Identification of *Penicillium* species in the South African litchi export chain

by

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DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Magister Scientiae to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other University.

Candice Leigh Johnston

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ABSTRACT

Penicillium species have been studied for over 200 years and the genus was first described by Link in 1809. Initially, morphological identification methods were used however, much diversity within the genus resulted in researchers seeking alternative techniques and approaches to improve accuracy. These methods involved biochemical analysis of secondary metabolites in conjunction with morphological examination. With the emergence of more accurate and rapid molecular identification tools, scientists embraced modern technology to address diversity challenges. In order to provide a more holistic approach towards the taxonomy of complex genera, morphological analysis remains an essential component in *Penicillium* identification. *Penicillium* species are omnipresent, dominant and problematic in postharvest environments. They are known to cause major losses in export markets due to fruit decay. The aim of this study was to identify species within the South African litchi export chain and develop a rapid method for *Penicillium* identification. This study used morphological as well as molecular identification methods in order to develop PCR-RFLP restriction maps for a number of dominant *Penicillium* species. Seventeen species of *Penicillium* were identified using conventional morphological methodology and DNA sequencing, both of which are laborious and time-consuming. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism provided reliability and repeatability as well as being a cost-effective and rapid identification alternative. A combined phylogenetic study indicated that the taxonomic position of several species may need to be reconsidered. Fourteen species were differentiated from one another through digestion of the β-tubulin gene region with five restriction enzymes. Banding patterns correlated well with phylogenetic and biochemical data of related studies, indicating that this method holds promise as a rapid identification procedure for *Penicillium* species.

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SUMMARY

Seventeen dominant species of *Penicillium* were isolated throughout the South African litchi export chain and identified using morphological as well as molecular methods. Identification was done by DNA sequencing of the ITS and β-tubulin gene regions and PCR-RFLP using restriction enzymes *Bfa*I, *Apo*I, *Hae*III, *Hpa*II, *Lwe*I and *Tai*I. In descending order of dominance, these species were identified as *Penicillium crustosum, P. glabrum*, *P. chrysogenum*, *P. biourgeianum/bialowiezense*, *P. solitum*, *P. commune*, *P citrinum*, *P. citreonigrum, P. paneum* and *P. polonicum*, *P. expansum*, *P. brevicompactum*, *P. echinulatum*, *P. corylophilum*, *P. italicum, P. steckii* and *P. sumatrense*. Fourteen species were differentiated from one another through restriction digest of the β-tubulin gene region.

Results found in this study correlated well with Frisvad and Samson (2004) and Samson *et al.* (2004). Where species appeared to be related on a biochemical level (Frisvad and Samson, 2004), it was confirmed through phylogenetic studies of Samson *et al.* (2004). Results of this study were confirmed through phylogeny as well as PCR-RFLP fingerprinting. For example, *P. brevicompactum* and *P. bialowiezense* are related through their ability to produce mycophenolic acid (Frisvad and Samson, 2004). These two species form one clade in the phylogenetic study of Samson *et al.* (2004). However, only the β-tubulin gene region was investigated in that study. This compared to species differentiation through *Hpa*II digestion of the β-tubulin gene region together with results of the combined phylogenetic analysis (both ITS and β-tubulin gene regions), which indicated that the species belong in separate, yet closely related subclades. This highlights the need to analyse more than one gene region in a phylogenetic study.

Four species namely, *P. commune, P. crustosum, P. echinulatum* and *P. solitum* could not be differentiated from one another through PCR-RFLP. These isolates demonstrated intra- and interspecies variation within banding patterns, indicating some degree of relatedness between them. This was validated through combined phylogenetic analysis

of all groups in this study. The taxonomic position of these groups may need to be reconsidered. These species were frequently isolated within the litchi export chain, which emphasises the need for their taxonomic resolution.

Another common species that was frequently isolated in this study is *P. glabrum*. Phylogenetic analysis placed the *P. glabrum* groups in two subclades, indicating the presence of at least two strains or subspecies. However, three distinct PCR-RFLP banding patterns developed. Little is known about this monoverticillate isolate, with regards to pathogenicity and resistance mechanisms. Similar results were found for *P. chrysogenum* groups, indicating the presence of two strains, which were differentiated through PCR-RFLP.

GENERAL INTRODUCTION

Penicillium species have been studied for over 200 years (Raper and Thom, 1949). Theories ranged from *Penicillium* originating from germinated yeast cells (1856) to yeast developing into *Mycoderma,* which in turn developed into *Penicillium* (1871) (Brefeld, 1875). Significant advances have been made in mycological studies although, there is still much to explore within this genus. *Penicillium* was first described by Link in 1809 (Brefeld, 1875) however; species diversity within this genus was greatly underestimated as all green penicillia were classified as *P. glaucum* (Raper and Thom, 1949; Pitt, 1979; Ramirez, 1982). It is estimated that the genus *Penicillium* may consist of more than 300 species (Pitt, 1991). Species diversity within this genus is high as Frisvad and Samson (2004) studied 58 taxa in *Penicillium* subgenus *Penicillium* alone.

Identification of *Penicillium* species initially focused on morphological methods incorporating the use of standardised media preparation and laboratory conditions (Thom, 1930; Raper and Thom, 1949; Pitt, 1973, 1979; Ramirez, 1982; Pitt, 1991). With much variability in colony morphology within this genus, morphological examination requires validation through alternative methodology (Colombo *et al.,* 2003; Marek *et al.*, 2003; Dean *et al.,* 2005). Alternative identification methods employed biochemical analysis of secondary metabolites such as mycotoxins (Frisvad and Filtenborg, 1983; Frisvad and Filtenborg, 1989) and exoenzymes (Cruickshank and Pitt, 1987), in conjunction with morphological examination. Morphological analysis remains an essential component in *Penicillium* identification; however molecular techniques are undeniably the future of mycological research.

Conidia are small, lightweight and static allowing for attachment onto almost any surface, which facilitates cross-contamination between surfaces and the surrounding environment (Anderson, 1956; Pitt, 1979; Morey *et al.,* 2003; Amiri *et al.*, 2005). As a dominant organism found in soil as well as the atmospheric environment, *Penicillium* is a significant genus particularly in fruit export. As early as 1880, *Penicillium* species were

noted as dominant decay agents of citrus fruit (Raper and Thom, 1949). Environmental weathering, harvesting, handling and packaging may cause the fruit surface to become damaged and as *Penicillium* is a classic wound pathogen (Anderson, 1956; Janisiewicz and Korsten, 2002), infection is a certainty.

Humidity is a major factor influencing conidial germination, as moisture may assist in attachment of the conidium to an appropriate substrate and is essential for development of the hyphal tip (Amiri *et al.,* 2005). Such humid conditions are found throughout export chains in areas cooled by Heating, Ventilating and Air Conditioning (HVAC) systems such as packhouses and cooled containers. It has been suggested that fungal growth within HVAC systems is highly probable as humid conditions are created around cooling coils (Chang *et al.,* 1996).

Litchi chinensis Sonn. (litchi) is a sensitive, exotic fruit grown only in select climates. This fruit has a thin, roughened pericarp that facilitates conidial attachment and it is easily desiccated and damaged, providing an ideal environment for *Penicillium* growth. Sulphur dioxide fumigation is currently the only method employed in controlling pericarp browning and fungal decay of litchi fruit (PPECB Export Directory, 2007). However, several species have reportedly developed resistance to the treatment (Jennings, 1993). In addition, when used in conjunction with hydrochloric acid, this process causes acidification of the pericarp that selects for fungal growth (Holcroft *et al.,* 1996; Lichter *et al.,* 2004). Litchi fruit has much appeal in European markets due to its seasonability, attractive red appearance and nutrient-rich, sweet-tasting aril (Lichter *et al.,* 2004; Sivakumar and Korsten, 2006).

During 2002/2003, seven thousand tonnes of South African litchi fruit was passed for export (PPECB Export Directory, 2007). However, currently only between one and two thousand tonnes of litchi fruit is exported annually, to destinations such as Central Europe (45.14%), the Middle East (35.1%), other parts of Africa (15.09%) and the United Kingdom (3.9%) (PPECB Export Directory, 2007). Factors such as dehydration, pericarp

browning and in particular, posthavest decay by fungal species such as *Penicillium,* cause a reduction in fruit quality that reduces export quantities. South Africa is one of few countries that have an ideal climate to cultivate such a fruit, and this should be used to its full potential. All efforts should focus on growth and expansion of this unique industry, through ensuring that high quality litchi fruit is exported with confidence, whereby benefiting the South African economy.

This dissertation will argue the importance of *Penicillium* species as prominent decay agents within the South African litchi export chain. This study was aimed at identifying and characterising dominant *Penicillium* species through the use of morphological and molecular methods. With much variability within this genus, a phylogenetic study serves to propose and clarify taxonomic positions of several species. It was essential to correctly identify species of this genus in order to determine their dominance and ecological role and to minimise losses for the South African litchi the fruit export industry.

Chapter 1

Literature review:

Penicillium species associated with exported fruit such as litchi

1. INTRODUCTION

"Thus the fungus obtains access everywhere; it is unavoidable as the air by which it is carried." – Prof. Oscar Brefeld (1875).

Penicillium is a saprophytic organism with an ecological role of decomposing dead and decaying matter (Raper and Thom, 1949). Small, resistant and lightweight conidia ensure the survival and prevalence of this organism in the environment (Brefeld, 1875). Identification of *Penicillium* species may seem an intimidating task, as species in this genus are diverse and variable (Peterson, 2000).

This genus is of particular importance in the agricultural industry, as *Penicillium* is a dominant decay agent of many crops, particularly fruit. *Penicillium* has been identified as a dominant decay agent of citrus fruit as early as 1880 (Raper and Thom, 1949). Studies have since focused mainly on other fruits such as table grapes (Franck *et al.,* 2005), apples (Janisiewicz *et al.,* 2003; Amiri and Bompeix, 2005), litchis (De Jager *et al.*, 2003; Jacobs and Korsten, 2004; Lichter *et al.,* 2004), peaches (Karabulat and Baykal, 2002) and pears (Lennox *et al.,* 2003).

The history of the methods used in *Penicillium* identification, *Penicillium* as a dominant decay agent, an air contaminant and fruit pathogen will be discussed in this review. Health and safety aspects of *Penicillium* and current methods used in its control will also be included. The focus will be on litchi fruit, as South Africa is a prominent litchi producing and exporting country (Hoger, 1997; Ghosh, 2001).

2. HISTORY OF *PENICILLIUM* IDENTIFICATION

The history of *Penicillium* species identification is depicted in Figure 1. Only significant events that contributed to currently used identification methodology are referred to. Methodologies used will not be elaborated on in this section although relevant references

are indicated. *Penicillium* identification can be grouped under three main developmental phases, which will be discussed individually within the time frame of events (Figure 1).

2.1 MORPHOLOGICAL DATA

Penicillium belongs to: Class – Ascomycetes, Order – Plectascineae, Family – Aspergillaceae, Genus – *Penicillium* (Raper and Thom, 1949). *Penicillium* derives its name from the Latin word "*penicillus*" meaning "little brush" (Pitt, 1979). This genus was first described by Link (1767-1851) in 1809 (Figure 1) as discussed by Raper and Thom (1949); Pitt (1979) and Ramirez (1982). Link also described the first three species of the genus namely, *P. glaucum, P. candidum* and *P. expansum* (Raper and Thom, 1949). Much difficulty has been encountered in correctly identifying *Penicillium* species for instance; Link classed all green penicillia as *P. glaucum* in 1824. Many mycologists followed this trend during the early era of science (Raper and Thom, 1949). Charles Thom (1872-1956) made exceptional contributions to the methodology behind identifying *Penicillium* species. In 1910, his work *"Cultural Studies of Species of Penicillium"* emphasised the need for standardised media in culture examination (Raper and Thom, 1949). Thirty-six species were described, of which 13 were new species while nine were not assigned names as they were insufficiently described (Hasselbring, 1910; Pitt, 1979) (Figure 1). In 1930, Thom developed a monograph – *"The Penicillia"* - in which he incorporated all material on *Penicillium* taxonomy to date. Again, he emphasised the need for standardised media and laboratory growth conditions, as well as observing colony growth characteristics (Figure 1) (Thom, 1930; Raper and Thom, 1949).

In 1928, Prof. Alexander Fleming discovered penicillin originally isolated from *P. notatum* (Figure 1). In the midst of World War II (1939-1945), the need arose for mass production of this antibiotic (Faddis, 1947). *Penicillium chrysogenum* was identified as the species with the greatest ability to produce large quantities of penicillin (Swann, 1983; Figure 1). With an abundance of research being done on *Penicillium* species from

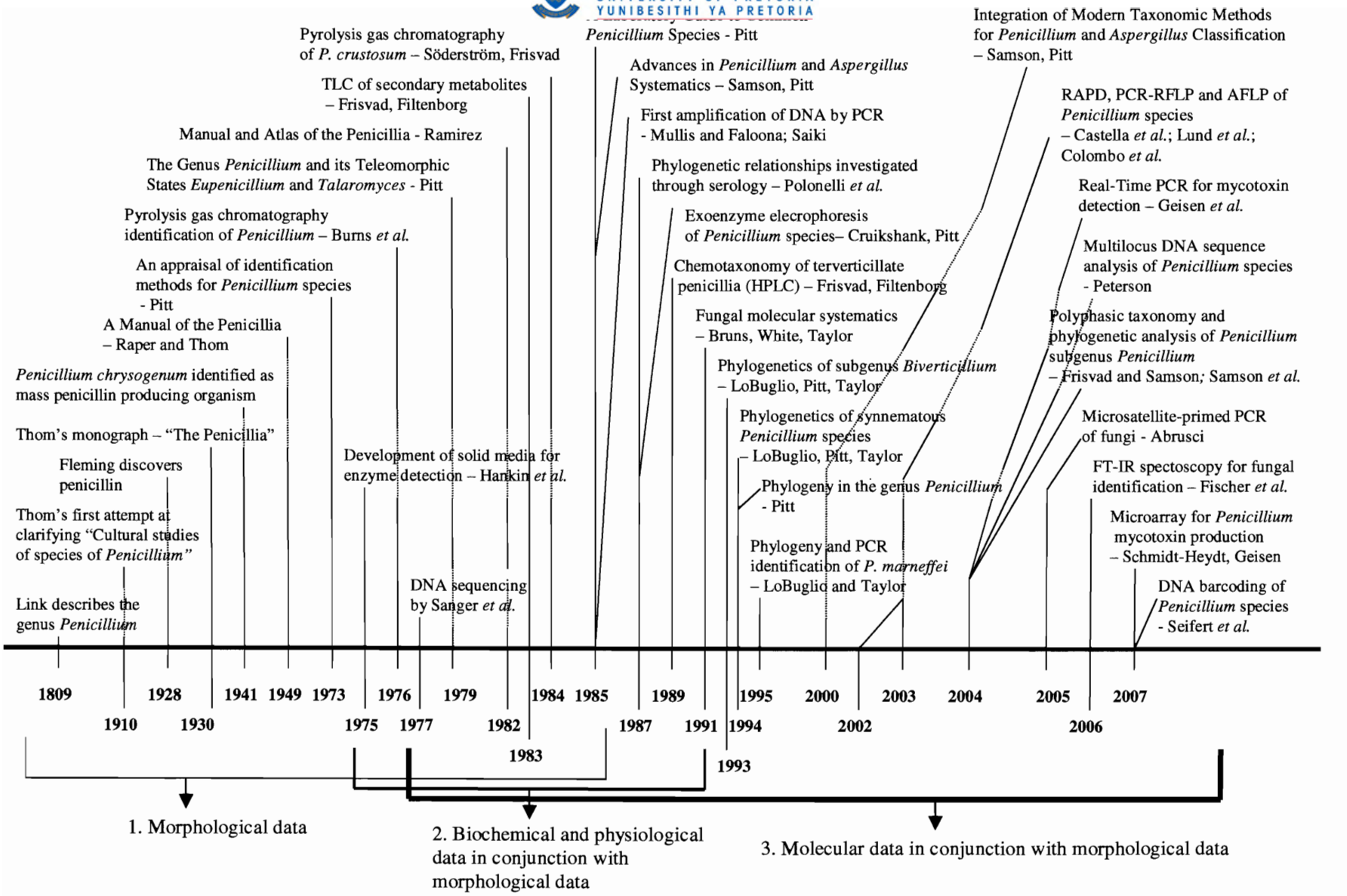

Figure 1: A timeline of significant events contributing to current identification methods of *Penicillium* species. Three developmental phases are indicated – 1. Morphological data 2. Biochemical and physiological data in conjunction with morphological data, 3. Molecular data in conjunction with morphological data.

