

MATERIALS AND METHODS

Fungal isolates

Diseased clove trees at eighteen different locations in northern Sulawesi (Fig. 1) were sampled during September 2001 and December 2002. These sites included Toliangoki,

Lahendong, Leilum, Kiawa, Rumoong, Tumpaan, Munte, Kombi, Lalumpe, Tulap, Kakas, Tinoor, Kumelembuai, Motoling, Tambelang, Poopo, Koka and Kembesr (Chapter 4, Fig. 1). Trees were examined for signs of insect infestation and fungal infection (Fig. 1). Larval tunnels were inspected on site, with the aid of a 10-x magnification hand lens, for fungal fruiting structures.

Adult and juvenile beetles, larvae and breeding galleries of *H. semivelutina*, as well as stem sections cut from infested trees were collected for further study. All samples were stored in plastic bags and transported to the laboratory. Dry bark samples were sprayed with distilled water and the bags sealed to create a moist environment, conducive to the sporulation of fungi. Reference specimens of *H. semivelutina* were stored in ethanol and are maintained in the collection of the School of Land, Water & Crop Sciences, University of Sydney, Australia.

Ascomata typical of a *Ceratocystis* sp. commonly developed on wood samples and isolations were made directly from these structures. Isolations were also made from ascomata that formed on the pieces of wood placed between carrots as described by Moller & De Vay (1968). Ascospore droplets at the apices of the ascomatal necks were transferred to 2 % Malt Extract Agar (MEA) (20 % w/v) (Biolab, Midrand, South Africa). Ascospore masses were transferred from the primary isolation plates onto 2 % MEA supplemented with streptomycin sulphate (0.001 g vol^{-1}, SIGMA, Steinheim, Germany) and Thiamine (0.001 g vol^{-1}, SIGMA, Steinheim, Germany) to obtain pure cultures and to encourage sporulation. All isolates used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1) and representative isolates have been deposited in the Centraalbureau voor Schimmelcultures (CBS), Baarn, Netherlands. Holotype material of the new *Ceratocystis* sp. from Sulawesi, consisting of dried cultures on MEA is kept at the National Fungal Herbarium (PREM), Pretoria, South Africa (Table 1).

DNA extraction

Mycelium from actively growing cultures on 2 % MEA plates, for each representative isolate chosen (Table 1) was scraped into Eppendorf tubes and lyophilised for 2 days. The

lyophilised mycelium was placed in liquid nitrogen and ground to a powder using a glass rod. DNA was extracted using the method described by Barnes *et al.* (2001).

PCR amplification

Both ITS regions (ITS1 and ITS2) including the 5.8S gene of the rDNA operon of all selected isolates (Table 1) were amplified using primers ITS1 and ITS4 (White *et al.* 1990) at an annealing temperature of 55 °C. Part of the β-tubulin gene was amplified using primers βt1a and βt1b (Glass & Donaldson 1995) at an annealing temperature of 56 °C. The EF1-α gene was amplified with the primers EF1-728F and EF1-986R (Carbone & Kohn 1999) at an annealing temperature of 58 °C.

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 water. 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 as noted above) for 40 s and 72 °C for 45 s. A 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 °C completed the programme. Amplification of the respective fragments was confirmed by electrophoresis in a 2 % agarose (Roche diagnostics, Mannheim, Germany) gel containing ethidium bromide and visualised under UV light. After amplification, products for each gene were purified using Sephadex columns following the manufacturer's guidelines (1 g in 15 ml H_2O, Sigma, Steinheim, Germany).

DNA Sequencing and analyses

Purified PCR amplicons were sequenced in both directions using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit, following the manufacturer's protocols (Applied BioSystems, Foster City, California). Sequencing of the respective gene areas was done using the same primers as those used for the PCR reactions. Sequence products were cleaned using the same technique as used for the PCR reactions. Sequence reactions were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California,

U.S.A) and sequence electropherograms were analysed using Sequence Navigator version 1.0.1 (Applied BioSystems, Foster City, California).

The sequences obtained for the *Ceratocystis* sp. from clove were compared with those of morphologically similar *Ceratocystis* spp. that are available in GenBank (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 option based on parsimony with tree bisection reconnection was used to obtain the most parsimonious tree. Confidence intervals using 1000 bootstrap replicates were calculated. *Ceratocystis virescens* (Davids.) Moreau was used as the out-group. A partition homogeneity test (Swofford 2002) was used to determine whether the sequence data sets for the three different gene 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 using the MCMC procedure were generated, sampling every 100^{th} tree and printing every 10^{th} tree. To avoid including trees that might have been sampled before convergence of the Markov chain, a number of trees (5000) were discarded. For the analysis of the ITS gene sequence, gamma rate heterogeneity was set, and no codon specific sites were included. For the β-tubulin and Elongation Factor sequences, codon specific sites were specified with a site-specific substitution rate and the site partition was treated as a by-codon.

Morphology and cultural characteristics

The growth rate of isolates CMW 11424, CMW 11443 and CMW 11449, representing the *Ceratocystis* sp. from clove was determined on 2 % MEA. Prior to the growth studies, the isolates were grown for two weeks at 20 °C. Mycelial plugs were taken from actively growing cultures using a 5 mm cork borer and single plugs were transferred to the centres of 90 mm Petri dishes containing 2 % MEA. Five plates for each isolate were incubated at 4 °C as well as at temperatures ranging from 10 °C to 35 °C, at five-degree intervals. Two measurements of colony diameter at right angles to each other were made every second day, for 16 days and averages were computed. The entire experiment was repeated once.

Morphological characteristics were described from 14-day-old cultures, on 2 % MEA supplemented with Streptomycin Sulphate (0.001 g.vol^{-1}, Sigma, Steinheim, Germany). For microscopic examination, fungal structures were mounted in lactophenol. Fifty measurements were made for each taxonomically relevant structure of isolate CMW 11424, and 10 measurements were made for each of the other isolates (CMW 11436, CMW11443, CMW 11449). Ranges, averages, and standard deviations of the corresponding measurements were calculated. Microscopic observations were made using a Carl Zeiss microscope and the photographic images were captured with a Zeiss Axio Vision camera system. Colour descriptions were determined using the colour charts of Rayner (1970).

RESULTS

Fungal isolates

A *Ceratocystis* sp. was the only fungus consistently found associated with *H. semivelutina* larval tunnels or isolations made from the discolouration associated with them. In total, 120 isolates of the fungus were collected from 22 different trees at 18 sites. Some of the isolates were obtained directly from ascomata in the tunnels or associated wood that had been incubated (Fig. 1). Others originated from carrot baiting of the discoloured wood.

PCR amplification

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

DNA Sequencing and analysis

Partition homogeneity tests for the sequence data sets of all three genes resulted in a P-value of 0.05. The dataset had a value equal to the required value and the three genes could thus be combined. The combined sequences of the ITS and β-tubulin and EF1- α genes resulted in a dataset of 1383 characters (Appendix). Of these characters, 804 were constant while 292 characters were parsimony-uninformative and 287 were parsimony-informative. Analysis of this dataset resulted in three most parsimonious trees (Fig. 2.), with a tree length of 825, a

consistency index (CI) of 0.8994, a homoplasy index (HI) of 0.1006, a retention index (RI) of 0.8588 and a rescaled consistency index (RC) of 0.7724. The posterior probability of the branch nodes of the combined datasets, generated with the Bayesian inference programme supported the bootstrap values.

The posterior probability for the branch nodes in the tree was 100 % for the *C. pirilliformis* Barnes & Wingfield, *C. fimbriata* and *C. albofundus* Wingfield, De Beer & Morris clades respectively. Isolates of the *Ceratocystis* sp. from clove resided in a discrete clade that grouped separately from all the other clades, with its own posterior probability of 100 % (Fig. 2).

Species of *Ceratocystis* included in the analyses resided in four well-resolved clades (Fig. 2). The *Ceratocystis* sp. from clove in Sulawesi did not group with any known *Ceratocystis* spp. It formed a single, well-supported sub-clade with a bootstrap support of 98 %. The other sub-clades represented isolates of *C. fimbriata*, *C. albofundus* and *C. pirilliformis* respectively (Fig. 2). This result was confirmed using the Bayesian analysis, with a branch node possibility of 100 % (Fig. 2). DNA-based comparisons thus show that the *Ceratocystis* sp. from Sulawesi represents a previously undescribed species of *Ceratocystis*.

Morphology and cultural characteristics

Three different culture morphologies were observed for the *Ceratocystis* sp. from dying *S. aromaticum* trees (Chapter 4). For the purpose of this chapter, only one culture group was chosen to represent this species. The isolates reached 90 mm in 16 days at the optimum temperature for growth of 25 °C. Growth at 10 °C was slow with cultures reaching only an average 10 mm in 16 days, and at 30 °C growth was slow reaching an average of 45 mm in 16 days. Colony colour differed at different temperatures. At 15 °C cultures had a distinct dark, olive green (23m) colour (Fig. 3a) while at 20 °C it had a white to buff brown colour (17'"I) (Fig. 3b). Cultures incubated at 25 °C had a honey colour (19 "k) (Fig. 3c.) and cultures at 30 °C had a hazel colour (11'k) (Fig. 3d). Cultures produced a fruity odour, similar to that produced by *C. fimbriata*.

Morphological characteristics of the *Ceratocystis* sp. from clove were most similar to those of *C. fimbriata.* The ascomatal bases of the teleomorph were black and globose and ornamented

with hyphal hairs (Fig. 4a). The ostiolar hyphae were divergent (Fig. 4b), exuding sticky masses of hat-shaped ascospores (Fig. 4c). The anamorph of the *Ceratocystis* sp. is a typical *Thielaviopsis* sp., with phialidic conidiogenous cells (Fig. 4d). Typical cylindrical and barrel-shaped conidia were observed (Fig. 4e & g). Cultures produced chlamydospores, either singly or in chains (Fig. 4f). This fungus could easily be distinguished from *C. albofundus* (Wingfield *et al.* 1996) and *C. pirilliformis* (Barnes *et al.* 2003), the two other *Ceratocystis* spp. with hat-shaped ascospores in the *C. fimbriata* complex, based on ascomatal colour and shape.