1940, Raper and Thom (1949) developed *"A Manual of the Penicillia"* (Figure 1). In this manual, 99 valid species were divided into sections (monoverticillata, asymmetrica, biverticillate-symmetrica and polyverticillata) and described with various taxonomic keys (Raper and Thom, 1949; Ramirez, 1982). Although many new species have been described since, it remains the taxonomic standard for identification of *Penicillium* species as many other publications are based on this body of work (Pitt, 1973, 1979; Ramirez, 1982; Pitt, 1991).

In 1973, Pitt's *"An appraisal of identification methods for Penicillium species: Novel taxonomic criteria based on temperature and water relations"* standardised the methods used in *Penicillium* identification (Figure 1) (Pitt, 1973). Recipes for two ideal culture media (Czapek Yeast Autolysate Agar (CYA) and Malt Extract Agar (MEA)) were included as well as respective incubation periods and temperatures, inoculation techniques and important microscopic characteristics. These guidelines have set a standard in *Penicillium* identification and are still in use today (Ramirez, 1982; Pitt, 1991).

2.2 BIOCHEMICAL AND PHYSIOLOGICAL DATA IN CONJUNCTION WITH MORPHOLOGICAL DATA

Initial differentiation between fungal taxa through chemotaxonomy was done by Hankin and Anagnostakis (1975), through the development of nine various culture media for enzyme production (Figure 1). A number of *Penicillium* species have been analysed through pyrolysis gas-liquid chromatography (GLC) as an alternative to morphological identification methods (Burns *et al.*, 1976; Söderström and Frisvad, 1984). Burns *et al.* (1976) effectively distinguished between 11 species of *Penicillium* through GLC and claimed this method was effective in strain differentiation. Söderström and Frisvad (1984) focused primarily on isolates of *P. crustosum* compared with their mycotoxin profiles (Figure 1). Through this method, they were able to differentiate between three strains of *P. crustosum.*

In 1979, Pitt published a monograph *"The Genus Penicillium and its Teleomorphic States Eupenicillium and Talaromyces"* (Pitt, 1979) (Figure 1). Although several faults were brought to light in the review by Robert Samson (Samson, 1981), the current method of morphologically identifying *Penicillium* species is described in detail. Species of *Eupenicillium* (Ludwig) and *Talaromyces* (Benjamin) were described and the genus was divided into four subgenera namely *Aspergilloides* [Dierckx], *Penicillium* [Sect. *Asymmetrica* Raper and Thom], *Biverticillium* [Dierckx] and *Furcatum* [Pitt] (Pitt, 1979).

Carlos Ramirez developed a *"Manual and Atlas of the Penicillia"* in 1982, which was a colour atlas and extensive description of all *Penicillium* species identified at that time (Figure 1). This manual addressed the issue of colony characteristics and colour descriptions through full colour images of plates used in the identification process (Ramirez, 1982). A disadvantage of this publication was that Ramirez reverted to the section nomenclature used by Raper and Thom (1949), as opposed to the newly developed subgenera classification of Pitt (1979) (Ramirez, 1982; Paden, 1984).

Samson and Pitt collaborated on *"Advances in Penicillium and Aspergillus Systematics"* in 1985. This manual covers the terminology and methodology in identification, taxonomy and taxonomic issues surrounding variability within the genus (Samson and Pitt, 1985) (Figure 1). Soon after this publication, John Pitt developed *"A Laboratory Guide to Common Penicillium Species"* (Pitt, 1991), which contains the current methodology for identification of *Penicillium* species (Figure 1). In this manual, 48 of the most commonly occurring *Penicillium* species are described and the identification process is simplified by the use of taxonomic keys. Ten species of *Eupenicillium* and six *Talaromyces* species were also included. This manual is highly acclaimed in the Reviewed Works of *Mycologia* (G.C.H., 1987).

As more species of *Penicillium* were described, it became evident that morphological identification alone was inadequate. Analysis of mycotoxins and other secondary metabolites as a method of species differentiation became a focal point (Frisvad and Filtenborg, 1983; Frisvad and Filtenborg, 1989) (Figure 1). These studies employed

Thin-Layer Chromatography and High-Performance Liquid Chromatography (HPLC) respectively. A variation of HPLC, Reversed Phase High Performance Liquid Chromatography was used to study the relatedness between certain *Penicillium* species using serological antigens (Polonelli *et al.*, 1987) (Figure 1). A promising method of exoenzyme electrophoresis was developed by Cruickshank and Pitt (1987) (Figure 1). This was performed on several *Penicillium* species belonging to subgenus *Penicillium.* Results correlated moderately well with the classifications of Pitt (1979) (Cruickshank and Pitt, 1987).

2.3 MOLECULAR DATA IN CONJUNCTION WITH MORPHOLOGICAL DATA

A dawning of a new era began with the first enzymatic amplification of DNA with the polymerase-catalysed chain reaction (PCR) by Saiki *et al.* (1985; 1988) and Mullis and Faloona (1987). This presented scientists with a magnitude of new possibilities within molecular biology. Understanding of identification and taxonomic schemes of *Penicillium* species has greatly been facilitated by the implementation of this methodology in mycological studies.

In 1991, Bruns and co-workers developed a summary of methods used to date for investigating the molecular evolution of various fungi (Bruns *et al.*, 1991) (Figure 1). Methods include DNA-DNA hybridization; restriction enzyme analysis (RFLP); DNA sequence analysis and electrophoretic karyotyping. A method to determine the genetic sequence of nucleic acids using dideoxynucleotide chain termination was developed by Sanger *et al.* (1977) (Figure 1). Applications of this technique were greatly improved by the introduction of the PCR methodology. Sequence analysis contributed to further phylogenetic studies and identification of *Penicillium* species particularly with the creation of global, electronic databases i.e. GENBANK (www.ncbi.nlm.nih.gov). Phylogenetic studies of *Penicillium* species from this time period include that of the subgenus *Biverticillium, P. marneffei* as well as the synnematous species *P. duclauxii, P. clavigerum* and *P. vulpinum* (LoBuglio *et al.*, 1993, 1994; LoBuglio and Taylor, 1995) (Figure 1).

In 1995, Pitt bridged the gap between the three developmental phases in his publication *"Phylogeny in the genus Penicillium: a morphologist's perspective"* (Pitt, 1995) (Figure 1). He proposed a hypothetical phylogeny for *Penicillium* species based on morphological, physiological and biochemical as well as molecular data. Samson and Pitt (2000) developed a manual that incorporates both molecular and morphological identification methods for *Penicillium* species (Figure 1). This guide serves to standardise identification techniques in which variable factors such as temperature, media composition and media preparation, may greatly influence results. Modern molecular taxonomic methods employed to differentiate between species of *Penicillium* are presented in the form of research articles. Common gene regions analysed include ITS and β-tubulin (Peterson, 2000; Seifert and Louis-Seize, 2000; Skouboe *et al.*, 2000).

Older methodologies such as Amplified Fragment Length Polymorphism (AFLP), Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Random Amplification of Polymorphic DNA (RAPD) are still widely used today to characterise strains and isolates of *Penicillium* (Figure 1). Castella *et al.* (2002) used AFLP, RAPD and sequencing of the Internal Transcribed Spacer (ITS) gene region to classify isolates of *P. verrucosum* into two groups based on their ability to produce mycotoxins, specifically ochratoxin A. Lund *et al.* (2003) used RAPD and AFLP techniques, together with morphology and secondary metabolites profiles to study the diversity and distribution of *P. commune* isolates within cheese production areas. Through such genetic analyses, contamination points occurring throughout the production area could be traced. Colombo *et al.* (2003) used PCR-RFLP to identify species of the *P. aurantiogriseum* group and such a method may be applied to detection of mycotoxigenic strains of *Penicillium* species. Other methods successful in detecting mycotoxin production in *Penicillium* species as well as other fungal genera include Real-Time PCR (Geisen *et al.*, 2004) and microarrays (Schmidt-Heydt and Geisen, 2007) (Figure 1).

Novel methods for identifying fungal genera are being implemented. These include microsatellite-primed PCR, which employs the use of specific primers to amplify desired microsatellite regions (Abrusci, 2005). Fourier-transform infrared (FT-IR) spectroscopy

allows for differentiation between species and strains of *Penicillium* and *Aspergillus* (Fischer *et al.,* 2006). DNA barcoding has been implemented in the identification of *Penicillium* species by Seifert *et al.* (2007). This method involves amplification and subsequent phylogenetic analysis of the mitochondrial cytochrome *c* oxidase 1 (*CO1)* gene region. Results showed this gene region to have a lower divergence than that of the ITS and β-tubulin resulting in a high taxonomic resolution of *Penicillium* species (Figure 1). The discovery of alternative gene regions is essential to the advancement of phylogenetic studies. Peterson (2004), recommended the use of multilocus DNA sequence analysis in order to achieve a fully representative phylogenetic study, as sequencing of single gene regions of isolates may not be able to differentiate between closely related species (Figure 1).

Several studies have been done to resolve taxonomic issues surrounding species of *Penicillium* subgenus *Penicillium* (Frisvad and Samson, 2004; Samson *et al.,* 2004). This subgenus is rich in species diversity and it is economically valuable in our daily lives (Peterson, 2004). Thom (1930), illustrates this (for *Penicillium* species in general) by, *"They rot our fruit,... injure our stored grain,... contaminate our pantries,... discolour fibres, wood,... stored paper and sometimes our books. In the laboratory they infest... every kind of culture operation, bacteriological, mycological, or phanerogamic".* Frisvad and Samson (2004) studied this subgenus intensely through polyphasic taxonomy. They were able to divide the species into various unique sections and series, and characterise *Penicillium* subgenus *Penicillium* in a stable taxonomic system. Morphological characters were examined and secondary metabolite profiles determined. Samson *et al.* (2004) studied the taxonomy of this subgenus by using partial β-tubulin sequences and confirmed the results of Frisvad and Samson (2004).

All three developmental phases involve the use of morphological data. This development is essential to our understanding of *Penicillium* species and identification thereof. In general, research tends to focus on specific species or subgenera of *Penicillium,* particularly subgenus *Penicillium.* Currently, few studies are focusing on several gene regions for phylogenetic analysis. In addition, little work has been done on PCR-RFLP

analysis of a number of species from different subgenera. In Peterson *et al.* (2004), three gene regions were investigated, focusing however, only on three closely related terverticillate species. Although other subgenera may not be as diverse, studies in these areas may resolve some taxonomic issues surrounding this genus as a whole.

3. IDENTIFICATION AND CHARACTERISATION OF *PENICILLIUM* SPECIES

3.1 MORPHOLOGICAL IDENTIFICATION

Morphological identification employs the use of three different culture media, incubated at three different temperatures as described by Pitt (1973; 1991). Microscopy is used in identification because defining characteristics can be used to distinguish between various teleomorphic and anamorphic species. Microscopic slides are made of each isolate on MEA and CYA, as characteristics may differ on these media. The subgenus (verticillate nature) can be determined by the number of branch points (rami) between the phialide (which bears the conidia on the tip) and the stipe (hyphal stalk). Isolates with one such branch point are monoverticillate, two branches - biverticillate, three branches - terverticillate and four branches - quarterverticillate (Figure 2; Pitt, 1991). Monoverticillate isolates are classified into subgenus *Aspergilloides* and terverticillate and quarterverticillate isolates are grouped into subgenus *Penicillium.* Biverticillate isolate classification is more complex with growth characteristics playing a major role in the separation of these isolates into either the *Furcatum* or *Biverticillium* subgenus (Pitt, 1979; 1991).

Due to certain *Penicillium* species having similar characteristics when grown on particular media, methods of identification cannot be limited to morphological examination alone (Dupont *et al.,* 1999; Colombo *et al.,* 2003; Marek *et al.*, 2003; Dean *et al.,* 2005). Conversely, isolates of a single species may appear different if grown on various media (Raper and Thom, 1949). This is adequately described by Raper and Thom (1949) by *"...a mold grown in the presence of a fermentative sugar may show one*

aspect; whereas, the same mold, if grown on a leather shoe or some other nitrogen-rich substrate, may assume a very different appearance". Morphological identification is tedious, time-consuming and accuracy of the procedure is sometimes questionable (Pitt, 1979; Dupont *et al.,* 1999; Colombo *et al.,* 2003; Marek *et al.*, 2003; Dean *et al.,* 2005). More precise, in-depth methods such as molecular identification also need to be employed to ensure accuracy. Species or strains that are morphologically similar may differ genotypically. These differences can only be detected through molecular identification techniques (Mitchell *et al.*, 1995; Vogler and Bruns, 1998). Identification of *Penicillium* species should therefore focus on the implementation of both morphological and molecular identification methods.

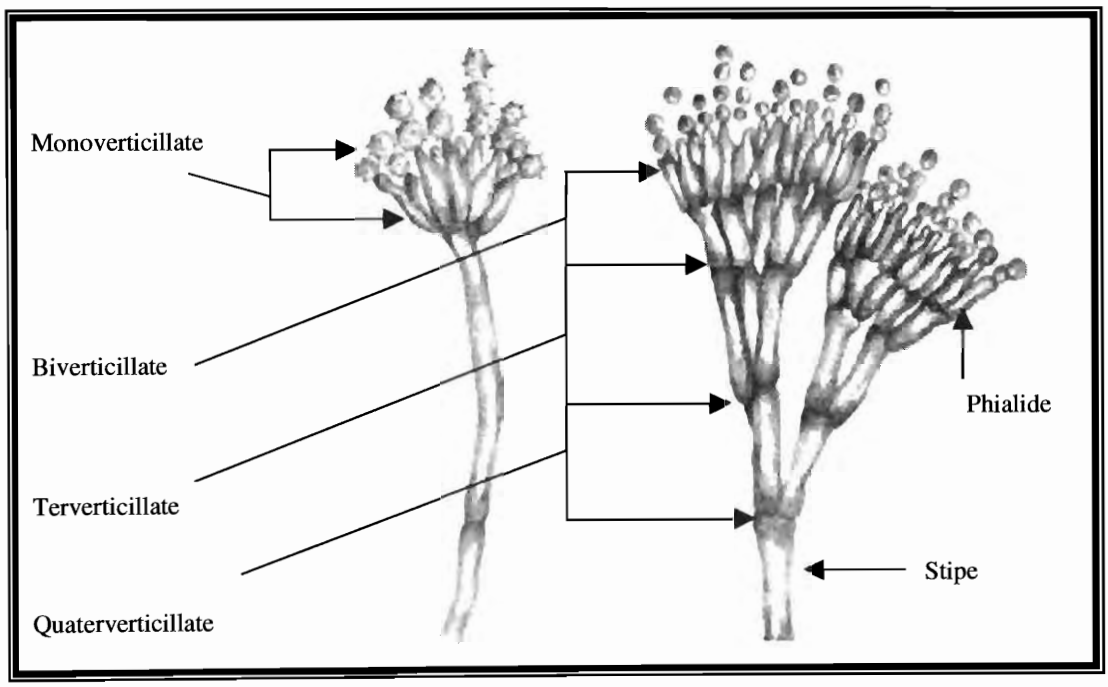

Figure 2: Diagram of a monoverticillate and quaterverticillate penicillus indicating the verticillate nature (subgenus) of an isolate according to the number of branch points between phialide and stipe (Adapted from Pitt, 1991).

3.2 MOLECULAR IDENTIFICATION

Molecular identification provides a more accurate and definitive method for distinguishing species, based on minor differences in genetic material (Fairbanks and

Anderson, 1999). Fingerprinting methods generally used for identification purposes include Random Amplified Polymorphic DNA (RAPD) (Hadrys *et al.,* 1992; Lund *et al.,* 2003), Amplified Fragment Length Polymorphic fingerprinting (AFLP) (Vos *et al.,* 1995; Majer *et al.,* 1996; Castella *et al.,* 2002), Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) (LoBuglio and Taylor, 1995; Sequerra *et al.,* 1997; Colombo *et al.,* 2003; Latouche *et al.,* 2003), microarrays (Schmidt-Heydt and Geisen, 2007), DNA barcoding (Seifert *et al.,* 2007) and DNA sequencing (Vogler *et al.,* 1998; Samson *et al.,* 2004). Variations on PCR identification methods include multiplex PCR assays (Dean *et al.* 2005) and Real-Time PCR (Geisen *et al.* 2004).