There were no macroscopic morphological differences between the *Ceratocystis* sp. from clove and *C. fimbriata*. However, the diameter of ascomatal bases of *C. fimbriata* is 121-255 µm (Upadhyay 1981) while those of the *Ceratocystis* sp. from clove are considerably larger (217-261 µm). The ascomatal necks of *C. fimbriata* are 950 µm long (Upadhyay 1981) while those of the clove fungus range from 849 to 1071 µm in length. The ascomatal necks of *C. fimbriata* are 18-35 µm wide (Upadhyay 1981) in contrast to those of the *Ceratocystis* sp. from clove that are much wider (44-54 µm). The ostiolar hyphae of *C. fimbriata* are 18-75 µm long (Upadhyay 1981), while those of the *Ceratocystis* sp. are consistently shorter (33-43 µm long). *Ceratocystis fimbriata* has ascospores that are 2-2.5 µm wide (Upadhyay 1981) while those of the clove fungus are 3-4 µm. The conidiophores of *C. fimbriata* are 35-130 µm (Upadhyay 1981) and those of the *Ceratocystis* sp. from clove are 53-81 µm long. The barrel-shaped conidia are narrower for *C. fimbriata* 6-8 µm (Upadhyay 1981) than the *Ceratocystis* sp. from clove, which are 5-15 µm in width.

TAXONOMY

The *Ceratocystis* sp. isolated from the tunnels and infections associated with *H. semivelutina* infesting clove in Sulawesi is morphologically distinct from all other described species of *Ceratocystis*. Sequence data for three different gene regions support the morphological differences observed. The following description is, therefore, provided describing it as a new taxon, *C. polychroma prov. nom.*

Ceratocystis polychroma, Van Wyk, Liew & Wingfield

Etymology: *polychroma* reflecting the different colours of cultures at different temperatures.

Stat.conid.: *Thielaviopsis*

(Fig. 3-4)

Coloniae ad 15 °C olivaceo-virides, infra olivaceae, ad 20 °C albae vel fulvo-brunneae, inframellinae, ad 25 °C supra infraque mellinae, et ad 30 °C supra infraque avellinae. *Mycelium* plerumque in medio immersum; mycelium aerium album adest. *Crescit* optime ad 25 °C, nullo incremento supra 35 °C, deminuto ad 10 °C. *Hyphae* leves, in septis non constrictae, 3–5 µm. *Bases ascomatum* atrobrunneae vel nigrae, globosae, hyphis ornatae, bases (208-) 217-261 (-269) µm diametro. *Colla ascomatae* basi atrobrunnea vel nigra, apicem versus laetescentes, (837-) 849-1071 (-1187) µm longa, basi (42-) 44-54 (-57) µm lata, apice (15-) 16-18 (-20) µm lata. *Hyphae ostiolares* divergentes, hyalinae, (31-) 33-43 (-46) µm longae. *Asci* non visi. *Ascosporae* lateraliter visae cucullatae, aseptatae, hyalinae, in vagina investitae, cum vagina 5-7 x 3-4 µm, sine vagina 4-5 x 3-4 µm. Ascosporae in massis mucilagineis fulvo-luteis in apicibus collorum ascomatum cumulantes. *Anamorpha Thielaviopsis*: conidiophora in mycelia singula, hyalina, basibus tumidis, apicem versus angustata, 53-81 (-103) µm longa, basi 4-6 µm lata, apicibus 3-4 µm lata. Evolutio *conidii* phialidici per parietes annulares faciendas, *conidia* duarum formarum: conidia primaria hyalina, aseptata, cylindrica, (13-) 16-24 (-26) x 3-5 µm; conidia secondaria hyalina, aseptata, doliiformia, 9-11 x 6-8 µm, in catenis portata. *Chlamydospora* elliptica, parietibus crassis, levia, "argus" brunnea, 11-14 x 8-14 µm, in agaro inclusa, singula vel in catenis facta.

Typus: **Sulawesi**: Toliangoki, isolated from larval tunnel of *Hexamitoderma semivelutina* (Coleoptera: Cerambycidae) on *Syzigium aromaticum*, December 2002, *E. C. Y. Liew*, (PREM 57818 – holotypus, living culture: CMW 11424).

Colonies olive green (23m), reverse olive (21"m) at 15 °C (Fig. 3a), at 20 °C colonies white to buff brown (17"'I), reverse honey (19"k) (Fig. 3b), at 25 °C colonies honey (19"k), reverse honey (19"k) (Fig. 3c), and at 30 °C colonies are hazel (11'k), reverse hazel (11'k) (Fig. 3d) in colour. *Mycelium* mostly submerged in medium, sparse white aerial mycelium present. *Optimal temperature* for growth 25 °C, no growth above 35 °C, diminished growth at 10 °C. *Hyphae* smooth, not constricted at septa, 3-5 µm wide. *Ascomatal bases* dark brown to black, globose, ornamented with hyphae, bases (208-) 217-261 (-269) µm in diameter. *Ascomatal necks* dark brown to black at base, becoming light brown towards the apex, (837-) 849-1071

(-1187) µm long, (42-) 44-54 (-57) µm wide at the base, (15-) 16-18 (-20) µm wide at the apex. *Ostiolar hyphae* divergent, hyaline, (31-) 33-43 (-46) µm long. *Asci* not observed. *Ascospores* cucullate in side view, aseptate, hyaline, invested in sheath, 5-7 x 3-4 µm with sheath, 4-5 x 3-4 µm without sheath. Ascospores accumulated in buff-yellow (19d) mucilaginous masses on the apices of ascomatal necks. *Thielaviopsis anamorph*: conidiophores occurring singly on mycelia, hyaline, swollen base tapering towards the apex, 53-81 (-103) µm long, 4-6 µm wide at base, 3-4 µm wide at the apices. Phialidic *conidium* development through ring wall building, *conidia* of two types: primary conidia hyaline, aseptate, cylindrical (13-) 16–24 (-26) x 3-5 µm, secondary conidia hyaline, aseptate, barrel-shaped 9-11 x 6-8 µm, borne in chains. *Chlamydospores* oval, thick walled, smooth, argus brown (13m), 11-14 x 8-14 µm, embedded in agar, formed singly or in chains, terminally.

Additional specimens examined: **Sulawesi**: Kiaea, isolated from larval tunnels of *H. semivelutina* on *S. aromaticum*, December 2002, *E. C. Y. Liew*, (culture CMW 11443, PREM 57820); same collecting data (culture CMW 11419, PREM 57817); Rumoong, isolated from larvae tunnel of *H. semivelutina* on *S. aromaticum*, December 2002, *E. C. Y. Liew*, (culture CMW 11449, PREM 57821).

DISCUSSION

Ceratocystis polychroma prov. nom. represents a new taxon that is consistently found in the larval tunnels of *H. semivelutina* on dying clove trees in Sulawesi. This fungus can also easily be isolated from the extensive red streaked discolouration of the living wood that is found associated with the borer. Morphologically, *C. polychroma prov. nom.* most closely resembles *C. fimbriata*. This explains why Liew *et al.* (2003) tentatively identified the fungus as *C. fimbriata*. Both species have characteristic globose to oval ascomatal bases covered with hyphae, and hat-shaped ascospores accumulating in slimy masses at the apices of the ascomatal necks. *Ceratocystis polychroma prov. nom.* can, however, be distinguished from *C. fimbriata* and all other *Ceratocystis* spp. based on morphology, growth in culture and DNA-based comparisons.

Ceratocystis polychroma prov. nom. produces colonies that are white to green in colour whereas isolates of *C. fimbriata* are typically olivacious green in culture. *Ceratocystis*

fimbriata cultures tend to produce obvious aerial mycelium, which is different to *C. polychroma prov. nom.* that produces a sparse white mat of mycelium on the surface of cultures. The bases of the ascomatal necks are much wider in *C. polychroma prov. nom.* than in *C. fimbriata* and the barrel-shaped conidia are also much wider in the former than the latter species.

Together with *C. polychroma prov. nom.*, there are seven *Ceratocystis* spp. with hat shaped ascospores. Other species include *C. fimbriata* (Halstead 1890, Upadhyay 1981), *C. moniliformis* Hedgc. (Davidson 1935, Hunt 1956), *C. albofundus* (Morris, Wingfield & De Beer 1993, Wingfield *et al.* 1996), *C. moniliformopsis* Yuan & Mohamm. (Yuan & Mohammed 2002), *C. pirilliformis* (Barnes *et al.* 2003) and *C. acericola* Griffin (Grylls & Siefert 1993). Of these fungi, only *C. fimbriata* (Upadhyay 1981), *C. pirilliformis* (Barnes *et al.* 2003) and *C. polychroma prov. nom.* produce chlamydospores. The ascomatal bases of *C. pirilliformis* (Barnes *et al.* 2003) are pear-shaped and thus unique. *Ceratocystis moniliformis* and *C. moniliformopsis* both have short conical spines covering their ascomatal bases, which are absent in *C. polychroma prov. nom.* and other species with hat-shaped ascospores (Davidson 1935, Yuan & Mohammed 2002). *Ceratocystis acericola* can be distinguished from all the above species by the absence of ostiolar hyphae (Upadhyay 1981).

Ceratocystis polychroma prov. nom. and *C. fimbriata* are clearly similar and they are also phylogenetically closely related. Sequence data for the ITS regions of the ribosomal DNA operon alone showed that *C. polychroma prov. nom.* is different to *C. fimbriata*. By adding sequence data for two other gene regions, we were able to gain substantial additional support for the view that *C. polychroma prov. nom.* represents a unique group, although it resides in the clade including *C. fimbriata*, *C. pirilliformis* and *C. albofundus*. This clade is strongly separated from the *C. coerulescens* clade (Witthuhn *et al.* 1998), which also includes species with hat-shaped ascospores.

Ceratocystis polychroma prov. nom. is closely associated with damage to clove trees by the cerambycid beetle, *H. semivelutina*. Association with an insect is not unusual amongst *Ceratocystis* spp., that require wounds for infection and are known to be vectored by insects (Iton 1966, Seifert, Wingfield & Kendrick 1993). Numerous species of *Ceratocystis,* such as *C. fimbriata*, produce fruity aromatics and are thus attractive to casual insects such as picnic

beetles (Coleoptera: Nitidulidae) and flies (Diptera) that transport them to freshly made wounds on trees (Moller & De Vay 1968, Hinds 1972). This is very different to species such as *C. polonica* Siemaszko, *C. laricicola* Redfern & Minter and *C. rufipenni* Wingfield, Harrington & Solheim that do not produce fruity aromas, but are specifically associated with the bark beetles, *I. typographus* L., *I. cembrae* Heer and *Dendroctonus rufipennis* Kirby respectively (Redfern *et al.* 1987, Wingfield *et al.* 1997, Yamaoka *et al.* 1997). Although *C. polychroma prov. nom.* is closely associated with an insect, we do not believe that this borer acts as a vector for the fungus. This is because cerambycid beetles are ecologically poorly adapted to transmit such fungi (Wingfield 1987). Adult borers that emerge from dying clove trees and that might be carrying *C. polychroma prov. nom.* ascospores never again enter trees. Rather, they mate and female insects insert an ovipositor under the bark. This would not easily allow for the transmission of spores on their bodies, which do not come into close contact with the wood. One possibility is that they carry mites or other phoretic animals that might act as secondary vectors as suggested for vectors of the pine wood nematode *Bursaphelechus xylophilus* Steiner & Buhrer (Wingfield 1987). Alternatively, other insects not specifically associated with *H. semivelutina* might enter the relatively long-lived galleries of this borer and thus act as vectors for the fungus. Further studies of insects associated with the galleries of *H. semivelutina* are planned to resolve this question.

Ceratocystis polychroma prov. nom. resides in a genus of very well known pathogens of woody plants (Kile 1993). Its association with dramatic dieback of cloves and the very characteristic discolouration of woody tissue associated with woodborer damage suggests that it contributes to tree death. However, pathogenicity of the fungus remains to be demonstrated. This process is somewhat frustrated by the high value of single trees that belong to small-scale farmers. Nonetheless, pathogenicity tests are planned for the future and these will substantially enhance our understanding of the serious dieback disease of clove trees in Sulawesi.

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

Species	Isolate no.	Alternative numbers	GenBank accession nr.	Date of isolation	Host	Geographical origin	Associated insect	Collector(s)
C. fimbriata	CMW 2218[a]	None	AF395680[d] N/A[e] AY528974[f]	1991	*Platanus* sp.	France	None	Grosclaude, C.
"	CMW 2219[a]	"	AF395679[d] N/A[e] AY528975[f]	"	"	"	"	"
C. albofundus	CMW 5329[a]	"	AF388947[d] N/A[e] N/A[f]	1999	*Acacia mearnsii*	Uganda	"	Roux, J.
"	CMW 5943[a]	"	N/A[d] N/A[e] N/A[f]	2000	"	"	"	"
C. pirilliformis	CMW 6569[a]	"	N/A[d] N/A[e] AY528982[f]	"	*Eucalyptus nitens*	Australia	"	Wingfield, M.J.
"	CMW 6579[a]	"	N/A[d] N/A[e] AY528983[f]	"	"	"	"	"
C. polychroma prov. nom	CMW 11424[a, b, c]	PREM 57818 CBS N/A	AY528966[d] AY528970[e] AY528978[f]	2002	*Syzygium aromaticum*	Sulawesi, Indonesia	*Hexamitodera semivelutina*	Liew, E. C. Y. & Wingfield, M. J.
"	CMW 11436[a]	PREM 57819 CBS N/A	AY528967[d] AY528971[e] AY528979[f]	"	"	"	"	"
"	CMW 11443[a, b, c]	PREM 57820 CBS N/A	N/A[d] N/A[e] N/A[f]	"	"	"	"	"
"	CMW 11449[a, b, c]	PREM 57821 CBS N/A	AY528968[d] AY528972[e] AY528980[f]	"	"	"	"	"
"	CMW 11455[a]	PREM 57822 CBS N/A	AY528969[d] AY528973[e] AY528981[f]	"	"	"	"	"
C. virescens	CMW 3276[a]	None	AY528984[d] AY528990[e] AY528991[f]	1963	*Quercus* sp.	U. S. A.	None	Hinds, T.

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

[a, b, c, d, e, f] Isolates marked with [a] CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, those marked with [b] were sequenced, those marked with [c] represents isolates that were used for morphological descriptions, GenBank accession numbers that are marked with [d] reprenesnt the ITS sequences, those marked with [e] represent the β-tubulin sequences and those marked with [f] represent the Elongation Factor sequences.

Figure 1. Symptoms of the disease caused by *C. polychroma prov. nom.* in Sulawesi, Indonesia, a) diseased and dead *Syzygium aromaticum* trees, b) internal symptoms showing sap stain in the wood, c-d) tunnels in the wood caused by *Hexamitodera semivelutina* larvae along with sap stain damage, e) the *H. semivelutina* larvae isolated inside the *S. aromaticum* trees.


a

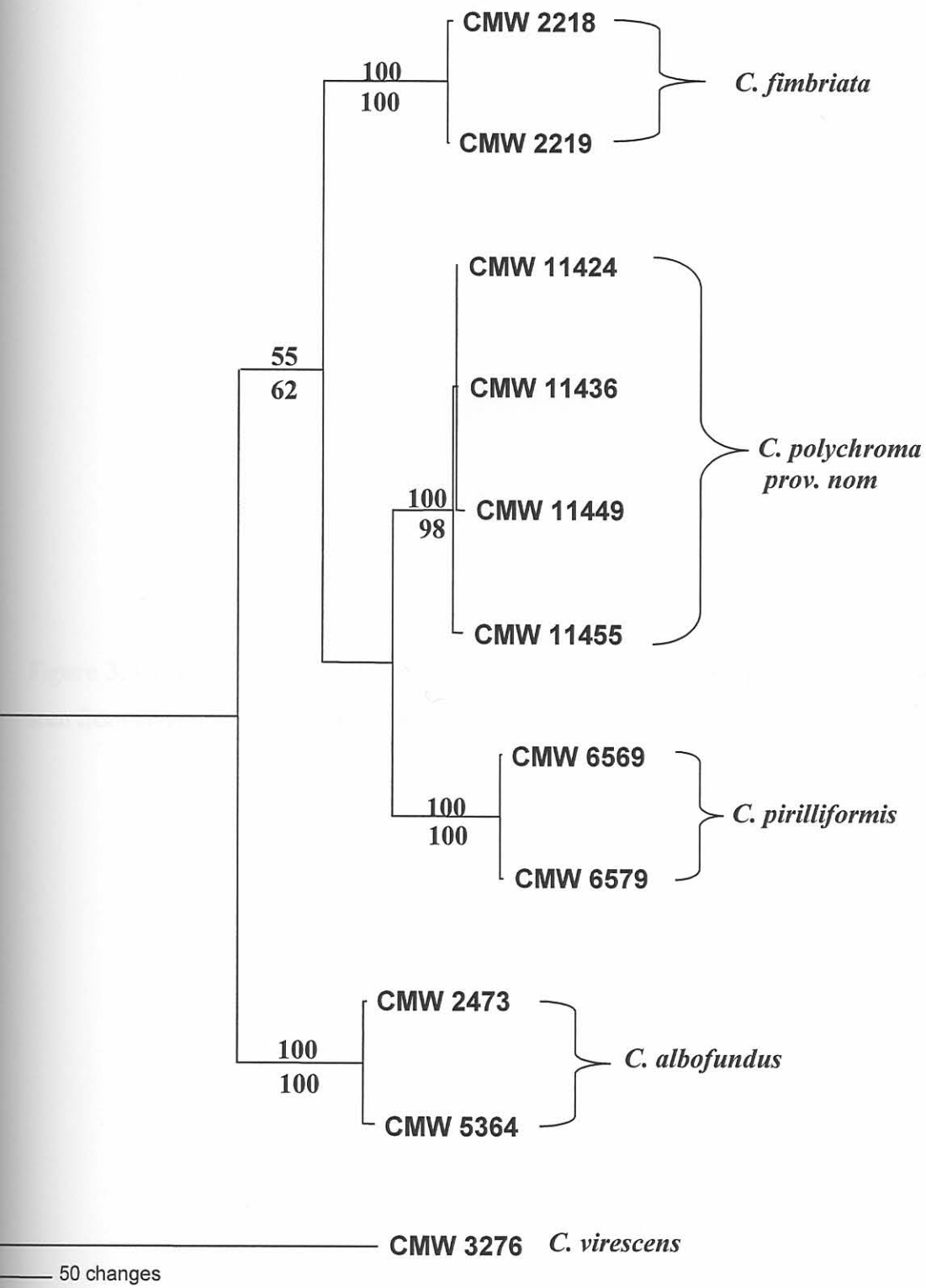

b


c


d


e

Figure 2. A phylogenetic tree based on the combined sequence data for three gene regions; ITS, β-tubulin and Ef1-α. The phylogram was obtained using the heuristic search option based on parsimony. Bootstrap values are indicated above the branches while Bayesian values are indicated below the branches. *Ceratocystis virescens* was treated as the out-group.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100
100
CMW 2219
C. fimbriata
CMW 11424
55
62
CMW 11436
C. polychroma
prov. nom
100
98
CMW 11449
CMW 11455
CMW 6569
100
100
C. pirilliformis
CMW 6579
CMW 2473
100
100
C. albofundus
CMW 5364
CMW 3276
C. virescens
50 changes

Figure 3. Growth and morphology of *C. polychroma prov. nom.* at different temperatures as seen from above and below. a) 15 °C, b) 20 °C, c) 25 °C, d) 30 °C.