Fungal identification can be rapidly performed as the ITS gene region (White *et al.,* 1990) forms part of the highly conserved 5.8S ribosomal DNA. This region is present in most fungal species (Mitchell *et al.,* 1995; Seifert and Louise-Seize, 2000; Skouboe *et al.,* 2000) and commonly used primers are readily available. Alternative regions such as beta tubulin (β-tubulin) are less conserved and can provide greater resolution between closely related species if required (Glass and Donaldson, 1995). Beta-tubulin and other tubulin proteins, form part of microtubules that are essential components of the cytoskeleton and mitotic spindles (Thon and Royse, 1999). Dupont *et al.* (1999) and Colombo *et al.* (2003) effectively differentiated between phenotypically similar species of *Penicillium* using β-tubulin and ITS gene regions respectively. Restriction enzymes tested previously for efficiency to differentiate various *Penicillium* species through PCR-RFLP include *Alu*I, *Ava*I, *Bam*HI, *Bgl*I, *Clo*I, *Eco*RI, *Hae*III, *Hind*III, *Hinf*I, *Hpa*II, *Mbo*I, *Mse*I, *Rsa*I and *Taq*I (Seifert, 2000; Colombo, 2003).

Sequencing in conjunction with fingerprinting techniques has been used extensively in identification of various fungal genera and species (Chen *et al.,* 1996; Cooke and Duncan, 1997; Samson *et al.,* 2004). Sequencing allows for further investigations into the relatedness of organisms through phylogenetic studies and single base changes within the DNA sequence may represent intraspecific variation (Peterson, 2000). In short, numerous studies have been done to resolve the taxonomic issues surrounding *Penicillium* species (LoBuglio *et al.,* 1994; Skouboe *et al.,* 1999; Peterson, 2000; Seifert

et al., 2000; Frisvad and Samson, 2004; Peterson, 2004; Samson *et al.,* 2004; Wang and Zhuang, 2007).

4. *PENICILLIUM* IN THE ATMOSPHERE

Conidia are a major air contaminant and can result in allergic reactions, asthma, mucous membrane irritation, bronchitis, hyper-sensitivity pneumonitis, organic dust toxic syndrome, conditions resulting from activation of the immune response and many other respiratory disorders in sensitive individuals (Buttner and Stetzenbach, 1993; Calderon *et al.*, 2002; Airaksinen *et al.,* 2004; Portnoy *et al.*, 2004). Bioaerosols contain fungal material such as viable as well as non-viable conidia, mycelial particles and proteins (Portnoy *et al.*, 2004). Conidia need not be viable in order to cause hypersensitivity reactions, as the antigens alone may induce a reaction (Buttner *et al.,* 1993). Sick Building Syndrome (SBS) can be described as sub-clinical symptoms caused by an inadequate ventilation system within a working area (Gupta *et al.,* 2007). Gupta *et al.* (2007) investigated the effect of SBS on employees in an office building. Worker symptoms include headaches, fatigue, congestion, dizziness and nausea.

Previous studies have shown indoor bioaerosol concentrations to be up to four times greater than that of outdoor air (Sawane and Saoji, 2004; Jo and Seo, 2005; Lee and Jo, 2005). This is important as the majority of daily activities take place indoors. It can be attributed to air-flow occurring from outdoors to indoors, which results in an accumulation of particles carried in the air. This difference appears to increase during wet weather. Rain tends to cleanse the outside atmosphere of bioaerosols while the indoor humidity increases, which promotes spore germination (Bhati and Gaur, 1979; Sawane and Saoji, 2004). Bioaerosols may settle on indoor surfaces but are easily dislodged and recirculated. Undisturbed indoor air has a velocity, which is estimated at 0.142 m/s (Airaksinen *et al.,* 2004). Previous studies have shown the minimum air velocity required to dislodge *Penicillium* conidia from conidiophores to be 0.5 m/s. Should activity within the room increase, the velocity of the air surrounding surfaces on

which the spores have settled increases. Thus, human exposure to an abundance of fungal material occurs.

Air conditioning (HVAC) systems have been shown to reduce indoor spore counts if windows remain closed and filters are regularly maintained (Streifel *et al.,* 1987). However, these maintenance procedures seldom occur at regular intervals. Chang *et al.* (1996) demonstrated that fungal growth in HVAC systems is highly probable due to the design and functioning of these units. Bioaerosols penetrate and settle onto duct materials and humid conditions are created around the cooling coils promoting fungal growth (Chang *et al.,* 1996). Cleaning guidelines for HVAC systems have been published by the National Air Duct Cleaners Association (NADCA) to assist in "Understanding Microbial Contamination in HVAC Systems" (Foarde *et al.,* 1997; NADCA, 2004).

Dispersal of fungal material may also be facilitated through cleaning (Franke *et al.*, 1997). Previous studies have shown spore concentrations to be particularly high on carpeted surfaces (Franke *et al.*, 1997; Buttner *et al.*, 2002). Vacuuming is a commonly employed method to clean such surfaces, which further aggravates spore dispersal into the surrounding atmosphere (Buttner and Stetzenbach, 1993). High pressure steam-cleaning is commonly used to clean storage areas such as fruit packhouses however; there are several disadvantages to this method. Firstly, no chemicals that are effective in reducing the inoculum are used during the steam-cleaning process. Secondly, the cleaning technique used creates air currents that disturb fungal material that has settled onto surfaces. Finally, steam cleaning involves the use of moisture and this creates ideal conditions for germination of *Penicillium* conidia.

5. *PENICILLIUM* IN THE ENVIRONMENT

Known for producing vast amounts of small, lightweight and resistant conidia, *Penicillium* species have the ability to survive, develop and reproduce in adverse conditions, due to this effective survival ability. Major contributing survival factors

include the efficiency of spore attachment, extended spore viability and colonisation of almost any surface (Anderson, 1956; Pitt, 1979; Morey *et al.,* 2003; Amiri and Bompeix, 2005).

Environments that contain a high concentration of Penicillium spores include the surrounding atmosphere, air-conditioning units, walls, floors and wet and humid surfaces and environments (Morey *et al.,* 2003). *Penicillium* species are reliant on a passive method of spore dispersal (Dobbs, 1942; Franke *et al.,* 1997; Buttner *et al.*, 1999) and where growth is concealed (e.g. within wall structures), slight disturbances may cause conidia to dislodge and circulate in the air in the room. In cooled indoor environments, the approximate velocity of air exiting cooling units is 2.8 m/s (Buttner *et al.*, 1999). As discussed previously, the minimum air velocity required to dislodge *Penicillium* conidia from conidiophores is 0.5 m/s (Pasanen *et al.,* 1991). This indicates the ease with which *Penicillium* spores may dislodge into surrounding environments resulting in a decrease in indoor air quality (Morey *et al.,* 2003; Gupta *et al.,* 2007).

The surface structure of a *Penicillium* conidium consists of various patterns of rodlets that vary between species. Electron micrographs have shown roughened surface textures of conidia (Hess *et al.,* 1968). This roughened texture may play a role in conidial attachment. Moisture may assist *Penicillium* spore attachment in two ways (Amiri *et al.,* 2005). Firstly, hydration causes the weight of the spore to increase and secondly, moist conditions are required for the development of the hyphal tip. These factors positively influence spore attachment by making it less likely for the spore to re-enter the atmosphere, allowing for initiation of germination mechanisms. In the presence of appropriate nutrients, moisture and oxygen, conidia attach to an appropriate surface. Conidia then undergo a change in surface properties and chemical composition, enhancing host attachment. Troy and Koffler (1969) demonstrated an increase in hexose sugars and chitin in the walls of *P. chrysogenum* conidia when grown on suitable media. When exposed to moisture, polysaccharides and glycoproteins present on the outside wall of conidia assist in host attachment (Agrios, 1997). In addition, glucan composition of the cell now resembles that of higher plants (Troy and Koffler, 1969), so the conidial

chemistry is similar to that of its host. Nuclear mitosis is initiated and subsequent germination follows.

Several factors influence germination of the conidia. It may be controlled by a number of sensory activators and molecular signaling pathways (Osherov and My, 2001). Water activity plays an essential role in conidial germination and to a lesser extent, temperature and pH (Sautour *et al.*, 2001). Germination is followed by rapid hyphae extension and growth (Anderson, 1956) (Figure 3).

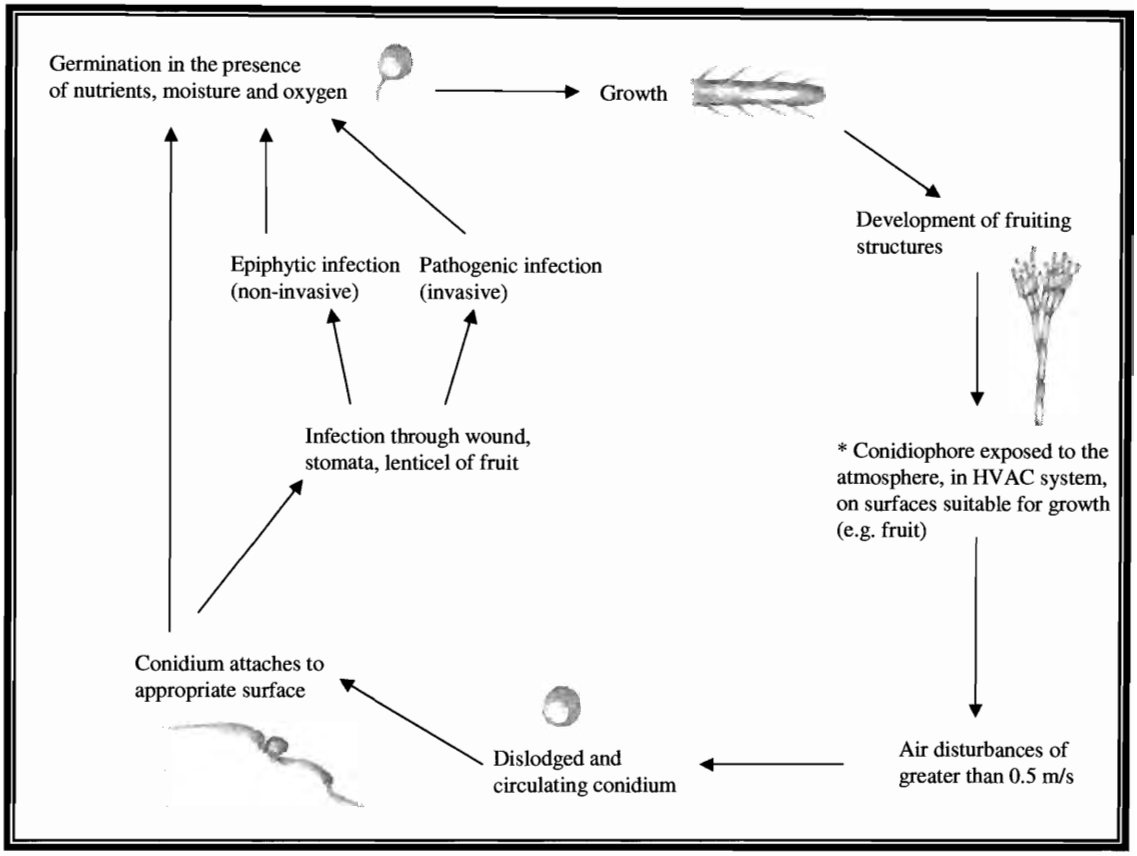

Figure 3: The lifecycle of *Penicillium* species.
Starting point is indicated by *. Conidia present on conidiophores are exposed to atmospheric disturbances. A condium is dislodged from a fruiting structure and it circulates in the atmosphere until in comes into contact with a suitable host and substrate. Infection of fruit occurs through wounds, lenticels and stomata. Some species will initiate a pathogenic infection while others will remain epiphytic. Germination occurs which is followed by rapid growth, colonisation and development of fruiting structures.

6. *PENICILLIUM* ASSOCIATED WITH FRUIT

6.1 IMPORTANCE OF *PENICILLIUM* IN THE PREHARVEST ENVIRONMENT

A delicate balance of microbial populations exists on plant surfaces, which changes throughout the different developmental phases (Korsten, 2006). As *Penicillium* is a common soil inhabitant (Pitt, 1991) reliant on air disturbances for spore dispersal, its presence on the phyllo- and fructoplanes may be anticipated (Agrios, 1997; Korsten, 2006). Although *Penicillium* is a common postharvest fungus, most pathogenic infections occur preharvestly during fruit development. Several species of *Penicillium* have been indicated to be pathogenic to a number of different plant hosts. These include *P. expansum* (Link), *P. italicum* (Wehmer), *P. digitatum* (Sacc.), *P. solitum* (Westling), *P. viridicatum* (Westling), *P. rugulosum* (Thom) and occasionally *P. hirsutum* (Dierckx) (Garber *et al.,* 1965; Pitt, 1991). Following preharvest infection, disease development may remain latent to allow for suitable growth conditions, such as an increase in sugar concentrations and water activity (Dantigny *et al.,* 2007).

Preharvest parameters that promote and facilitate infection of litchi fruit by *Penicillium* species include environmental factors such as warm winds (to facilitate spore dispersal), high rainfall conditions and pest damage (Gilbert, 1978; De Jager *et al.,* 2003; Jiang *et al.,* 2003). Environmental conditions, extensive exposure of the fruit to harsh sunlight (Ghosh, 2001) and pest infestation may cause the pericarp to be desiccated, wounded or cracked (Gilbert, 1978). Desiccation of the fruit is associated with mycological decay as micro-crack formation is initiated in the pericarp. These micro-cracks as well as lenticels, stomata and associated pericarp wounds, serve as entry points for pathogens (Cooke and Rayner, 1984; Underhill and Simons, 1993; Coates *et al.*, 1995, Agrios, 1997; Sivakumar *et al.,* 2005, Neri *et al.*, 2006).

Penicillium species are one of the most dominant decay agents affecting litchi fruit. The litchi pericarp provides an ideal environment for conidial attachment and fungal growth, even under cold storage conditions (Underhill and Simons, 1993). Many protrusions on

the litchi pericarp give it a particularly rough texture (Sivakumar *et al.,* 2005), whereby conidia require minimal energy to attach to the fruit. Distinguishing between healthy and *Penicillium* infected fruit may be difficult during preharvest growth development as micro-cracks are difficult to detect (Coates *et al.*, 1995). The litchi pericarp is only 1-3 mm thick (Underhill and Simons, 1993) and the flesh of the fruit provides an abundance of nutrients and sugars as well as a low pH, which selects for fungal growth, in particular that of *Penicillium* species (Lichter *et al.,* 2004; Tournas *et al.,* 2005).

6.2 IMPORTANCE OF *PENICILLIUM* IN THE POSTHARVEST ENVIRONMENT

Penicillium is one of the most commonly known storage fungal species (Adams and Moss, 2003). Most of the spoilage and decay of fruit caused by this organism takes place during storage, when plant defenses no longer play a role (Tournas *et al.,* 2005). Primarily, contamination of fruit such as litchi, apple and pear occurs post-harvestly during storage, with potential for growth and development of the fungus during storage and transport (Marín *et al.*, 2006).

Following fruit harvest, the microbial population of the phylloplane is altered (Korsten, 2006). In terms of microbial communities, *Penicillium* is classified as an *r*-strategist (Atlas and Bartha, 1998). This strategy is characterised by high reproductive rates, and the ability to thrive in environments which are sparsely populated and not resource limiting (Atlas and Bartha, 1998). As *Penicillium* is an opportunistic pathogen, it will thrive and develop rapidly, should the microbial balance of the fructoplane change due to postharvest treatments such as sulphur dioxide fumigation (Korsten, 2006).

Dependant on the host specificity of *Penicillium* species, growth may proceed in two ways. Fruit may develop epiphytic *Penicillium* growth as is seen with most species on various fruit (Korsten, 2006). This condition is predominantly a cosmetic drawback since the fungus colonises the fructoplane and does not affect the fruit internally. It does however; affect the export potential and market value of the fruit (Korsten, 2006). Alternatively, growth conditions permitting, a physical pathogenic infection of the fruit

may occur, either pre- or postharvestly. Pathogenic infection of fruit by *Penicillium* species is characterised by physical invasion of the tissue. Enzymes such as pectinases and cellulases are produced, which facilitates tissue degradation (Atlas and Bartha, 1998).

The importance of *Penicillium* infection of fruit becomes apparent once it is understood how rapidly cross-contamination occurs between stored fruit, as well as between the surrounding environments (Anderson, 1956; Coates *et al.*, 1995). *Penicillium* is an aggressive pathogen exhibiting ideal physical fitness to spread, infect and colonise various environments given the correct growth conditions. If a wound is present on a neighbouring, uninfected fruit, mycelia may directly penetrate and infect this fruit (Anderson, 1956). Due to *Penicillium* being a classical wound pathogen (Janisiewicz and Korsten, 2002; Neri *et al.*, 2006); intact fruit may escape infection even if surrounded by heavily contaminated fruit.

Postharvest decay of litchi fruit by *Penicillium* species is characterised by general softening of the fruit (with no apparent indentations or wrinkling of the fruit surface) and a watery texture of decaying areas (Anderson, 1956). Fruit has a mouldy taste on consumption and in humid storage conditions; blue-green fruiting bodies become apparent (Anderson, 1956). Once removed from cold storage, rapid decay of the fruit occurs (Holcroft and Mitcham, 1996). The characteristic earthy odour of fruit contaminated with *Penicillium* is caused by the compound geosmin. *Penicillium expansum* (Link), as well as eight other *Penicillium* species are known to produce this compound (Whitfield, 1998).

6.3 *PENICILLIUM* SPECIES ASSOCIATED WITH FRUIT SURFACES

Penicillium preferentially colonise fruit as opposed to other hosts such as vegetables as it is an acid-tolerant fungus thriving in environments of low surface pH (Lichter *et al.*, 2004; Tournas and Katsoudas, 2005; Korsten, 2006). Common hosts for *Penicillium* as a dominant decay agent include apples (Amiri and Bompeix, 2005; Conway *et al.*, 2005), citrus (Brown *et al.*, 2000; Palou *et al.*, 2003), pears (Lennox *et al.*, 2003), peaches

(Droby *et al.*, 2003), table grapes (Franck *et al.*, 2005) and litchi (De Jager and Korsten, 2003; Jacobs and Korsten, 2004; Korsten, 2006). Certain species appear to have host preferences such as *P. digitatum* and *P. italicum* occurring predominantly on citrus fruit (Pitt, 1991; Palou *et al.*, 2003).

Species which have previously been reported on litchi include *P. aurantiogriseum* (Dierckx), *P. brevicompactum* (Dierckx), *P. chrysogenum* (Thom), *P. citreonigrum* (Dierckx), *P. citrinum* (Thom), *P. corylophilum* (Dierckx), *P. decumbens* (Thom), *P. expansum* (Link), *P. fellutanum* (Biourge), *P. glabrum* (Westling), *P. janthinellum* (Biourge), *P. rugalosum* (Thom), *P. solitum* (Westling) and *P. viridictum* (Westling) (De Jager and Korsten, 2003; Jacobs and Korsten, 2004). The range of species having been isolated from different studies is an indication of moderate diversity within the genus as well as consistency between species and their ability to colonise different environments. All these species are common soil saprophytes (Domsch and Gams, 1970), many of which may cause decay by serving as an initial inoculum source.

7. HEALTH AND SAFETY ASPECTS OF *PENICILLIUM*

With *Penicillium* having such a wide environmental distribution, these species act as hygiene indicators of potential cross-contamination points of fruit during export. Some species play a significant role in food safety through the production of mycotoxins. Patulin is produced by *P. expansum,* which is commonly found on deciduous fruit, as well as various other species of *Penicillium* and *Aspergillus* (Sommer *et al.*, 1974; Sweeney and Dobson, 1998). *Penicillium expansum* is able to survive adverse conditions, as it is a psychrophile (Pitt, 1973; Sweeney and Dobson, 1998). This indicates that *P. expansum* should be a dominant isolate found under cold storage conditions. Storage of apples at 0°C does not prevent the production of patulin (Sommer *et al.*, 1974). In addition, patulin is a heat stable toxin that is not destroyed during fruit processing involving heat (Sommer *et al.*, 1974). The maximum levels of patulin permitted by the European Union in apples intended for direct consumption is 25 µg/kg (European Commission, 2003).

Citrinin was first isolated from *P. citrinum* (Thom) (Hetherington and Raistrick, 1931). *Penicillium expansum* and *P. verrucosum* (Dierckx) (Pitt, 1991) and species of *Aspergillus* and *Monascus* (Xu *et al.,* 2006) are also known producers of this mycotoxin. Due to routine mycotoxin detection not being very well developed, there is no legal limitation and supporting legislation concerning citrinin in foodstuffs (Xu *et al.,* 2006). *Penicillium* research however, has become more focused in this area in recent years with the development of mycotoxin detection methods such as Thin Layer Chromatography, High-Performance Liquid Chromatography, enzyme immunoassays (Xu *et al.,* 2006), microarrays (Schmidt-Heydt and Geisen, 2007) and Real Time PCR (Geisen *et al.,* 2004). Mycotoxin detection is of particular importance in the fruit juicing industry as Tournas *et al.* (2006) detected *Penicillium* species in pasteurized fruit juice.

8. CONTROL OF *PENICILLIUM* SPECIES

Several methods are commonly used for *Penicillium* disease control of fruit including fungicides, SO_2 fumigation and biological control. However, an ideal method in control is to prevent injury to the fruit, since *Penicillium* species are classic wound pathogens (Anderson, 1956). Care should therefore be taken during picking, handling, packaging and transport to minimise wounding of the fruit (Korsten, 2006). Fruit should also be visually inspected for wounds and *Penicillium* growth during the packing and repacking processes. Infected fruit should promptly be discarded and removed from the facility to prevent release of conidia into the environment.

Maintaining the cold chain during fruit export is essential in ensuring a high quality product (Korsten, 2006). Fruit should be refrigerated as soon as possible after harvest, and kept at low temperatures until sold to the consumer (Sivakumar *et al.,* 2005; Korsten, 2006). Refrigeration causes a reduction in the metabolism of the fruit and cooled conditions are less favourable for disease development (Korsten, 2006). For optimal results it is recommended that litchi fruit be stored at 0 - 1°C throughout the export chain (PPECB Export Directory, 2007).

To prevent browning and maintain the characteristic red colour of the litchi pericarp, fruit is treated post-harvestly. Fruit is fumigated with sulfur dioxide gas, which initially bleaches the pericarp. The red colour of the pericarp returns after dipping in diluted hydrochloric acid (Lichter *et al.,* 2004). Sulfur dioxide treatment and other fungicide applications are currently the only commercially used methods to prevent pericarp browning and control of fungal growth on litchi in South Africa (Jiang *et al.,* 2003; PPECB, 2007). Sulphur dioxide fumigation and hydrochloric acid dipping appear to have little or no effect in the reduction of fruit decay (Holcroft and Mitcham, 1996; Lichter *et al.,* 2004). On the contrary, it may initiate fungal decay of the fruit by providing a suitable growth environment, devoid of competitive microorganisms that were eliminated during the fumigation process. In addition, previous studies have shown that SO_2 treatment of fruit causes lenticels to open (Amiri *et al.,* 2005) and may promote damage of the fruit (Franck *et al.,* 2005). Such treatments and harsh environmental factors cause the development of micro-cracks in the pericarp (Gilbert, 1978, Underhill and Simons, 1993, Sivakumar *et al.,* 2005) and subsequently promote decay by acid tolerant fungi such as *Penicillium* species (Lichter *et al.,* 2004). It is essential to develop alternative postharvest control methods as maximum residue limits for sulphur set by the European Union is 10 ppm (Jiang *et al.,* 2003). Some *Penicillium* species appear to be resistant to sulphur dioxide treatment. *Penicillium egyptiacum* (Beyma) conidia for example, appear to be unaffected by exposure of 10-100 ppm gaseous sulfur dioxide (Jennings, 1993).

Alternative control measures to sulfur dioxide treatment are being tested for use both at pre- and postharvest levels. Examples include microwave power in the control of *P. expansum* (Karabulut and Baykal, 2002), ozone gas treatment in the control of *P. digitatum* and *P. italicum* (Palou *et al.*, 2003), biological control (De Jager *et al.,* 2003) and Controlled Atmosphere (CA) packaging (Beaudry, 1999; Sivakumar and Korsten, 2006; Conway *et al.,* 2005). Controlled atmosphere packaging has shown promise in reducing fungal decay as well as maintaining fruit quality (Beaudry, 1999; Sivakumar and Korsten, 2006). This method is safe for consumers however, the pericarp becomes brown once the fruit is removed from the packaging and exposed to the surrounding

atmosphere. This reduces the appeal of the fruit to European markets. It is however, necessary to determine the effect of these treatments on *Penicillium*, in order to implement the most effective integrated control solution.

9. CONCLUSION

Identification of *Penicillium* species has evolved over three developmental phases. However, with great advances in the field of molecular biology, the basis of identification still requires morphological techniques. Raper and Thom (Thom, 1930; Raper and Thom, 1949), followed by Pitt (Pitt, 1973; 1979), took the first steps in developing the identification guidelines using standardised media and growth conditions.

Penicillium conidia are a major air contaminant, thriving in closed, humid environments (Chang *et al.,* 1996; Foarde *et al.,* 1997). Humid conditions are often found in litchi packhouses and areas cooled by commercial HVAC systems. *Penicillium* has the ability to survive in chilled environments such as cooled containers and cold storage facilities. Effective spore attachment mechanisms play an essential role in the infection, growth, development and propagation of this filamentous fungus (Agrios, 1997; Amiri *et al.,* 2005). Cross-contamination is easy between fruit and other crops, and decay is rapid if fruit is not stored within appropriately cooled conditions.

South Africa is a major litchi producing and exporting country, focusing on ensuring quality and extending shelf life of the fruit. Quantities of litchi fruit passed for export however, has decreased over the years. Major contributing factors that negatively impact on fruit quality are pericarp browning and postharvest decay. Studies have found moderate species diversity within the *Penicillium* genus (De Jager *et al.,* 2003) as well as consistency between the species found during export (Jacobs and Korsten, 2004).

Penicillium can affect the health and safety of the consumer especially if effective control measures are not followed. Several species produce mycotoxins affecting the safety of the product. Sulfur dioxide exposure is the most commonly employed postharvest

treatment of litchi fruit. This treatment has no apparent effect on reducing *Penicillium* inoculum. The presence of *Penicillium* species on the fruit may indicate inappropriate handling at various stages in the supply chain. Workers involved in the above aspects are often in a position to minimize wounding which can result in fruit losses. Employees should be adequately trained in careful picking and basic hygiene, as fruit damage is the key to *Penicillium* infection. Exporters must be aware of the potential risk of cross-contamination and infection of the fruit and how rapidly it may occur. Care must also be taken when storing various fruit crops together and adequate cleaning routines should be in place to minimize cross-contamination between products and from season to season.

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Chapter 2

Isolation and identification of *Penicillium* species in the South African litchi export chain

1. INTRODUCTION

Penicillium species have a natural ecological role of decaying organic matter, thus this genus is a dominant saprophytic fungus found in fruit export. *Penicillium* species have high reproductive rates (Atlas and Bartha, 1998), indicating their ability to thrive and dominate in nutrient-rich environments such as litchi fruit. In order to ensure high quality fruit, effective cold chain management is crucial. Coldrooms and cooled-containers are essential in delivering high-quality fruit to the consumer however; these environments are highly condusive to the spread of conidia and subsequent survival of *Penicillium* species.

Most studies rely on the use of DNA sequencing for identification of *Penicillium* species, although this method is costly, time-consuming and database information may not be entirely accurate (Ciardo *et al.,* 2007). Previous studies have made use of PCR-RFLP to successfully differentiate between several *Penicillium* species. LoBuglio and Taylor (1995) used PCR-RFLP to analyse nucleotide differences between different isolates of *P. marneffei* in order to develop species-specific primers. Flórez *et al.* (2007) used PCR-RFLP to distinguish between a number of *Penicillium* species isolated from cheese. Both these studies analysed the ITS gene region, although it is well established that this region is highly conserved (Glass *et al.,* 1995; Skouboe *et al.,* 2000; Samson *et al.,* 2004; Wang and Zhuang, 2007).

With much diversity within the genus *Penicillium*, it is essential to develop molecular identification methods that are rapid, repeatable and reliable. The aim of this study was to identify *Penicillium* species within the South African litchi export chain and develop a PCR-RFLP method with which species may be easily differentiated from one another. The use of PCR-RFLP is ideal as it is a method that is quick and easy to perform and it can easily be applied to routine screening of *Penicillium* isolates.

2. MATERIALS AND METHODS

2.1 ISOLATION OF *PENICILLIUM* ISOLATES

Sampling occurred over three litchi-producing and exporting seasons – 2004/2005, 2005/2006 and 2006/2007 – during December to February. Local samples originated from four different litchi packhouses in Limpopo Province (South Africa) as well as cold storage facilities at a harbour (Western Cape Province, South Africa). International samples originated from a port in Holland, two distribution centres in Holland and France and two re-packing facilities in the United Kingdom (U.K.) and Belgium. Fungal isolates were obtained from air samples, fruit wash-water and swabs taken from surfaces in contact with fruit or boxes while the products moved through the export chain. Surfaces from which transport swabs (Medical Wire and Equipment Co., Bath, U.K.) were taken include pickers' and packers' hands; boxes and crates in orchards and packhouses; walls, floors and flaps in packhouses, cold rooms and repacking areas and packlines (bands, rollers, sorters and dip tanks).

Locally, sampling was done at the beginning as well as the end of the litchi season, while international sampling was done once during the season. Sampling procedures however, were identical at all locations. Sampling was done with five replicates per sampling location.

In local orchards, pickers' hands, crates and picking bags were sampled using 10 workers per site. In packhouses, wasterooms and pre-cool rooms; boxes and crates; packers' hands and various areas of the packline were sampled. Walls and floors of packhouses were sampled with ten replicates. In coldrooms, walls, floors and flaps were sampled at one to three sampling points dependant on the size of the facility.

Coldrooms, containers and repacking areas were sampled internationally. Coldroom walls, floors and flaps were sampled, while walls and floors of containers were sampled.

In the repacking area, walls; floors; repackers' hands and boxes were sampled. Data was intended to indicate which *Penicillium* species occurred within the litchi export chain.

A SAS Compact Surface Air System (PBI International, Italy) was used for air sampling. This system has an air capacity of 30 litres per unit (U), which is 20 seconds. Prior to use, the operating system was optimised for cold rooms as well as areas of ambient temperature to ensure countable fungal colonies (unpublished data). For low temperature areas, such as coldrooms, 6 U was used for sampling, while in areas of ambient temperature, 1 U was used due to the inoculum load being lower in cooled environments.

All swabs were transported in cooler boxes in order to minimise temperature fluctuations. Swabs were promptly processed by aseptically placing the swab in nine ml Ringers' solution (Merck, Johannesburg, South Africa) and mixed well using a vortex shaker (Labotec, Johannesburg). Dilution series were performed and 100 µl of each suspension was plated out on Malt Extract Agar (MEA) (Merck) plates amended with D(+)-glucose (Merck) as well as Standard 1 Nutrient Agar (STD1) (Merck). Petri dishes containing both agar types were incubated at 25°C however; MEA plates were incubated for 96 h while STD1 plates were incubated for 48 h. Air sample plates of MEA and STD1 were incubated at 25°C for five days. Single, representative colonies were identified and conidia thereof plated out to obtain pure cultures. Total counts were not regarded as part of this study and data will not be presented as such.

Due to the vast number of isolates obtained, the cultures were grouped according to similar cultural characteristics to make handling and identification manageable. These characteristics included colony size, shape, colour, texture and formation, mycelia and reverse plate colouration and exudate production. Each group was assigned a number and a representative isolate was randomly chosen. Groups containing only one isolate were not included in this study.

All isolates were preserved for future reference. Cultures were freeze-dried in duplicate as well as preserved on Potato Dextrose (PDA) (Merck) agar slants and in sterile water.

Cultures are maintained in the fungal culture collection of the Microbiology and Plant Pathology Department, University of Pretoria, Pretoria, South Africa.

2.2 MORPHOLOGICAL IDENTIFICATION AND CLASSIFICATION

2.2.1 CULTURE PREPARATION

Vials containing 9 ml Ringers' solution were sterilized. Three to four agar blocks of approximately 5 mm X 5 mm in size were cut aseptically from representative cultures, added to the vials and vortexed. Ten microliters of the spore suspension was inoculated onto three agar types at three points equidistant from each other. Five Petri dishes were inoculated per isolate according to the guidelines of Pitt (1991). One complex Malt Extract Agar (MEA) [malt extract powder, peptone, glucose and bacteriological agar] plate and one 25% Glycerol Nitrate Agar (G25N) [Czapek concentrate ($NaNO_3$, KCl, $MgSO_4.7H_2O$, $FeSO_4.{}_7H_2O$, $ZnSO_4.{}_7H_2O$, $CuSO_4.{}_5H_2O$), K_2HPO_4, yeast extract powder, glycerol and bacteriological agar] plate per isolate were incubated at 25°C for seven days. Three Czapek Yeast Extract Agar (CYA) [Czapek concentrate, K_2HPO_4, yeast extract powder, sucrose and bacteriological agar] plates were incubated at 5°C, 25°C and 37°C respectively for seven days (Pitt, 1991).