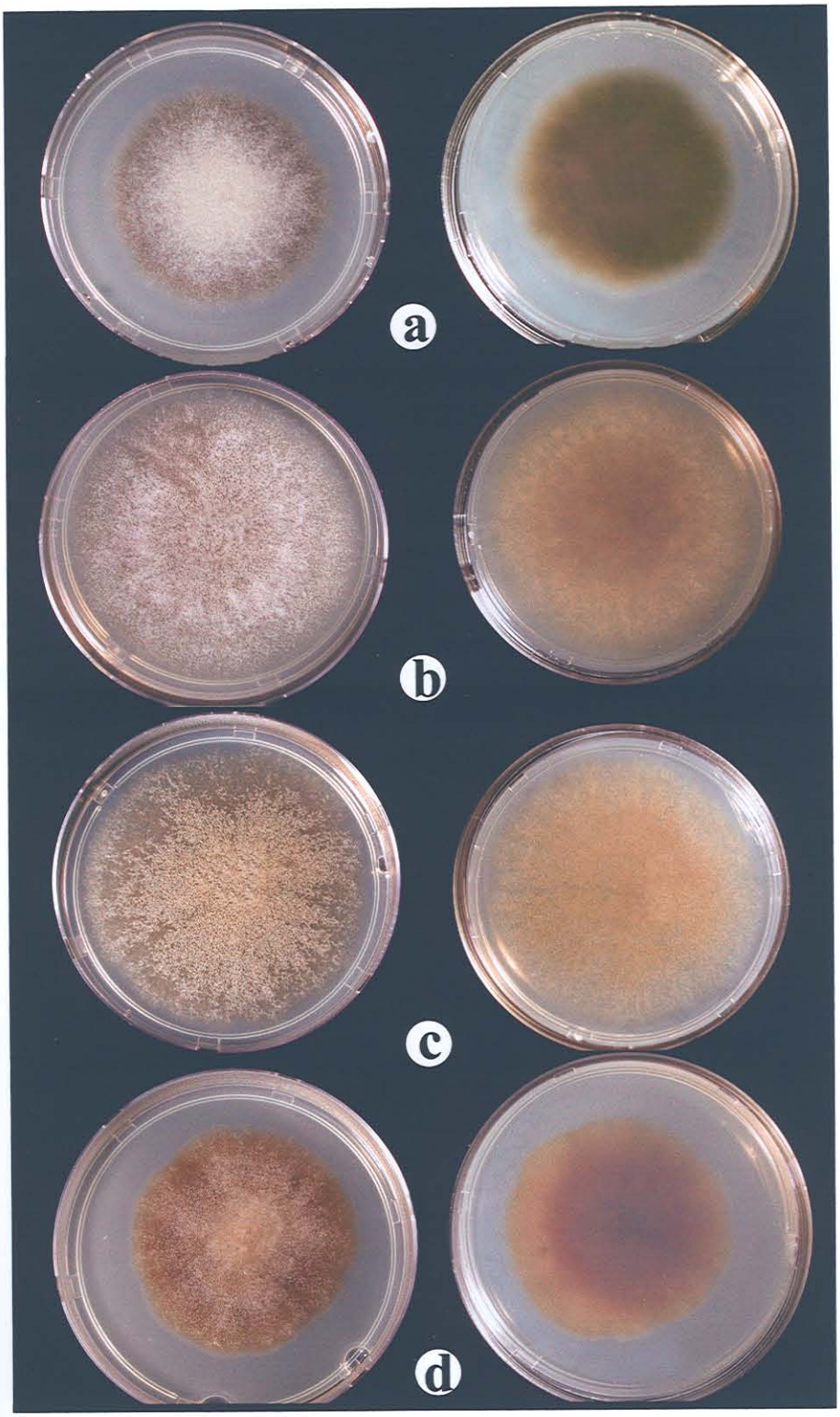
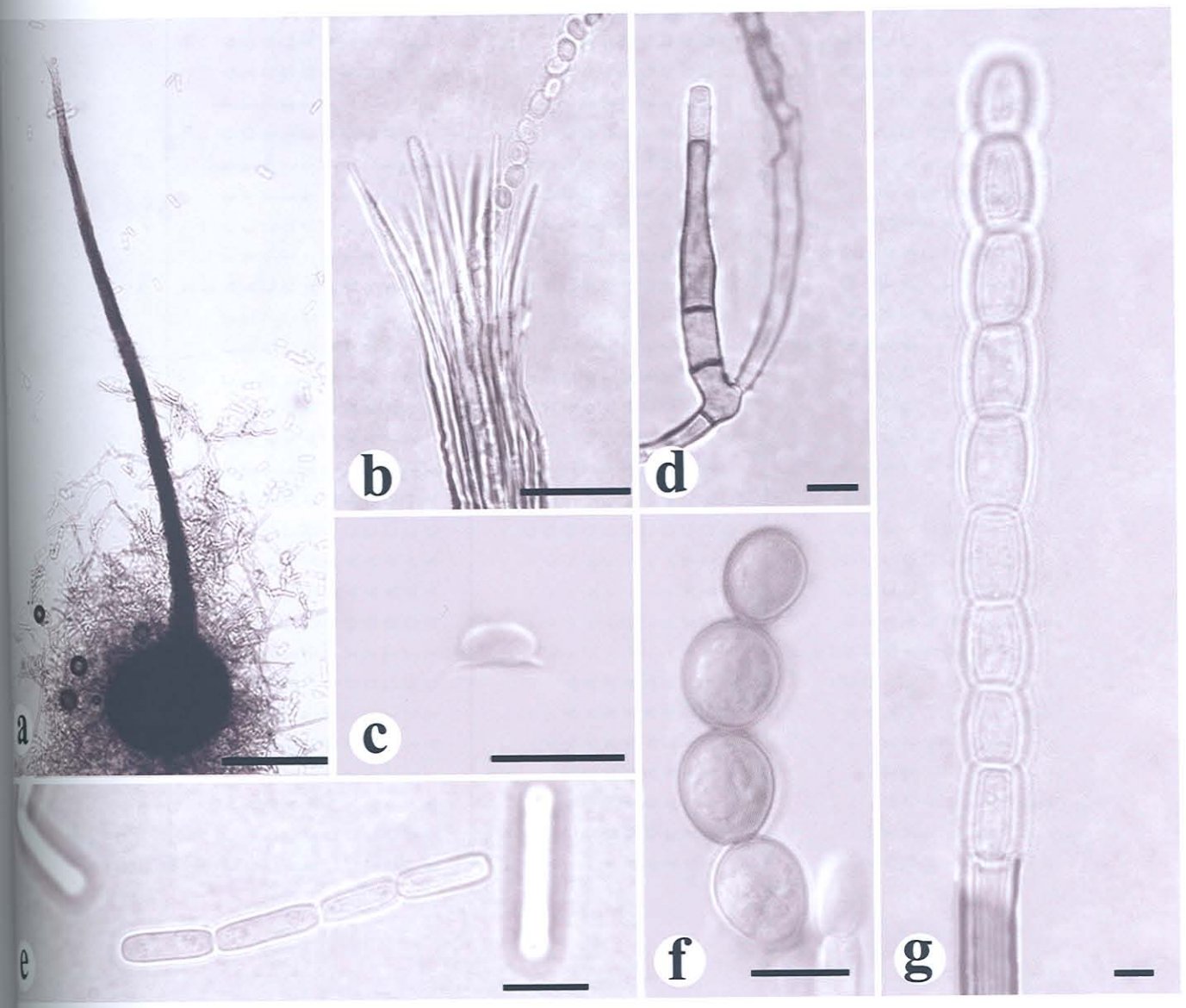
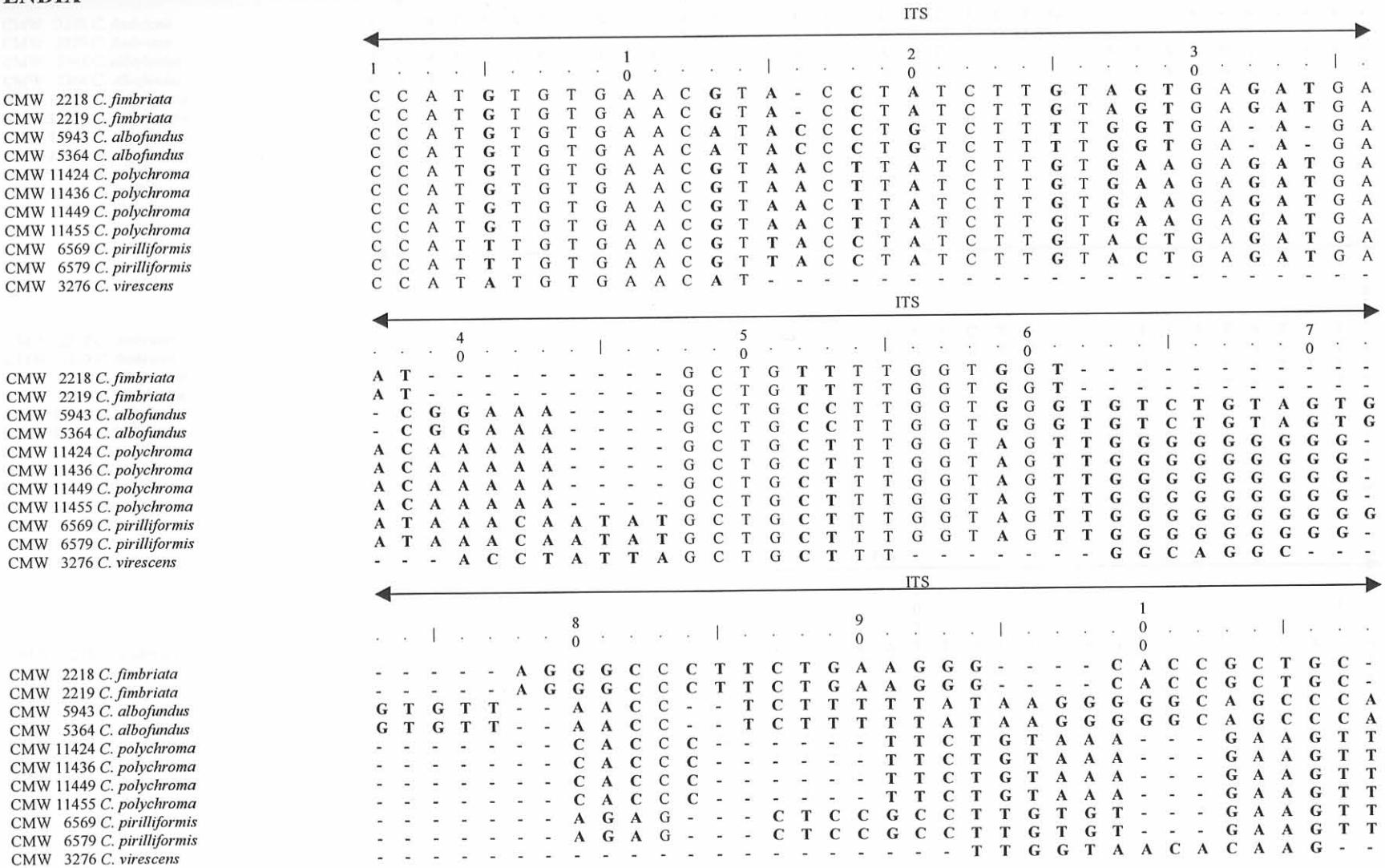