2.2.2. CULTURE EXAMINATION

2.2.2.1 MEASUREMENT OF COLONY GROWTH

On the seventh day of incubation, growth and spore germination on the 5°C incubated plate was examined using a stereomicroscope (Zeiss, Germany). Growth at 25°C and 37°C was only visually examined (Pitt, 1991). Diameters of distinct colonies were measured in millimeters on the reverse side of the Petri dish. Each colony that developed from the suspension droplet was measured twice across the widest points. A maximum of six measurements were taken per plate. Colonies where growth was inhibited, or those

that developed from stray droplets were disregarded (Pitt, 1991). All measurements were documented.

2.2.2.2 COLONY CHARACTERISTICS

Colony characteristics were assessed visually and stereomicroscopically. Important characteristics included colony texture and colour, conidia production, exudate production and colour thereof, pigmentation of mycelium or exudate, diffusion of the pigment into the medium, sclerotia production and buckling of the medium. Other unique characteristics were also noted and all information was documented.

2.2.2.3 MICROSCOPY

A compound microscope (Zeiss) was used for the examination of fruiting structures and conidia. Bright field microscopy of *Penicillium* species requires staining with lactofuschin (Carmicheal, 1955). Microscopic slide preparations were made from hyphal growth on each of the MEA and CYA plates due to certain characteristics being more apparent on certain media (Pitt, 1991). Important characteristics which were observed and noted include mono-, bi-, ter-, or quaterverticillate penicilli nature; stipe size, shape and texture; conidia shape, size, colour and texture; conidiation and the presence or absence of conidial chains (Pitt, 1991).

2.3 MOLECULAR IDENTIFICATION

2.3.1 SINGLE SPORE ISOLATIONS

Single spore isolations were performed on all representative isolates to ensure genetically homogenous DNA. It was performed by plating out 90 µl of sterile water and 10 µl of the spore suspension (described previously) for each isolate onto 90 mm Petri dishes containing 0.4 % water agar (Bacteriological agar, Merck). Plates were incubated for three to twelve hours at 25°C. Single spores were examined and isolated using a

stereomicroscope. Single spores were inoculated onto Petri dishes containing PDA, which were supplemented with chloramphenicol and incubated until growth was sufficient for DNA extraction. Isolations were repeated in triplicate.

2.3.2 DNA EXTRACTION

For extraction of total DNA from the mycelia and conidia of the isolates, the DNeasy® Plant Mini Kit from Qiagen (Southern Cross Biotechnology, Johannesburg) was used according to the manufacturer's specifications. A FastPrep® Instrument FP 120 (Bio 101® Systems, France) was used to lyse the cells at 4.0 m/s for 40 s. Mechanical disruption of the cells was facilitated by the use of 0.5g of 0.5 mm silica beads (Biospec Products Inc., Separations, Johannesburg). Total DNA extracts were visualised on a 1 % agarose gel (Whitehead Scientific, Johannesburg) stained with 0.01 % ethidium bromide. A 1 kilo-basepair (kb) Hyperladder I (Bioline, Celtic Molecular Diagnostics (Pty) Ltd., Cape Town, South Africa) molecular marker was included for size estimation. Extractions were observed under ultraviolet illumination in an electrophoresis gel-documentation system (Vilber Lourmat, OmniScience, Johannesburg).

2.3.3 POLYMERASE CHAIN REACTION

Primers chosen for amplification of the Internally Transcribed Spacer (ITS) gene regions of the 5.8S rDNA were ITS1 (5` - TTT CCG TAG GTG AAC CTG C – 3`) and ITS4 (5` - TCC TCC GCT TAT TGA TAT GC – 3`) (White *et al.,* 1990). A partial section of the beta-tubulin (β-tubulin) gene region was amplified with Bt2a (5` - GGT AAC CAA ATC GGT GCT GCT TTC – 3`) and Bt2b (5` - ACC CTC AGT GTA GTG ACC CTT GGC – 3`) primers (Glass *et al.,* 1995).

Amplifications of the ITS gene region were performed in a 50 µl reaction volume and the protocol per reaction contained 0.5 µl of genomic DNA, 5 µl 10X NH_4 reaction buffer, 2.5 µl 50mM magnesium chloride, 10 mM of each of the four dNTPs, 0 – 4% (of the final volume) stock dimethyl sulphoxide, 0.5 µl of each 15 µM oligonucleotide primer

and 1 unit (U) of *Taq* DNA polymerase. Sterile SABAX water was added to result in the final volume of 50 µl. A lesser volume of genomic DNA (0.15 - 0.2 µl) and 10 µM of each oligonucleotide primer (Bt2a and Bt2b) was used in the β-tubulin PCR reaction. Remaining components were used as in the ITS PCR.

Conditions for the PCR were optimised and PCR cycle profiles were performed using the 2700 Perkin-Elmer PCR thermocycler (Perkin-Elmer, Massachusetts, U.S.A). Cycling conditions for ITS amplification were: initial denaturation at 95°C for two min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 57°C for 45 s and primer extension at 72°C for 90 s, followed by a final extension at 72°C for seven min. Cycling conditions for β-tubulin amplification were 95°C for three min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for two min, followed by a final extension of 72°C for ten min.

Amplification products were visualised by electrophoresis using a 1 % (w/v) agarose gel stained with 0.01 % ethidium bromide. A 100 bp Hyperladder IV (Bioline) molecular marker was included for size estimation. Products were observed using ultraviolet illumination in an electrophoresis gel-documentation system (Vilber Lourmat).

2.3.4 SEQUENCING AND PHYLOGENETIC ANALYSIS

Purification of the PCR products was performed prior to sequencing. A QIAquick® PCR Purification Kit from Qiagen (Southern Cross Biotechnology) was used according to the manufacturer's specifications. Both forward and reverse strands of the ITS and β-tubulin amplicons were sequenced using the BigDye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, U.S.A). Components per sequencing reaction were 3 µl sterile water, 1 µl dilution buffer, 2 µl Bigdye® Reaction Mix, 1µl of a 2 µM primer (forward or reverse) and 3 µl purified PCR product resulting in a final volume of 10µl. Sequencing reactions were performed using the 2700 Perkin-Elmer PCR thermocycler (Perkin-Elmer). Sequencing cycle conditions were 96°C for one min, followed by 25 cycles of 96°C for 10 s, 50°C for five s, 60°C for four min and samples were held at 4°C.

Sequencing reactions were purified prior to analysis using the following clean-up protocol. Ten microlitres of sterile water was added to the sequencing reaction and centrifuged briefly for 30 s. Two microlitres of 3M sodium acetate was mixed with the reaction to facilitate DNA precipitation. Fifty microlitres of chilled absolute ethanol was added to the reaction and vortexed. This mixture was incubated for ten min on ice and centrifuged at 4°C (13 000 r.p.m) for 20 min. Following centrifugation, absolute ethanol was removed, 80 µl 70% ethanol was added and centrifuged for five min at room temperature, 6000 r.p.m. The 70% ethanol was removed and tubes were left exposed to allow any remaining ethanol to evaporate.

Samples were analysed using an ABI 3130 Genetic Analyzer (Applied Biosystems). Sequences were edited using Vector NTI Advance 9.1.0 software (www.invitrogen.com/bioinformatics (2004)) and consensus sequences were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) to clarify identification results. Sequences were deposited in Genbank and accession numbers were assigned to all isolates for both ITS and β-tubulin gene regions (http://www.ncbi.nlm.nih.gov.Genbank/submit.html).

Sequence data was edited further and aligned using Vector NTI Advance 9.1.0 (www.invitrogen.com/bioinformatics (2004)), Bioedit Multiple Sequence Alignment (Hall, 1999) and ClustalX 1.81 (Thompson *et al.,* 1997). Phylogenetic analysis of sequence data was done using PAUP (Phylogenetic Analysis using Parsimony) beta version 4.0b10 (Swofford, 1998). Missing data (?), gaps (-) and parsimony uninformative characters were excluded from the analysis. Fifty-three characters had a weight equal to one while 103 characters had a weight other than one. A Partition Homogeneity Test (PAUP 4.0b10) was performed with 100 replicates to test the congruence and combinability of the ITS and β-tubulin data sets (Huelsenbeck *et al.,* 1996). Both data sets were analysed separately and subsequently combined. A heuristic search for the most parsimonious trees was performed using a random addition sequence and Tree Bisection and Reconnection branch-swapping algorithm. Bootstrap re-sampling of the most parsimonious trees was performed using PAUP 4.0b10 with 1000 replicates

(Felsenstein, 1985). Groups with a confidence level greater than 70 % were retained for the consensus trees. *Furasium oxysporum* sequence data for ITS and β-tubulin gene regions was downloaded from Genbank (Accession numbers – EU073196 (ITS) and EF450110 (β-tubulin)) as outgroups for the respective data sets. Trees were rooted to the outgroup. Tree diagrams were viewed and edited using TreeView 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/rod.html) and Microsoft Powerpoint.

2.3.5 POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Sequence data was analysed with Vector NTI Advance 9.1.0 software (www.invitrogen.com/bioinformatics (2004)) to develop restriction maps of the ITS and β-tubulin gene regions. Restriction enzymes that could potentially differentiate between the various *Penicillium* spp. were identified. Enzymes, *Hae*III, *Apo*I (isochitzomer – *Xap*I), *Bfa*I (isochitzomer – *Fsp*BI), *Hpa*II, *Lwe*I (isochitzomer – *Bsc*AI) and *Tai*I (Fermentas, Inqaba Biotechnologies, Pretoria, South Africa) were used according to the manufacturers specifications and tested for efficacy in distinguishing between the various *Penicillium* species. Sterile water, 1U of restriction enzyme and 2 µl of the appropriate buffer was added per reaction to 18 - 20 µl of the PCR amplicons, resulting in a total volume of 30 µl. The reaction mixture was incubated at optimal temperature for three to four hours in a water bath. Products were left overnight to ensure complete product digestion. Fingerprint banding patterns were observed by loading 18 - 20 µl PCR-RFLP product on a 3 % (w/v) agarose gel stained with 0.01 % ethidium bromide. A 100 bp Hyperladder IV (Bioline) molecular marker was included in order to visually distinguish between fragment sizes in the banding pattern of a species. Visualization was done under ultraviolet illumination in an electrophoresis gel-documentation system (Vilber Lourmat).

3. RESULTS

3.1 ISOLATION OF *PENICILLIUM* ISOLATES

A total of 1542 *Penicillium* isolates were obtained over the 2004/2005, 2005/2006 and 2006/2007 sampling seasons. Cultures were grouped according to similar cultural characteristics and a total of 310 groups could be identified. Each of these groups had unique and defining characteristics. Of these groups, 57 contained isolates of atmospheric origin that were chosen for further study. In total, there were 919 isolates which represents 59.6 % of all isolates obtained in this study. The number of isolates per group is indicated in Table 1.

3.2 IDENTIFICATION AND CLASSIFICATION

3.2.1 MORPHOLOGICAL IDENTIFICATION AND POLYMERASE CHAIN REACTION

One representative isolate from each of the 57 groups was identified according to the methods described by Pitt (1991). The polymerase chain reaction for the ITS and β-tubulin gene regions was optimised for all 57 groups. Amplified fragments were approximately 550-650bp and 450-500bp for the ITS and β-tubulin gene regions respectively.

3.2.2 SEQUENCE AND PHYLOGENETIC ANALYSIS

Edited sequence data for both ITS and β-tubulin gene regions was subjected to BLAST analysis and results are indicated in Table 1. Where more than one identification result is indicated, BLAST results were repeatedly inconclusive. See Appendix I and II for ITS and β-tubulin sequence alignments. No BLAST results for the β-tubulin gene region were obtained for those groups identified as *P. glabrum* through analysis of the ITS gene

region, as there were no records for the *P. glabrum β-tubulin gene partial cds sequence* in the Genbank database at that time.

The partition homogeneity test results indicated that the two data sets were combinable as P = 0.01 (P<0.05; *g1* = -0.284602). A total of 1056 characters were included in the analysis of the combined data sets. Of these characters, 902 were excluded, as they were parsimony uninformative. The remaining 154 characters were parsimony informative and included in the analysis. Following heuristic searches using PAUP* (Swofford, 1998), one tree was retained with significant consistency index (CI) and retention index (RI) values (CI = 0.6126; RI = 0.9200) (Figure 1) and a tree length of 278.38595. Figure 1 indicates clades I to V and various subclades to be discussed.

Table 1: Sequence identification results for all *Penicillium* groups analysed in this study

Group	Number of Isolates	Sequence Identification	*ITS and Beta-tubulin Accession Numbers	Group	Number of Isolates	Sequence Identification	ITS* and Beta-tubulin Accession Numbers
1	45	*P. biourgeianum* Zaleski *P. bialowiezense* Zaleski	*EU128590 EU128532	**30**	27	*P. glabrum* (Wehmer) Westling	*EU128619 EU128561
2	6	*P. chrysogenum* Thom	*EU128591 EU128533	**31**	3	*P. chrysogenum* Thom	*EU128620 EU128562
3	24	*P. biourgeianum* Zaleski *P. bialowiezense* Zaleski	*EU128592 EU128534	**32**	2	*P. polonicum* Zaleski	*EU128621 EU128563
4	4	*P. crustosum* Thom	*EU128593 EU128535	**33**	43	*P. glabrum* (Wehmer) Westling	*EU128622 EU128564
5	4	*P. glabrum* (Wehmer) Westling	*EU128594 EU128536	**34**	14	*P. commune* Thom	*EU128623 EU128565
6	7	*P. echinulatum* Raper and Thom	*EU128595 EU128537	**35**	3	*P. chrysogenum* Thom	*EU128624 EU128566
7	15	*P. polonicum* Zaleski	*EU128596 EU128538	**36**	2	*P. rolfsii* Thom *P. piscarium* Westling	*EU128625 EU128567
8	45	*P. glabrum* (Wehmer) Westling	*EU128597 EU128539	**37**	2	*P. expansum* Link	*EU128626 EU128568
9	72	*P. chrysogenum* Thom	*EU128598 EU128540	**38**	4	*P. paneum* Frisvad	*EU128627 EU128569
10	10	*P. solitum* Westling	*EU128599 EU128541	**39**	5	*P. polonicum* Zaleski	*EU128628 EU128570
11	39	*P. glabrum* (Wehmer) Westling	*EU128600 EU128542	**40**	4	*P. chrysogenum* Thom	*EU128629 EU128571
12	1	*P. glabrum* (Wehmer) Westling	*EU128601 EU128543	**41**	4	*P. glabrum* (Wehmer) Westling	*EU128630 EU128572
13	9	*P. glabrum* (Wehmer) Westling	*EU128602 EU128544	**42**	2	*P. sumatrense* von Szilvinyi	*EU128631 EU128573
14	2	*P. crustosum* Thom	*EU128603 EU128545	**43**	2	*P. steckii* Zaleski	*EU128633 EU128575
15	2	*P. glabrum* (Wehmer) Westling	*EU128604 EU128546	**44**	12	*P. citrinum* Thom	*EU128634 EU128576
16	4	*P. glabrum* (Wehmer) Westling	*EU128605 EU128547	**45**	2	*P. expansum* Link	*EU128635 EU128577
17	277	*P. crustosum* Thom	*EU128606 EU128548	**46**	32	*P. solitum* Westling	*EU128636 EU128578
18	27	*P. crustosum* Thom	*EU128607 EU128549	**47**	4	*P. biourgeianum* Zaleski *P. bialowiezense* Zaleski	*EU128637 EU128579
19	6	*P. glabrum* (Wehmer) Westling	*EU128608 EU128550	**48**	12	*P. expansum* Link	*EU128638 EU128580
20	11	*P. commune* Thom	*EU128609 EU128551	**49**	11	*P. citreonigrum* Dierckx	*EU128639 EU128581
21	11	*P. commune* Thom *P. solitum* Westling	*EU128610 EU128552	**50**	7	*P. citrinum* Thom	*EU128640 EU128582
22	8	*P. paneum* Frisvad	*EU128611 EU128553	**51**	16	*P. citreonigrum* Dierckx	*EU128641 EU128583
23	15	*P. paneum* Frisvad	*EU128612 EU128554	**52**	2	*P. brevicompactum* Dierckx	*EU128642 EU128584
24	5	*P. glabrum* (Wehmer) Westling	*EU128613 EU128555	**53**	3	*P. glabrum* (Wehmer) Westling	*EU128643 EU128585
25	5	*P. polonicum* Zaleski	*EU128614 EU128556	**54**	11	*P. brevicompactum* Dierckx	*EU128644 EU128586
26	6	*P. glabrum* (Wehmer) Westling	*EU128615 EU128557	**55**	2	*P. glabrum* (Wehmer) Westling	*EU128645 EU128587
27	10	*P. citrinum* Thom	*EU128616 EU128558	**56**	3	*P. corylophilum* Dierckx	*EU128646 EU128588
28	4	*P. biourgeianum* Zaleski *P. bialowiezense* Zaleski	*EU128617 EU128559	**57**	2	*P. italicum* Wehmer	*EU128647 EU128589
29	9	*P. chrysogenum* Thom	*EU128618 EU128560				

ITS accession numbers are indicated with a *. Where more than one identification result is indicated, BLAST analysis was inconclusive.

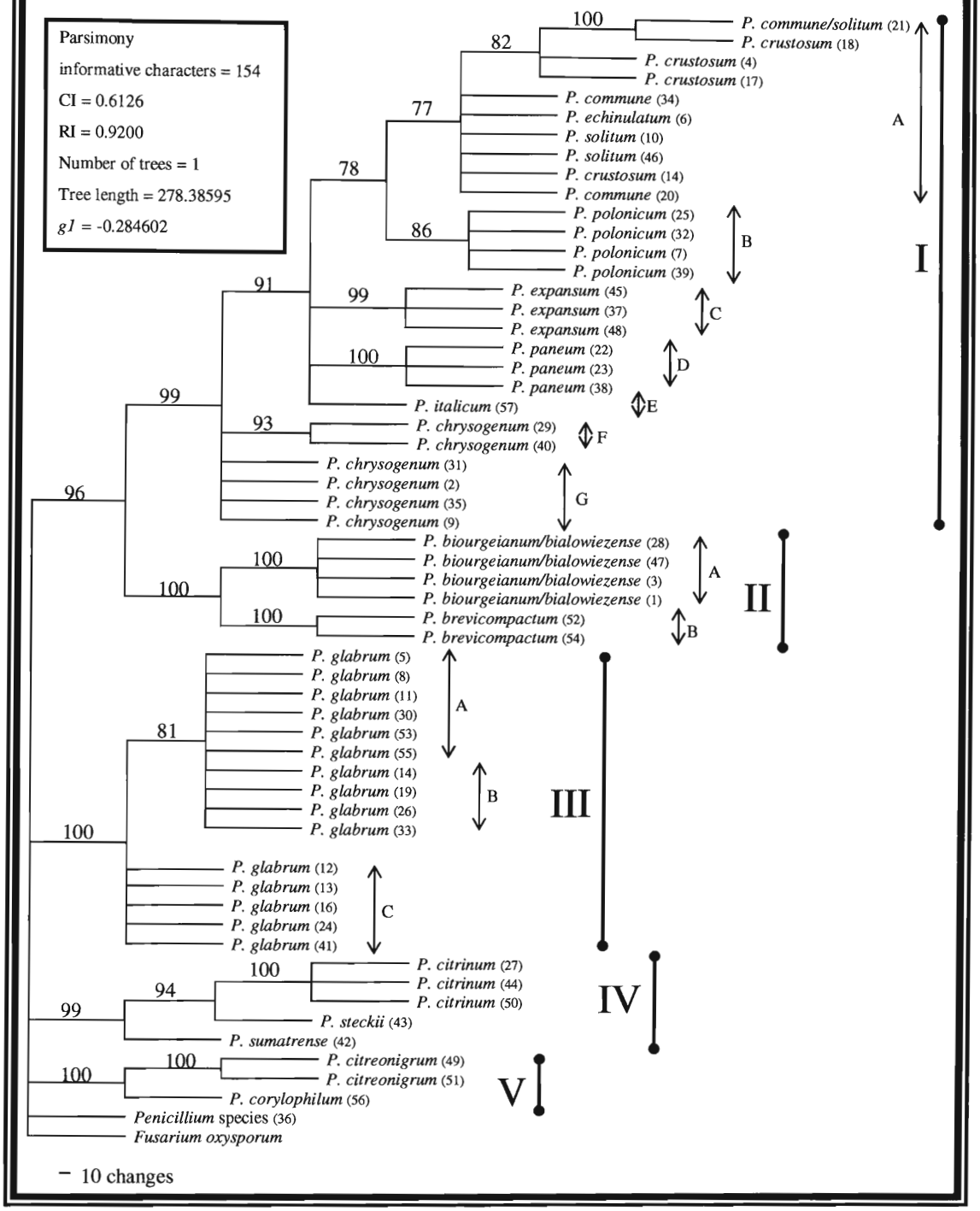

Figure 1: A phylogram generated after a heuristic search of the combined ITS 1, 5.8S and ITS 4 and partial β-tubulin sequence data of the 57 *Penicillium* groups analysed in this study. Group numbers are indicated in brackets. *Fusarium oxysporum* was included as an outgroup. Species names are indicated with the respective group numbers. Bootstrap values are indicated above each branch. Clades I to V are indicated with various sub-clades.

3.2.3 POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM

To distinguish between the various *Penicillium* species found in this study, restriction enzymes were tested for efficiency on both ITS and β-tubulin gene regions. Groups were divided according to species identification as well as verticillate nature (subgenus). Digestion of the β-tubulin gene region was effective in species and strain differentiation (Figures 2-10). The ITS gene region however, demonstrated limited taxonomic value for species differentiation using PCR-RFLP (Figure 12 and 13).

Digestion of the β-tubulin gene region of subclade I-A (Figure 1) with the *Hpa*II restriction enzyme resulted in several variable banding patterns, with minimal consistency found between species groups (Figure 2). This indicates relatedness between isolates as well as individual species. Approximate fragment sizes of each group are indicated in Table 2. In addition, identification of group 21 is unconfirmed as BLAST analysis of the ITS gene region resulted in *P. commune* while the β-tubulin gene region was *P. solitum* (Table 1). Minimal differentiation was found between subclades II-A and II-B when the ITS gene region was digested with *Apo*I (data not shown). Subclade II-B was identified as *P. brevicompactum* while subclade II-A had conflicting identification results for both ITS and β-tubulin gene regions. These groups were identified as *P. biourgeianum* and *P. bialowiezense* for the ITS and β-tubulin gene regions respectively (Table 1). Identification of species in these two subclades was confirmed through digestion of the β-tubulin gene region with *Hpa*II, as two unique banding patterns were displayed (Figure 3) – one representing subclade II-A and the other subclade II-B. Fragment sizes are indicated in Table 2.

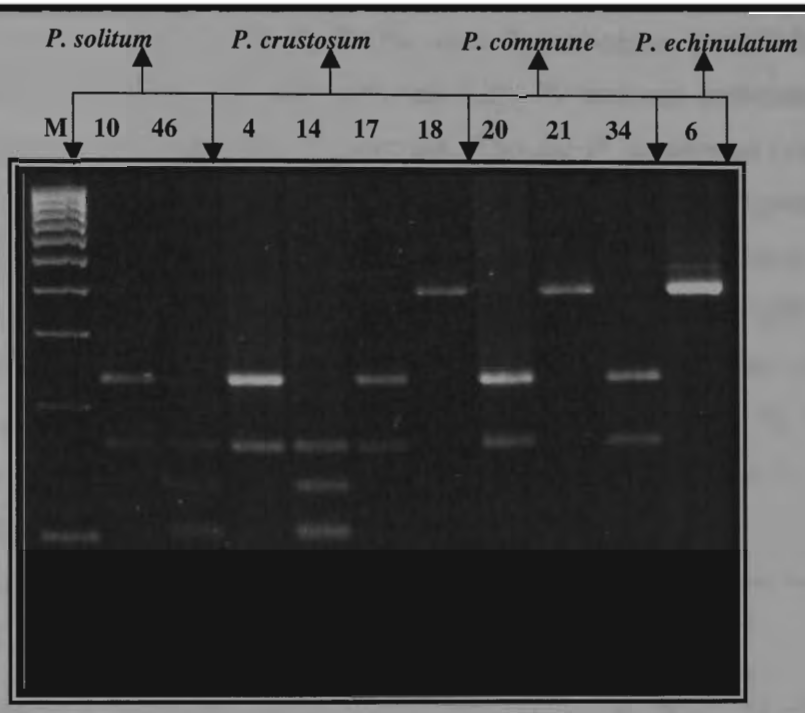

Figure 2: *Hpa*II digest of the β-tubulin gene region of subclade I-A grouped into species groups, showing inconsistency with the banding patterns of these species. Although identification of group 21 remains inconclusive, it was placed in the *P. commune* group (M = 100 bp marker).

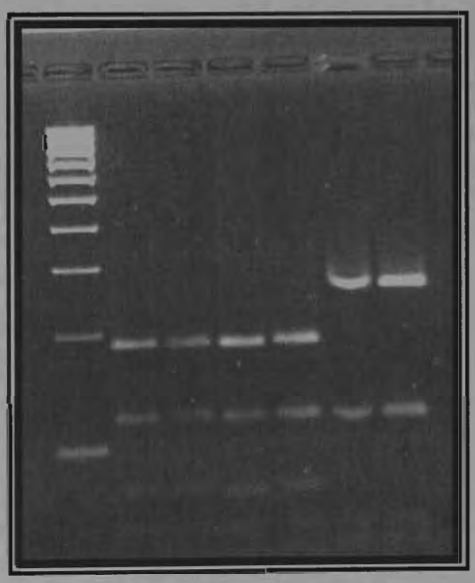

Figure 3: *Hpa*II digest of the β-tubulin gene region of subclades II-A and II-B. Subclade II-B (52, 54) was confirmed to be *P. brevicompactum*, while subclade II-A (1, 2 28, 47) remains unconfirmed between *P. biourgeianum* (ITS) and *P. bialowiezense* (β-tubulin) (M = 100 bp marker).

The remaining species used in PCR-RFLPs were *P. polonicum* (subclade I-B), *P. expansum* (subclade I-C), *P. paneum* (subclade I-D), *P. italicum* (subclade I-E), *P. chrysogenum* (subclades 1-F and I-G), *P. citrinum, P steckii*, *P. sumatrense* (clade IV), *P citreonigrum* and *P. corylophilum* (clade V) and unidentified group 36 (Figures 4 and 5) (Figure 1). Fragment sizes for all species are indicated in Table 2. Restriction digests of the β-tubulin gene region of these groups using *Bfa*I (isochitzomer – *Fsp*BI or *Mae*I) enabled the differentiation of several species from one another. *Penicillium polonicum, P. expansum* (Figure 4) and an unknown *Penicillium* species (group 36) (Figure 5) displayed unique banding patterns. These species groups were removed from the remainder of the identification process.

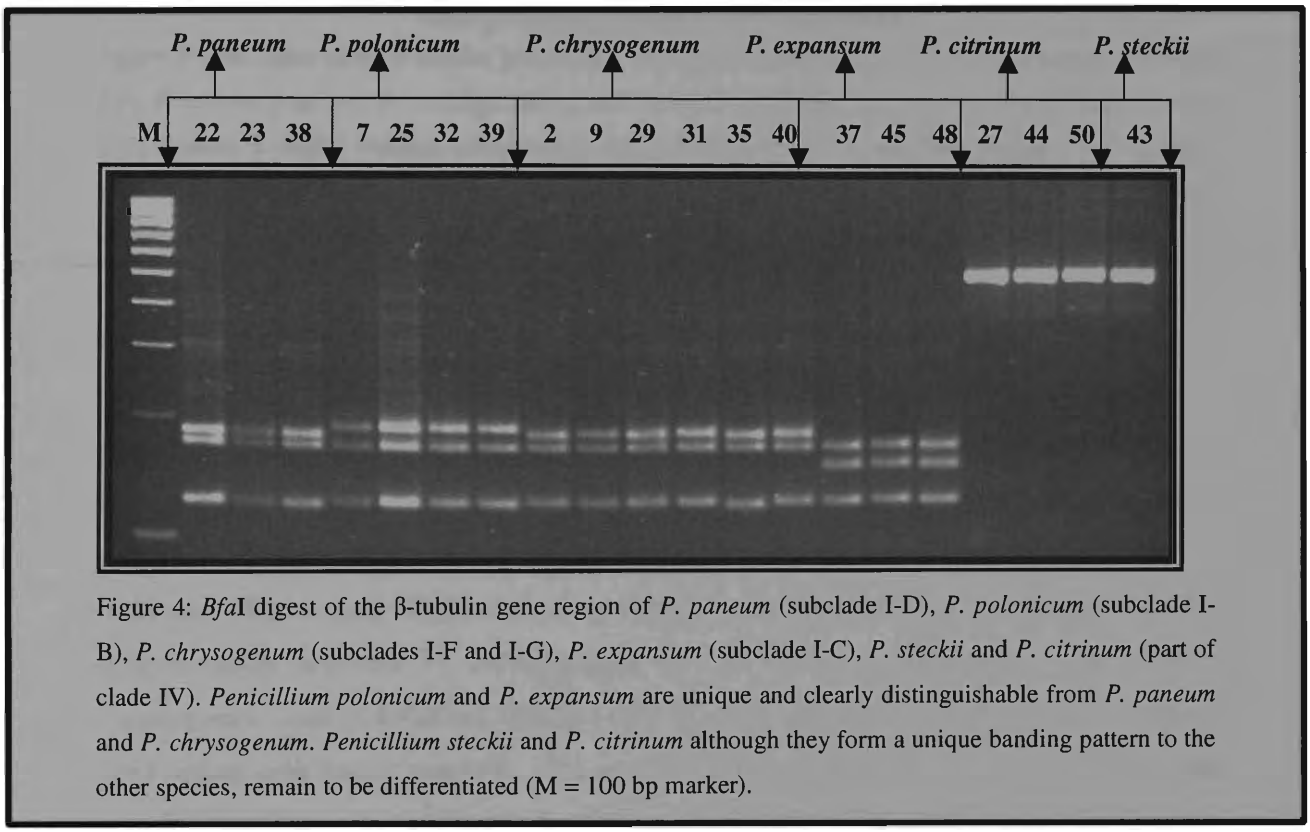

Figure 4: *Bfa*I digest of the β-tubulin gene region of *P. paneum* (subclade I-D), *P. polonicum* (subclade I-B), *P. chrysogenum* (subclades I-F and I-G), *P. expansum* (subclade I-C), *P. steckii* and *P. citrinum* (part of clade IV). *Penicillium polonicum* and *P. expansum* are unique and clearly distinguishable from *P. paneum* and *P. chrysogenum*. *Penicillium steckii* and *P. citrinum* although they form a unique banding pattern to the other species, remain to be differentiated (M = 100 bp marker).

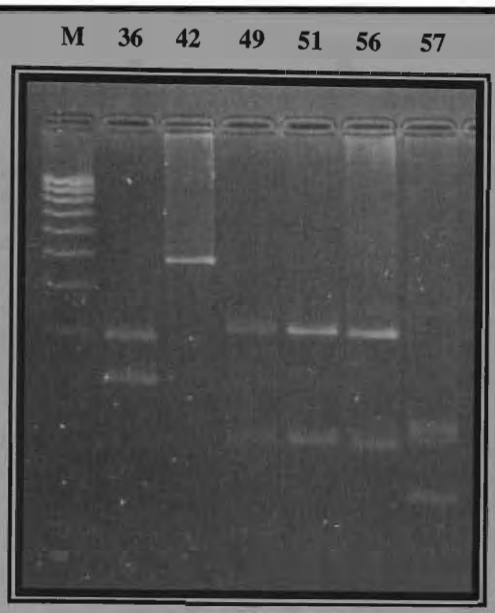

Figure 5: *Bfa*I digest of the β-tubulin gene region of groups 36 (unidentified), *P. sumatrense* (part of clade IV), *P. citreonigrum* and *P. corylophilum* (clade V) and *P. italicum* (subclade I-E). *Penicillium italicum* (57) displays a similar banding pattern to *P. paneum* and *P. chrysogenum* (See Figure 4). *Penicillium sumatrense* (42) displays a similar banding pattern to *P. citrinum* and *P. steckii* (See Figure 4). *Penicillium citreonigrum* (49, 51) displays a similar banding pattern to *P. corylophilum* (56) (M = 100 bp marker).