a
b
c
d

Figure 4. Morphological characteristics of *C. polychroma prov. nom.* (CMW 11424). a) Globose ascomatal base (scale bar = 200 µm), b) Divergent ostiolar hyphae (scale bar = 20 µm), c) Hat-shaped ascospore in side view (scale bar = 10 µm), d) Phialidic conidiogenous cell with emerging cylindrical conidium (scale bar = 5 µm), e) Cylindrical conidia in a chain (scale bar = 10 µm), f) Chain of chlamydospores (scale bar = 10 µm), g) Barrel-shaped conidia in a chain (scale bar = 5 µm).



APPENDIX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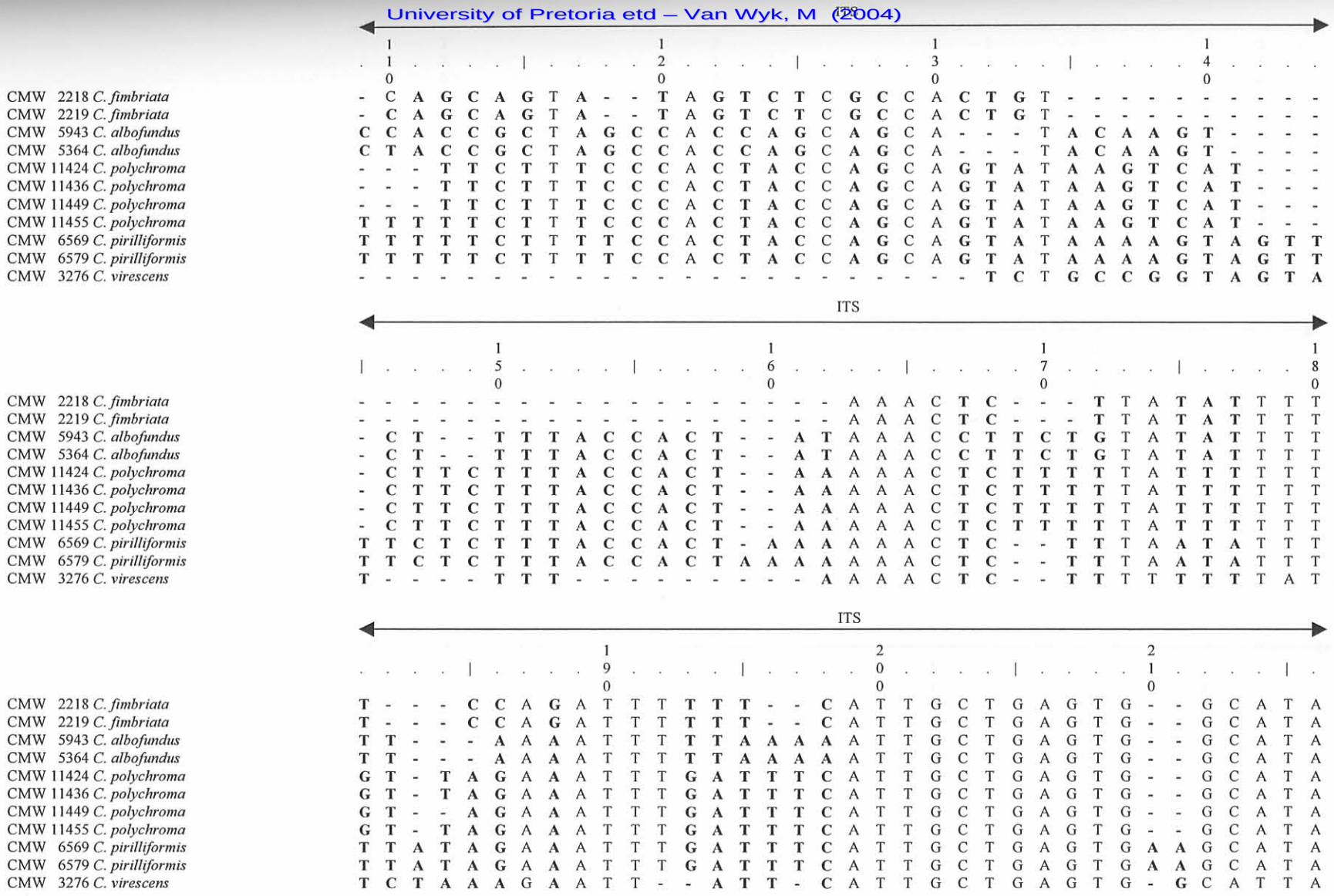
CMW 5943 C. albofundus
CMW 5364 C. albofundus
CMW 11424 C. polychroma
CMW 11436 C. polychroma
CMW 11449 C. polychroma
CMW 11455 C. polychroma
CMW 6569 C. pirilliformis
CMW 6579 C. pirilliformis
CMW 3276 C. virescens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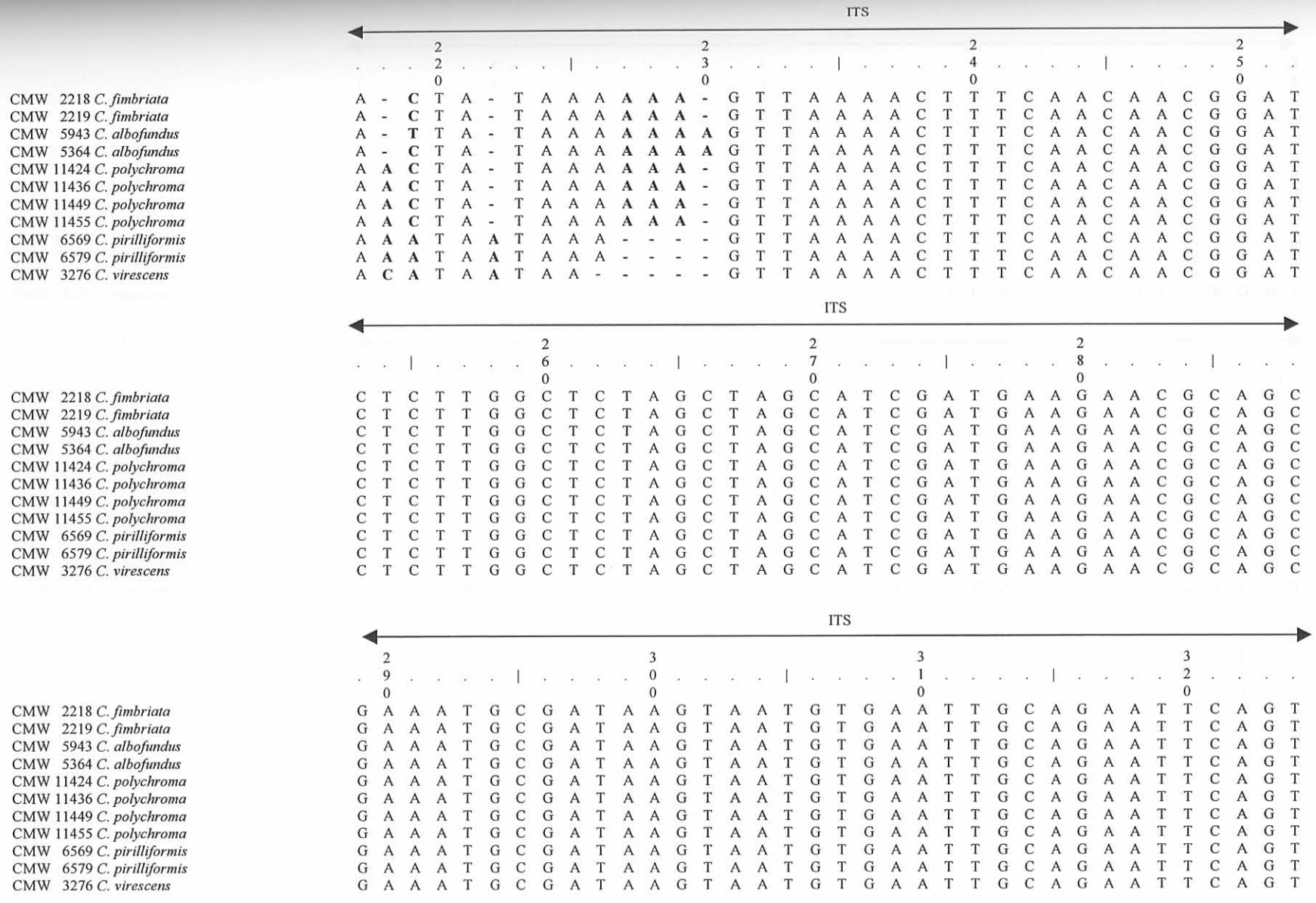
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CMW 11436 C. polychroma