Penicillium paneum, P. chrysogenum (Figure 4) and *P. italicum* (Figure 5) displayed similar banding patterns with *Bfa*I digestion of the β-tubulin gene region. The β-tubulin gene region of these species was subsequently digested with the restriction enzyme *Apo*I (isochitzomer – *Xap*I) (Figure 6). *Penicillium paneum* displayed a unique banding pattern, distinguishing it from the remaining two species, *P. chrysogenum* and *P. italicum.* Restriction digest of the β-tubulin gene region with *Hpa*II allowed for differentiation between *P. chrysogenum* and *P. italicum* (Figure 7). *Penicillium chrysogenum* could be further differentiated into two groups by digesting the β-tubulin gene region with *Lwe*I (Figure 8). These groups correspond to subclades I-F (29, 40) and I-G (2, 9, 31, 35) indicated in Figure 1. Fragment sizes for all species are indicated in Table 2.

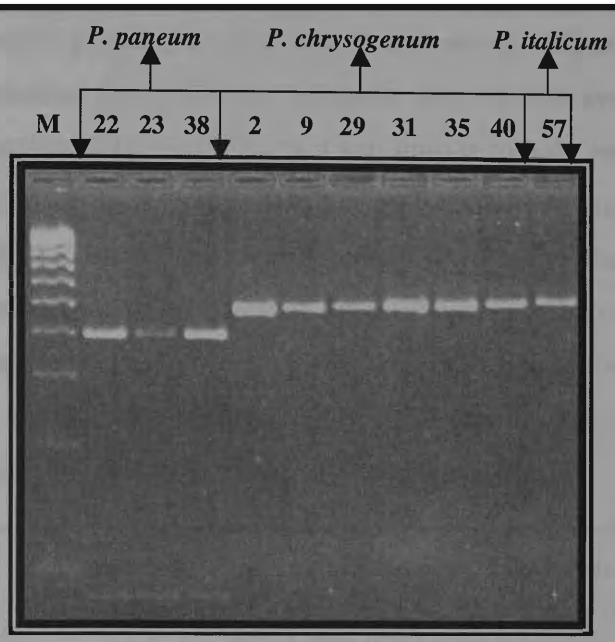

Figure 6: *Apo*I digest of the β-tubulin gene region distinguishing *P. paneum* (subclade I-D) from *P. chrysogenum* (subclade I-F and I-G) and *P. italicum* (subclade I-E) groups (M = 100 bp marker).

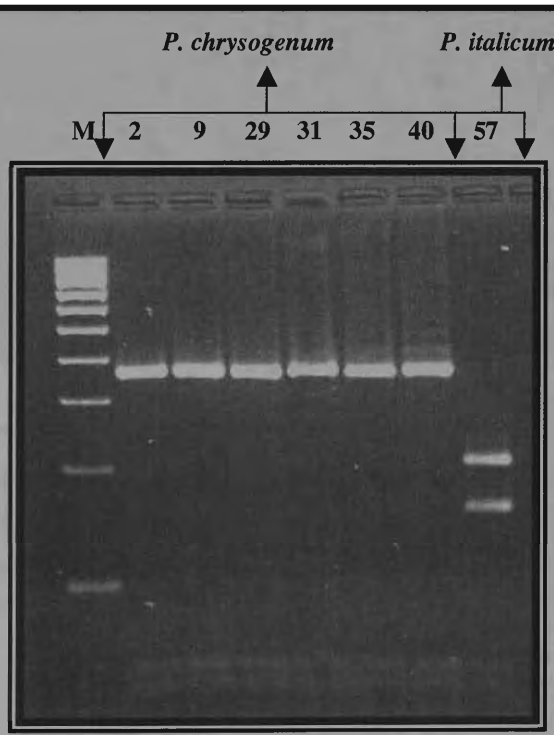

Figure 7: *Hpa*II digest of the β-tubulin gene region of *P. chrysogenum* (subclade I-F and I-G) and *P. italicum* (subclade I-E) groups (M = 100 bp marker).

Penicillium citrinum and *P. steckii* (Figure 4) and *P. sumatrense* (Figure 5) all clustered in Clade IV and demonstrated a similar banding pattern with no cleavage of the β-tubulin PCR product with restriction enzyme *Bfa*I, which was unique to only these three species. The β-tubulin gene region of these groups was treated with *Tai*I in order to distinguish between them (Figure 8). *Penicillium citreonigrum* and *P. corylophilum* (clade V) also had similar banding patterns with *Bfa*I digest of the β-tubulin gene region (Figure 5) and they appear to be phylogenetically related (Figure 1). These two groups were distinguished from one another through digestion of the β-tubulin gene region with *Apo*I (Figure 9). Fragment sizes for these species are indicated in Table 2.

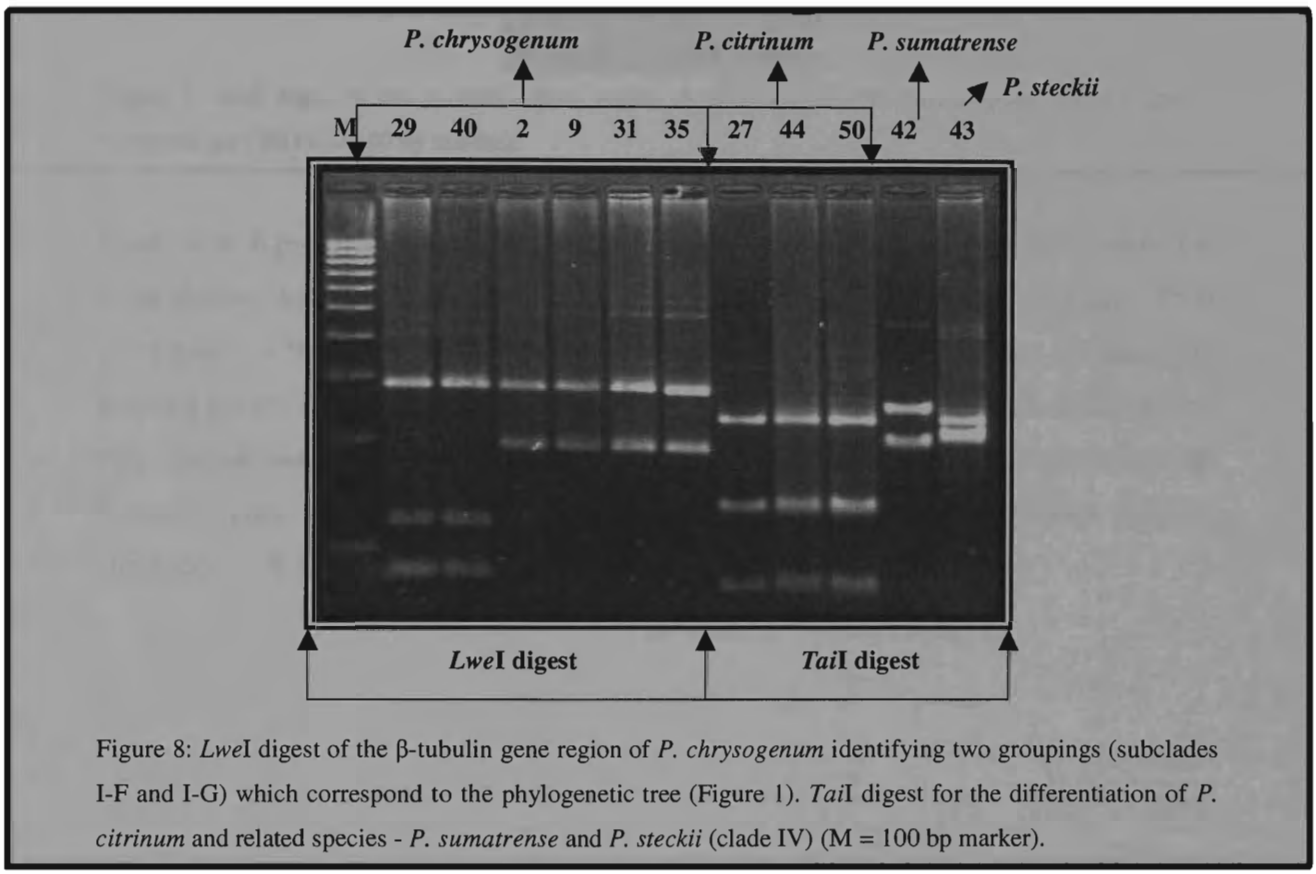

Figure 8: *Lwe*I digest of the β-tubulin gene region of *P. chrysogenum* identifying two groupings (subclades I-F and I-G) which correspond to the phylogenetic tree (Figure 1). *Tai*I digest for the differentiation of *P. citrinum* and related species - *P. sumatrense* and *P. steckii* (clade IV) (M = 100 bp marker).

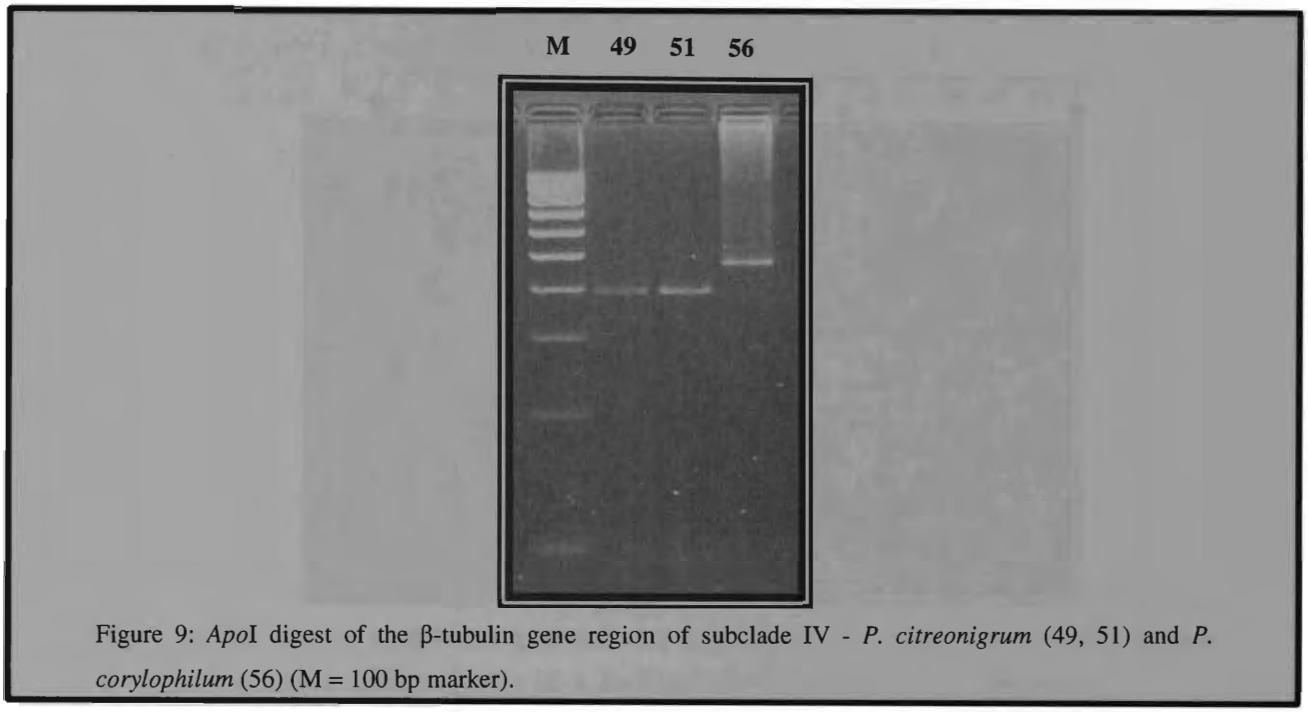

Figure 9: *Apo*I digest of the β-tubulin gene region of subclade IV - *P. citreonigrum* (49, 51) and *P. corylophilum* (56) (M = 100 bp marker).

Restriction digest of the β-tubulin gene region of *P. glabrum* groups with *Bfa*I resulted in three distinct banding patterns – A, B and C (Figure 10). Subclades III-A and III-B correspond to banding patterns A and B to form one *P. glabrum* clade. Groups with banding pattern C correspond to the second *P. glabrum* subclade (III-C) in the combined phylogenetic analysis (Figure 1 and 10). Figure 11 is a partial sequence alignment of the β-tubulin gene region of these groups indicating three sequence variations between subclades A, B and C. Fragment sizes are indicated in Table 2.

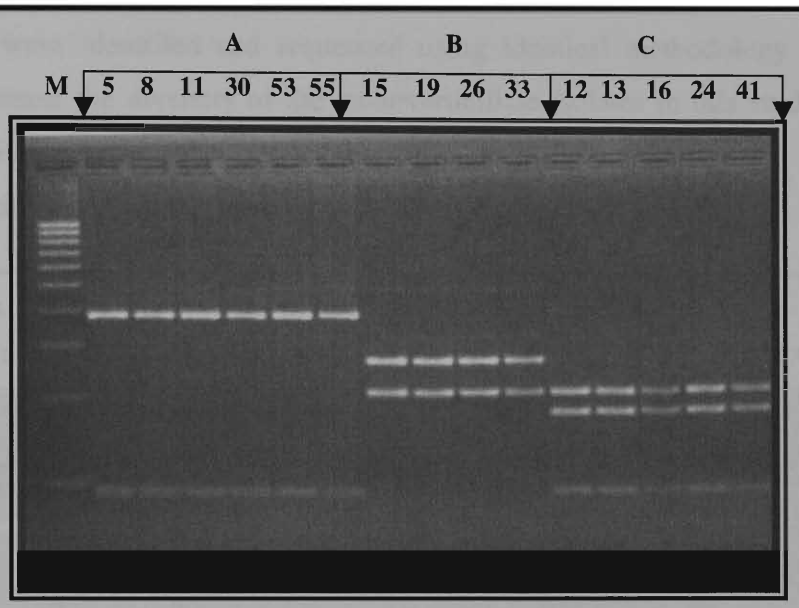

Figure 10: *Bfa*I digest of the β-tubulin gene region of *P. glabrum* groups. Three distinct banding patterns developed which correspond to subclades III-A, III-B and III-C in Figure 1 (M = 100 bp marker).

```
Group 5     GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT
Group 8     GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT
Group 11    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT   A
Group 30    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT
Group 53    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT
Group 55    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAACTT

Group 15    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAGCTT
Group 19    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAGCTT   B
Group 26    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAGCTT
Group 33    GAAACGGTTG CTGCTGGCCT ATCAAGATAA ACATTAGAGA AGCCTTTATA CTTCTAGCTT

Group 12    GTAACGGTTG CTGCTGGCCT ATCAAGATCA ACATTAGAGA AGCCTTTATA CTTCTAGCTT
Group 13    GTAACGGTTG CTGCTGGCCT ATCAAGATCA ACATTAGAGA AGCCTTTATA CTTCTAGCTT
Group 16    GTAACGGTTG CTGCTGGCCT ATCAAGATCA ACATTAGAGA AGCCTTTATA CTTCTAGCTT   C
Group 24    GTAACGGTTG CTGCTGGCCT ATCAAGATCA ACATTAGAGA AGCCTTTATA CTTCTAGCTT
Group 41    GTAACGGTTG CTGCTGGCCT ATCAAGATCA ACATTAGAGA AGCCTTTATA CTTCTAGCTT

            1                                2                              3
```

Figure 11: A partial β-tubulin alignment of *P. glabrum* groups indicating three sequence variations between subclades III-A, III-B and III-C (indicated on the right). Regions marked (1) and (2) indicate nucleotide substitutions in subclade III-C, which differ from subclades III-A and III-B. In region (3) however, subclades III-B and III-C share a nucleotide substitution.

Figure 12 represents the *Hae*III digestion of the ITS gene region of the monoverticillate groups *P. glabrum* and *P. citreonigrum* as well as two additional isolates namely, *P. thomii* (T*) and *P. citreonigrum* (C*) isolated from related studies (data not shown).

These isolates were identified and sequenced using identical methodology and were included to increase the diversity of the monoverticillate isolates in this study. These species were distinguished from one another through the ITS gene region. Fragment sizes for restriction digest of each species with *Hae*III is indicated in Table 3.