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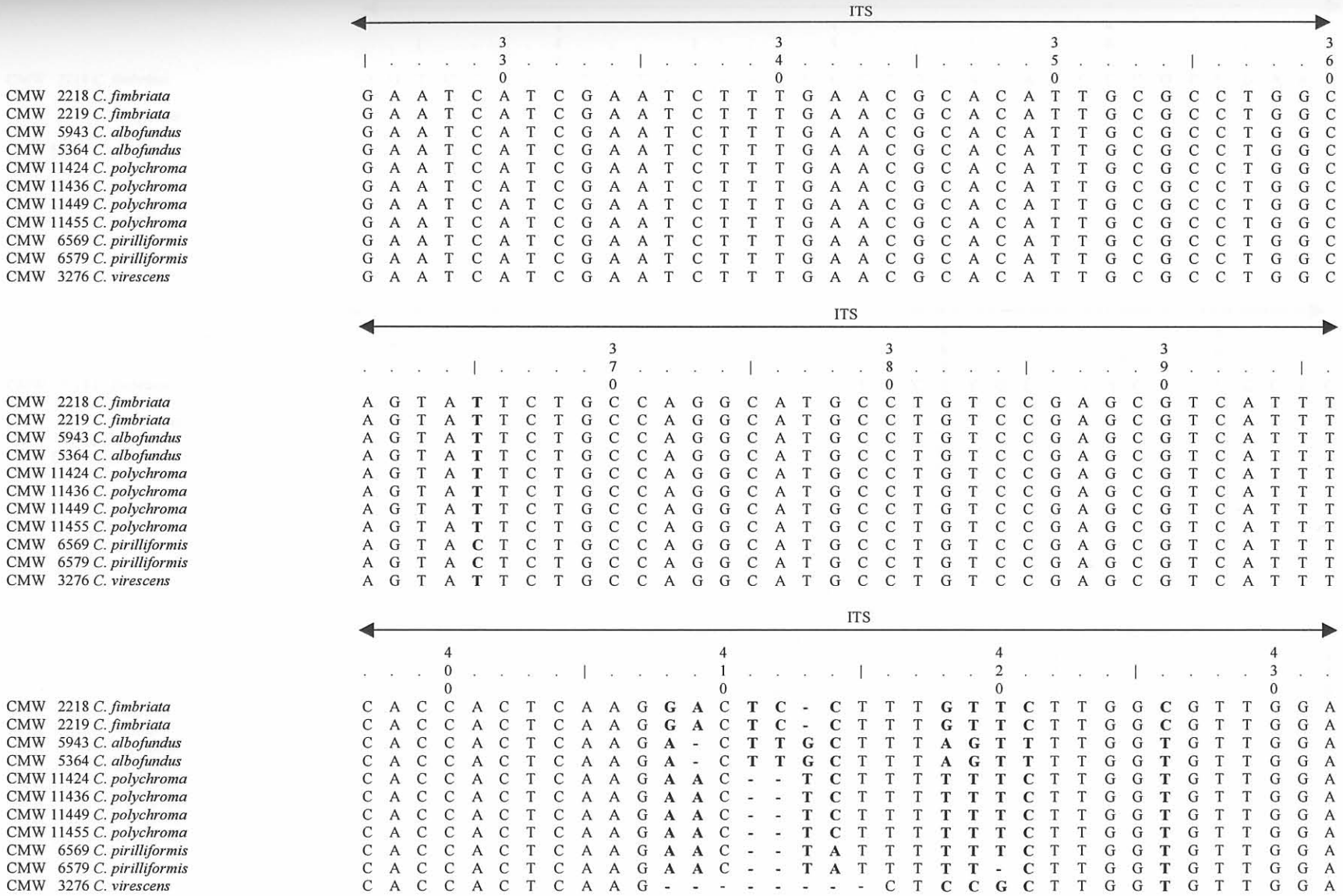
ITS
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CMW 2219 C. fimbriata
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CMW 5364 C. albofundus
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CMW 11436 C. polychroma
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CMW 6569 C. pirilliformis
CMW 6579 C. pirilliformis
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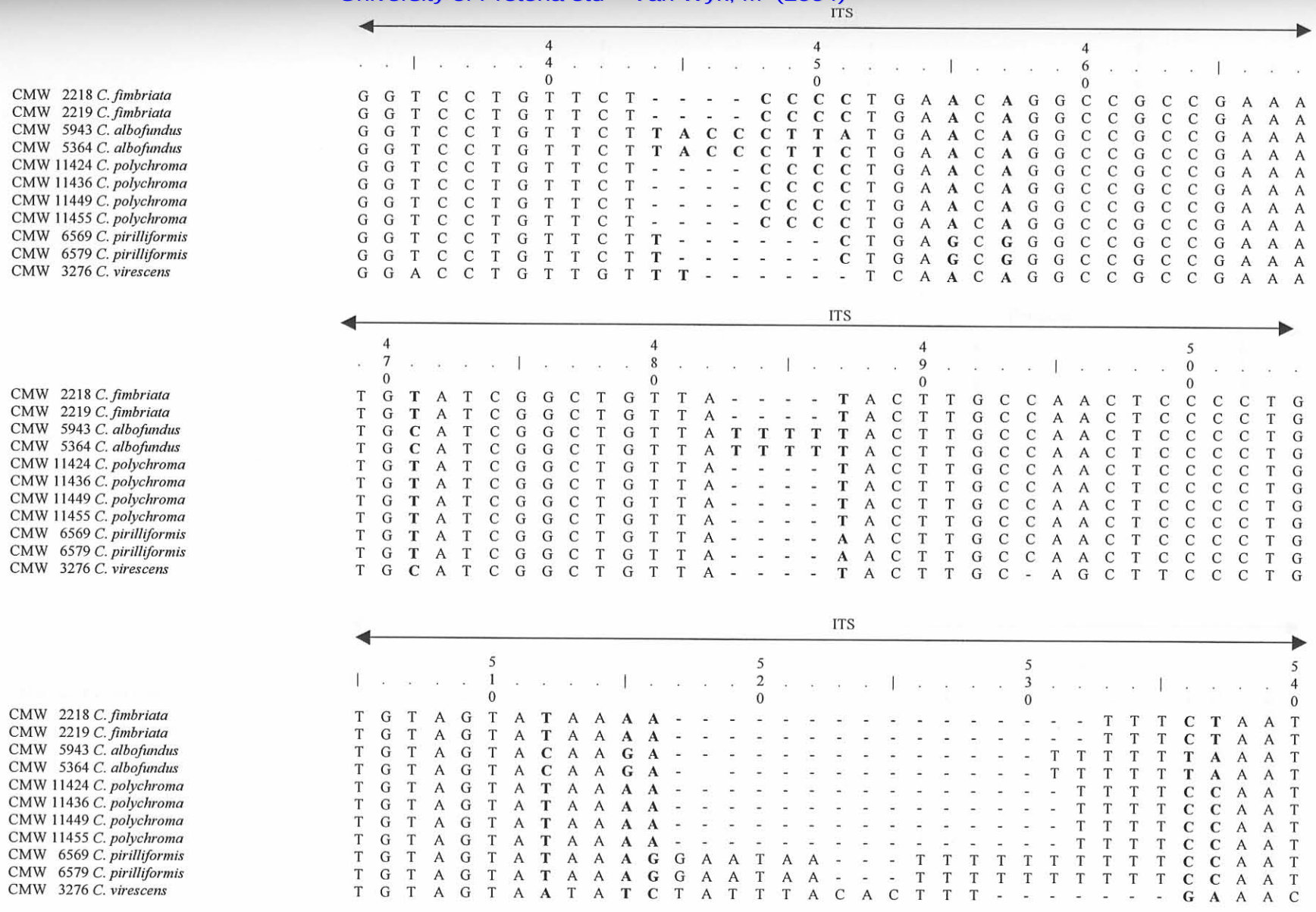
ITS
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CMW 2218 C. fimbriata
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CMW 5943 C. albofundus
CMW 5364 C. albofundus
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CMW 11436 C. polychroma
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CMW 6569 C. pirilliformis
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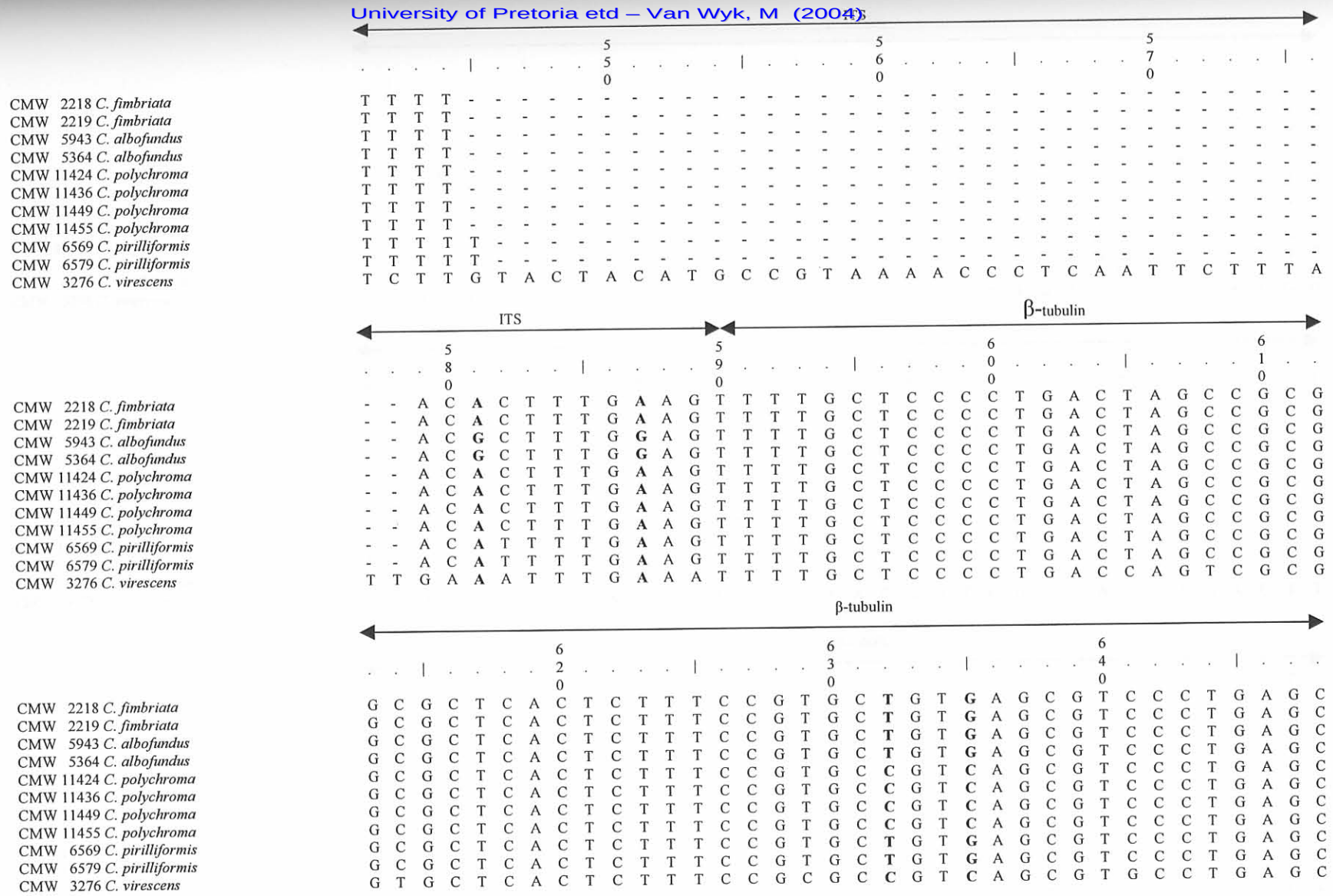


β-tubulin

Isolate	Sequence (650 at column 7, 660 at column 17, 670 at column 27)
CMW 2218 *C. fimbriata*	T T 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 G
CMW 2219 *C. fimbriata*	T T 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 G
CMW 5943 *C. albofundus*	T T 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 G
CMW 5364 *C. albofundus*	T T 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 G
CMW 11424 *C. polychroma*	T T 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 G
CMW 11436 *C. polychroma*	T T 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 G
CMW 11449 *C. polychroma*	T T 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 G
CMW 11455 *C. polychroma*	T T 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 G
CMW 6569 *C. pirilliformis*	T T 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 G
CMW 6579 *C. pirilliformis*	T T 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 G
CMW 3276 *C. virescens*	T G 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 G

β-tubulin

Isolate	Sequence (680 at column 1, 690 at column 11, 700 at column 21, 710 at column 31)
CMW 2218 *C. fimbriata*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 2219 *C. fimbriata*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 5943 *C. albofundus*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 5364 *C. albofundus*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 11424 *C. polychroma*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **C** T
CMW 11436 *C. polychroma*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **C** T
CMW 11449 *C. polychroma*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **C** T
CMW 11455 *C. polychroma*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **C** T
CMW 6569 *C. pirilliformis*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 6579 *C. pirilliformis*	C T G C T T C C G A C T T C C G C A A C G G C C G T T A C C T G A C **T** T
CMW 3276 *C. virescens*	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** T

β-tubulin

Isolate	Sequence (720 at column 5, 730 at column 15, 740 at column 25, 750 at column 35)
CMW 2218 *C. fimbriata*	G C T C T G C C A T C T T G T A **C** G T A T A C C C **C** - T T **G** **A** A A A G A
CMW 2219 *C. fimbriata*	G C T C T G C C A T C T T G T A **C** G T A T A C C C **C** - T T **G** **A** A A A G A
CMW 5943 *C. albofundus*	G C T C T G C C A T C T T G T A **C** G T A T A C C C - **T** T T **G** **A** A A A G A
CMW 5364 *C. albofundus*	G C T C T G C C A T C T T G T A **C** G T A T A C C C - **T** T T **G** **A** A A A G A
CMW 11424 *C. polychroma*	G C T C T G C C A T C T T G T A **T** G T A T A C C C - **T** T T **C** **G** A A A G A
CMW 11436 *C. polychroma*	G C T C T G C C A T C T T G T A **T** G T A T A C C C - **T** T T **C** **G** A A A G A
CMW 11449 *C. polychroma*	G C T C T G C C A T C T T G T A **T** G T A T A C C C - **T** T T **C** **G** A A A G A
CMW 11455 *C. polychroma*	G C T C T G C C A T C T T G T A **T** G T A T A C C C - **T** T T **C** **G** A A A G A
CMW 6569 *C. pirilliformis*	G C T C T G C C A T C T T G T A **C** G T A T A C C C **T** **T** T T **G** **A** A A - G A
CMW 6579 *C. pirilliformis*	G C T C T G C C A T C T T G T A **C** G T A T A C C C **T** **T** T T **G** **A** A A - G A
CMW 3276 *C. virescens*	G C T C T G C C A T C T T G T A **T** G T - - - - - - **T** **T** T T **C** **A** - - - - -

β-tubulin

									760										770										780							
CMW 2218 *C. fimbriata*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	G	C	C	T	C	C
CMW 2219 *C. fimbriata*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	G	C	C	T	C	C
CMW 5943 *C. albofundus*	C	T	A	G	C	C	C	A	T	G	C	T	T	G	T	T	T	T	C	T	T	T	G	T	A	C	A	T	G	T	A	C	-	T	-	C
CMW 5364 *C. albofundus*	C	T	A	G	C	C	C	A	T	G	C	T	T	G	T	T	T	T	C	T	T	T	G	T	A	C	A	T	G	T	A	C	-	T	-	A
CMW 11424 *C. polychroma*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 11436 *C. polychroma*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 11449 *C. polychroma*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 11455 *C. polychroma*	T	T	A	G	C	C	C	A	T	T	G	C	T	G	T	T	T	T	C	T	T	C	G	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 6569 *C. pirilliformis*	T	T	A	G	C	C	C	A	A	T	G	C	T	G	T	T	T	T	C	T	T	C	C	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 6579 *C. pirilliformis*	T	T	A	G	C	C	C	A	A	T	G	C	T	G	T	T	T	T	C	T	T	C	C	T	A	C	A	T	G	T	A	C	C	C	C	C
CMW 3276 *C. virescens*	-	-	-	-	-	C	C	A	A	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	A	T	A	T	T	T	G	T	C	T	A	C

β-tubulin

								790										800										810								
CMW 2218 *C. fimbriata*	T	C	T	G	T	T	G	C	T	C	A	T	G	C	A	A	C	T	A	T	G	C	T	T	T	C	T	A	T	G	A	C	C	A	T	T
CMW 2219 *C. fimbriata*	T	C	T	G	T	T	G	C	T	C	A	T	G	C	A	A	C	T	A	T	G	C	T	T	T	C	T	A	T	G	A	C	C	A	T	T
CMW 5943 *C. albofundus*	A	C	T	G	T	T	G	C	T	G	A	T	G	C	A	A	C	T	G	T	G	A	T	T	T	C	T	A	T	G	A	C	T	A	T	T
CMW 5364 *C. albofundus*	A	C	T	G	T	T	G	C	T	G	A	T	G	C	A	A	C	T	G	T	G	A	T	T	T	C	T	A	T	G	A	C	T	A	T	T
CMW 11424 *C. polychroma*	T	C	T	G	C	T	G	C	T	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 11436 *C. polychroma*	T	C	T	G	C	T	G	C	T	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 11449 *C. polychroma*	T	C	T	G	C	T	G	C	T	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 11455 *C. polychroma*	T	C	T	G	C	T	G	C	T	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 6569 *C. pirilliformis*	T	C	T	G	C	T	G	C	C	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 6579 *C. pirilliformis*	T	C	T	G	C	T	G	C	C	C	A	T	G	C	A	A	C	T	G	T	G	C	T	T	T	C	C	A	T	G	A	C	C	A	T	T
CMW 3276 *C. virescens*	A	T	T	A	-	-	G	T	T	C	A	T	G	-	-	-	-	-	-	-	-	-	T	T	T	G	C	A	T	G	G	A	T	C	T	T

β-tubulin

		820										830										840										850				
CMW 2218 *C. fimbriata*	T	G	C	T	A	A	C	C	C	T	T	T	T	T	T	C	T	T	C	C	C	C	T	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 2219 *C. fimbriata*	T	G	C	T	A	A	C	C	C	T	T	T	T	T	T	C	T	T	C	C	C	C	T	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 5943 *C. albofundus*	T	G	C	T	A	A	C	C	C	C	A	T	T	T	T	C	T	T	C	T	C	-	-	-	T	C	T	A	C	T	T	T	A	C	A	G
CMW 5364 *C. albofundus*	C	G	C	T	A	A	C	C	C	C	A	T	T	T	T	C	T	T	C	T	C	-	-	-	T	C	T	A	C	T	T	T	A	C	A	G
CMW 11424 *C. polychroma*	T	G	C	T	A	A	C	T	A	T	T	T	T	T	T	C	T	T	C	C	C	T	C	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 11436 *C. polychroma*	T	G	C	T	A	A	C	T	A	T	T	T	T	T	T	C	T	T	C	C	C	T	C	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 11449 *C. polychroma*	T	G	C	T	A	A	C	T	A	T	T	T	T	T	T	C	T	T	C	C	C	T	C	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 11455 *C. polychroma*	T	G	C	T	A	A	C	T	A	T	T	T	T	T	T	C	T	T	C	C	C	T	C	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 6569 *C. pirilliformis*	T	G	C	T	A	A	C	C	C	T	T	T	T	T	T	C	T	T	C	T	C	T	T	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 6579 *C. pirilliformis*	T	G	C	T	A	A	C	C	C	T	T	T	T	T	T	C	T	T	C	T	C	T	T	C	T	C	T	A	C	T	T	T	A	C	A	G
CMW 3276 *C. virescens*	T	G	C	T	A	A	C	A	C	C	T	C	T	T	T	C	T	T	T	C	G	T	C	-	-	-	-	-	-	T	T	T	A	T	A	G

β-tubulin

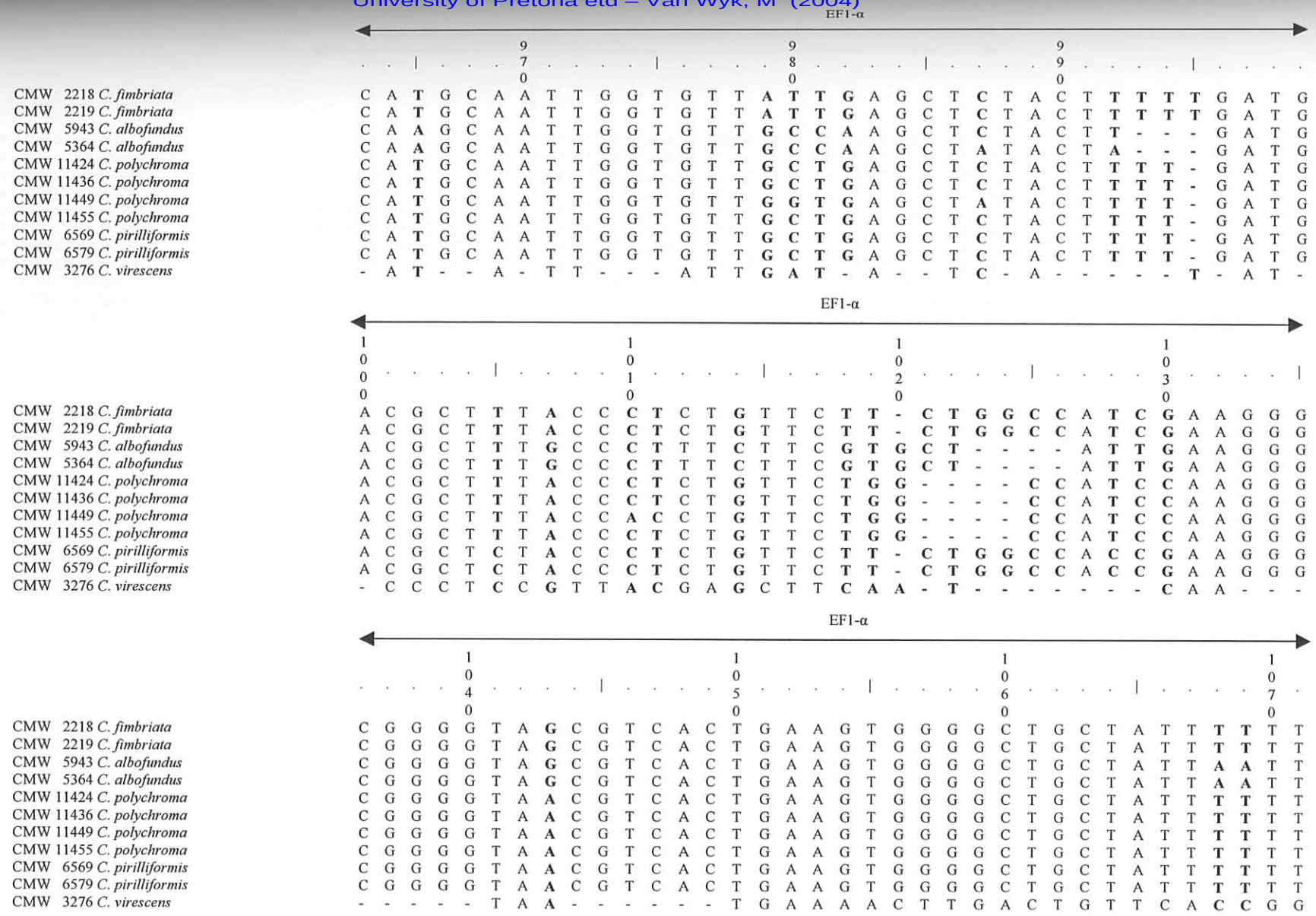
						860										870										880										890
CMW 2218 *C. fimbriata*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 2219 *C. fimbriata*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 5943 *C. albofundus*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 5364 *C. albofundus*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 11424 *C. polychroma*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 11436 *C. polychroma*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 11449 *C. polychroma*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 11455 *C. polychroma*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 6569 *C. pirilliformis*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 6579 *C. pirilliformis*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	C	G	A	A	G	A	C	C	A
CMW 3276 *C. virescens*	C	C	G	T	G	G	T	A	A	G	G	T	T	G	C	C	A	T	G	A	A	G	G	A	G	G	T	T	G	A	G	G	A	C	C	A

β-tubulin EF1-α

										900										910										920						
CMW 2218 *C. fimbriata*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 2219 *C. fimbriata*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**G**	A	T	C	A	T	**T**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 5943 *C. albofundus*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 5364 *C. albofundus*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 11424 *C. polychroma*	G	A	T	G	C	G	C	A	A	C	G	T	**T**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 11436 *C. polychroma*	G	A	T	G	C	G	C	A	A	C	G	T	**T**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 11449 *C. polychroma*	G	A	T	G	C	G	C	A	A	C	G	T	**T**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 11455 *C. polychroma*	G	A	T	G	C	G	C	A	A	C	G	T	**T**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 6569 *C. pirilliformis*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**A**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 6579 *C. pirilliformis*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**A**	A	T	C	A	T	**T**	G	A	G	A	A	G	T	T	C	G	A	G	A	A
CMW 3276 *C. virescens*	G	A	T	G	C	G	C	A	A	C	G	T	**C**	C	A	**G**	A	T	C	A	T	**C**	G	A	G	A	A	G	T	T	C	G	A	G	A	A

EF1-α

				930										940										950										960		
CMW 2218 *C. fimbriata*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 2219 *C. fimbriata*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 5943 *C. albofundus*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**A**	T	G	T	G	G	A	C	A	G
CMW 5364 *C. albofundus*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**A**	T	G	T	G	G	A	C	A	G
CMW 11424 *C. polychroma*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 11436 *C. polychroma*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 11449 *C. polychroma*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 11455 *C. polychroma*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**C**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 6569 *C. pirilliformis*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**A**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 6579 *C. pirilliformis*	T	A	A	G	T	C	T	C	C	C	C	T	A	T	T	T	**A**	C	C	C	T	C	A	T	T	G	**T**	T	G	T	G	G	A	C	A	G
CMW 3276 *C. virescens*	T	A	A	G	T	C	T	C	C	C	C	C	A	-	-	T	**C**	C	A	G	T	C	-	-	-	-	**T**	T	-	-	-	-	A	C	-	-



EF1-α

									1080										1090									
CMW 2218 *C. fimbriata*	T	-	-	-	-	-	C	T	A	A	A	T	G	**A**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 2219 *C. fimbriata*	T	-	-	-	-	-	C	T	A	A	A	T	G	**A**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 5943 *C. albofundus*	T	**T**	**T**	-	-	-	C	T	A	A	A	T	G	**G**	C	**T**	T	T	G	C	A	T	G	C	T	G
CMW 5364 *C. albofundus*	T	**T**	**T**	**G**	-	-	C	T	A	A	A	T	G	**G**	C	**T**	T	T	G	C	A	T	G	C	T	G
CMW 11424 *C. polychroma*	T	**T**	**T**	**T**	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 11436 *C. polychroma*	T	**T**	**T**	-	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 11449 *C. polychroma*	T	**T**	**T**	**T**	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 11455 *C. polychroma*	T	**T**	**T**	**T**	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 6569 *C. pirilliformis*	T	-	-	-	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 6579 *C. pirilliformis*	T	-	-	-	**C**	**T**	C	T	A	A	A	T	G	**G**	C	**G**	T	T	G	C	A	T	G	C	T	G
CMW 3276 *C. virescens*	T	**T**	**T**	-	**C**	-	G	-	A	A	T	T	C	**G**	T	**T**	G	G	T	T	G	G	-	A	T	C