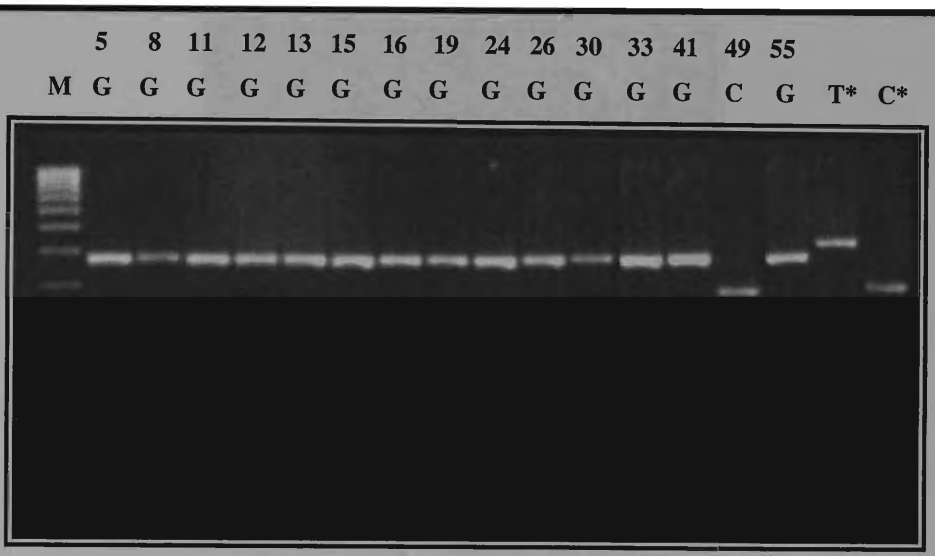

Figure 12: *Hae*III digest of the ITS region of monoverticillate isolates *P. glabrum* (G), *P. citreonigrum* (C) and *P. thomii* (T). Group numbers are provided for clarification. Isolates in the final two lanes, *P. thomii* (T*) and *P. citreonigrum* (C*) do not form part of this study (M = 100 bp marker).

Digestion of the ITS gene region of the biverticillate isolates (*P. citrinum, P. sumatrense, P. corylophilum, Penicillium* species (group 36) and *P. minioluteum* (PM)) with *Hae*III resulted in three distinct banding patterns (Figure 13). One banding pattern represents the subgenus *Biverticillium* [Dierckx] (*P. minioluteum*) while the remaining species form two similar, yet distinct banding patterns, both representing the subgenus *Furcatum* [Pitt]. One banding pattern represents *P. citrinum* and the other represents the remaining biverticillate groups of this study. Again, *P. minioluteum* was isolated from a related study (data not shown). Fragment sizes for each species are indicated in Table 3.

Group 36 appears to be unique in all PCR-RFLP analyses and remains to be identified to species level. In Figure 5, this group forms a unique banding pattern to all other groups analysed with *Bfa*I digestion of the β-tubulin gene region. Again in Figure 13, *Hae*III digest of the ITS gene region, group 36 is unique even though the ITS gene region is

highly conserved. Approximate fragment sizes are indicated in Table 3. Analysis of the β-tubulin gene region illustrated at basepair positions 460-474 and 489-499, a 8-15bp gap within the sequences of all groups except 36 (Appendix II).

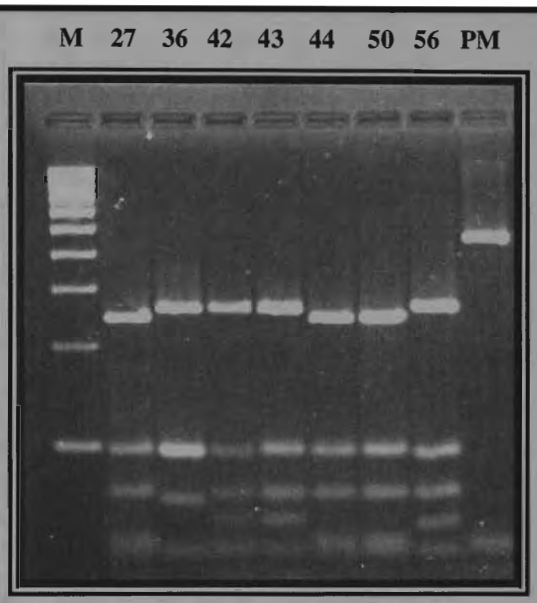

Figure 13: *Hae*III ITS digest of the biverticillate isolates: 27 – *P. citrinum*, 36 (unidentified), 42 – *P. sumatrense*, 43 – *P. steckii*, 44 and 50 – *P. citrinum* and 56 – *P. corylophilum* and *P. minioluteum* (PM). *Penicillium minioluteum* falls into subgenus *Biverticillium* but does not form part of this study (M = 100 bp marker).

Table 2: Reference table of beta-tubulin PCR-RFLP fragment size ranges of all species in this study with respective restriction enzymes

Beta Tubulin	*Bfa*I (bp)	*Apo*I (bp)	*Hpa*II (bp)	*Lwe*I (bp)	*Tai*I (bp)
P. chrysogenum (subclades I-F1 and I-F2)	170-175 145-155 122-124	uncut	345-355 51 30-40 24	273 100-105 82 265-275 245 195-200 180-190	
P. citreonigrum (part of clade IV)	295-310 160-170	375-390 75-90			
P. citrinum (part of clade III)	uncut				223-230 135 77 15-30 15
P. corylophilum (part of clade IV)	297 169	uncut			
P. expansum (subclade I-C)	155-160 135-140 124 27				
P. glabrum (A) (subclade III-A)	370-380 80-90				
P. glabrum (B) (subclade III-B)	255-265 190-195				
P. glabrum (C) (subclade III-C)	190-195 176 80-90				
P. italicum (subclade I-E)	172 170 123		203 143 51 44 24		
P. paneum (subclade I-D)	170-180 150-160 121	380-390 75-85			
P. polonicum (subclade I-B)	190-195 165-170 123				
P. steckii (part of clade III)	uncut				212 205 29 15
P. sumatrense (part of clade III)	uncut				241 205 29 15

Where no fragment size range is indicated, only one group has been analysed. Fragment sizes for individual groups 4, 6, 10, 14, 17, 18, 20, 21, 34, 46 (subclades I-A1, I-A2 and I-A3) are indicated separately.

Table 2 continued

Beta Tubulin	*Bfa*I (bp)	*Apo*I (bp)	*Hpa*II (bp)	*Lwe*I (bp)	*Tai*I (bp)
P. biourgeianum / P. bialowiezense (subclade I-G1)			182 110-120 70 51 25-35 22-24 6		
P. brevicompactum (subclade I-G2)			255 110-120 51 30-40 24		
36 – Unidentified *Penicillium* spp.	281 216				
10 – *P. solitum*			227 160 40-50 25-35		
46 – *P. solitum*			150–160 125–135 45-55 25-35		
4 – *P. crustosum*			227 160 40-50 25-35		
14 – *P. crustosum*			150–160 125–135 45-55 25-35		
17 – *P. crustosum*			227 160 40-50 25-35		
18 – *P. crustosum*			370-390 50-55 40-45		
20 – *P. commune*			227 160 40-50 25-35		
21 – *P. commune / P. solitum*			370-390 50-55 40-45		
34 – *P. commune*			227 160 40-50 25-35		
6 – *P. echinulatum*			370-390 50-55 40-45		

Where no fragment size range is indicated, only one group has been analysed. Fragment sizes for individual groups 4, 6, 10, 14, 17, 18, 20, 21, 34, 46 (subclades I-A1, I-A2 and I-A3) are indicated separately.

Table 3: Reference table of approximate ITS PCR-RFLP fragment size ranges of all species used in this study with *Hae*III restriction enzyme (Figures 2 and 3). Fragment sizes for *P. minioluteum* and *P. thomii* which are not part of this study, are also indicated

ITS	*P. citreonigrum*	*P. citrinum*	*P. corylophilum*	*P. glabrum*	*P. minioluteum*	*P. steckii*	*P. sumatrense*	*P. thomii*	192 – Unidentified *Penicillium* species
*Hae*III (bp)	283 110-115 68 54 25-30 20-30 15-25 10-20 10-15 5 5	240-245 93-96 65-70 20-30 15-25 10-20 5-10	258 83 69 54 27 26 25 12 5	371 85-95 65-70 20-40 5	443 45 44 33 10 9	258 69 55 49 29 25 13 11 5	260 69 53 31 18 5	410 85-95 65-70 20-40 5	256 95 94 63 25 5 2

4. DISCUSSION

Penicillium is one of the most dominant fungal species found in various local and international environments of the litchi export chain. This is clearly indicated by the number of isolates obtained in this study. Diversity was low within this genus as all groups identified fall within 18 species. The most dominant *Penicillium* species in this study (in descending order) were *P. crustosum* (310 isolates), *P. glabrum* (212 isolates), *P. chrysogenum* (97 isolates), *P. biourgeianum/bialowiezense* (77 isolates), *P. solitum* (42 isolates), *P. commune* (36 isolates), *P citrinum* (29 isolates), *P. citreonigrum* (27 isolates), *P. paneum* (27 isolates) and *P. polonicum* (27 isolates), *P. expansum* (16 isolates), *P. brevicompactum* (13 isolates), *P. echinulatum* (seven isolates), *P. corylophilum* (three isolates), *P. italicum* (two isolates), *P. steckii* (two isolates), *P. sumatrense* (two isolates) and group 36 (two isolates).

A common misconception in the litchi fruit industry is that *P. expansum* is the main causal agent of decay. Although it is a mycotoxin producer and broad-spectrum pathogen of many fruit (Pitt, 1991) including litchi (De Jager *et al.,* 2003), it was not found to be dominant in this study. Only 1.04% of isolates analysed were identified as this species. *Penicillium expansum* was regularly isolated from international coldroom air samples however, never from South African sampling points. This indicates that contamination of the fruit with *P. expansum* did not occur locally.

Penicillium crustosum was the most dominant species in this study. It is characterised by mass spore production, which are readily dislodged from fruiting structures (Pitt, 1991; Frisvad and Samson, 2004). This may justify the prevalence of this species within the atmosphere. Conidia that settle out of the atmosphere may come into contact with a substrate suitable for germination. With subsequent growth and fruiting structure development, dislodged conidia may form new colonies and the pathogens life cycle is repeated. *Penicillium crustosum* has been indicated to be weakly pathogenic on pome fruit (Pitt, 1991). This species was isolated locally and internationally and was dominantly found on fruit surfaces and particularly the atmosphere in cooled environments. *Penicillium crustosum* was more commonly isolated from fruit treated

with sulphur dioxide (25.5 % of all *P. crustosum* isolates). Previous reports have shown the development of sulphur dioxide resistance with *Penicillium* species (Jennings, 1993). This could support the conclusion that *P. crustosum* can survive sulphur treatments and thereafter colonise the surface of the fruit. De Jager and Korsten (2003) and Korsten (2006) indicated that SO_2 fumigation alters the balance of microbial populations present on the fruit surface, as most organisms are unable to survive the treatment. This allows for opportunistic pathogens such as *Penicillium* species to act as primary colonisers and thrive within environments of abundant nutrients and little competition from other microbial species.

Penicillium glabrum was the second most dominant species isolated from litchi fruit. Little is known about the role of *P. glabrum* in the export chain although it has previously been reported on litchi fruit (De Jager and Korsten, 2003; Jacobs and Korsten, 2004). It is however, a known mycotoxin producer (Frisvad and Thrane, 1995). No pathogenicity studies have been done for this species nor had any submissions for *P. glabrum β-tubulin gene partial cds sequence* been made into GENBANK. This species is known as a decay agent of several commodities including grapes, spices, nuts and dairy products (Hocking, 1994; Freire *et al.,* 2000; Overy *et al.,* 2003; Serra *et al.,* 2006); however this species is uncommon on fruit such as citrus and apples. Of the 212 *P. glabrum* isolates found in this study, 70 originated from air samples (approximately one third of the sample size), both locally and internationally. Culture examination for all groups showed moderate to extensive conidiogenesis. Over time, cultures demonstrated release of conidia from fruiting structures, much like that of *P. crustosum.* This clarifies why *P. glabrum* is a prominent air contaminant.

Considering culture grouping was done prior to identification, morphological variation within isolate groups was prevalent. This approach may have contributed to some incorrect identification of species. In a number of instances, isolates of the same species were placed within different morphological groups. Examples of this include *P. glabrum* being identified from 15 morphological groupings, *P. chrysogenum* from six groups, *P. polonicum* from four groups and *P. paneum, P. expansum* and *P. citrinum* from three

groups each. Such variation could have been eliminated through initial direct molecular methods such as DNA sequencing, which would have been impractical. Although moderate correlations were found between morphological groupings and sequencing identifications, exceptions were found in some groups mainly due to greater morphological variation within the species. These include morphological identification of *P. chrysogenum* while sequencing results implicated *P. polonicum*. Similar variations were found between *P. solitum, P. expansum* and *P. crustosum* species groups.

Although DNA sequencing is more reliable than morphological identification, there are several disadvantages associated with this method. Firstly, both DNA sequencing and morphological identification are time-consuming processes. Such time losses cannot be afforded by industry, as the litchi season is short and rapid identification is required. Secondly, there are several sequences in GENBANK that appear to be misidentified (Ciardo *et al.,* 2007). Lastly, sequencing is a costly procedure and the number of isolates may be high. Thus it is not a feasible and practical alternative. Due to these disadvantages, it is desirable to develop an alternative method that is rapid, repeatable and reliable and one that reduces identification costs.

The combined phylogenetic study was used in determining the PCR-RFLP banding patterns for each species, by differentiating between closely related species or strains. Subclade I-A consists of several species – *P. commune, P. crustosum, P. echinulatum* and *P. solitum*. Although restriction digest of the ITS gene region of these species showed no differentiation between them (data not shown), digestion of the β-tubulin gene region with *Hpa*II provided greater resolution and indicated some degree of relatedness between these groups. Seifert and Louis-Seize (2000) indicated *P. solitum*, *P. commune* and *P. crustosum* to be related, as they are the large-conidium species of the "*Penicillium aurantiogriseum*" group. Both *P. solitum* and *P. crustosum* are producers of viridicatin (Lund *et al.,* 1995). Several studies have shown relatedness between *P. crustosum, P. commune, P. solitum* and *P. expansum* (Peterson, 2000; Skouboe *et al.,* 2000; Samson *et al.,* 2004) however in this study, *P. expansum* forms part of subclade I-C. Skouboe *et al.* (2000), found identical ITS sequences for *P. solitum* and *P. echinulatum* while in this

study, although there were sequence variations within *P. solitum* sequences of groups 10 and 46, *P. echinulatum* (6) only differed from the consensus sequence with a single nucleotide insertion at basepair position 526. These species were isolated frequently in this study. The number of isolates for all four species was approximately one quarter (25%) of the total sample size. This indicates the importance of resolving the taxonomic status of these species. In addition, Seifert and Louis-Seize (2000) reiterated the importance of these species as they are dominant mycotoxin producers, particularly ochratoxin A. In this study, these species show intra- as well as interspecific variation between the related groups of this clade as a single base change between species may represent intraspecific variation (Peterson, 2000). Molecular fingerprinting for these species found in this study is inconsistent and the taxonomy of these groups may need to be reconsidered. If factors such as secondary metabolite production, growth rates and conidial production are investigated more closely, it may provide additional resolution (Frisvad and Samson, 2004).

Subclades I-B to I-G consist of species *P. polonicum, P. expansum, P. paneum, P. italicum* as well as *P. chrysogenum.* All these species are classified in subgenus *Penicillium* [Sect. *Asymmetrica* Raper and Thom], but they are grouped in various sections and serotypes (ser.). *Penicillium polonicum* (subclade I-B), a producer of the mycotoxin verrucosidin (Aranda *et al.,* 2002) clusters with subclade I-A and is classified in section *Viridicata*, ser. *Viridicata.* All species in subclade I-A are also classified in section *Viridicata. Penicillium commune* and *P. crustosum* however, are classified in ser. *Camembertii* while *P. echinulatum* and *P. solitum* form part of ser. *Solita* (Frisvad and Samson, 2004; Samson *et al.,* 2004). This validates relatedness between these species groups (Samson *et al.*, 2004).

It can be anticipated that *P. expansum* forms a central group in Clade I among other terverticillate species of this study, as *P. expansum* is the type species of genus *Penicillium* (Frisvad and Samson, 2004). *Penicillium expansum* and *P. italicum* are classified in section *Penicillium,* ser. *Expansa* and ser. *Italica* respectively and they are indicated to be related by Wang and Zhuang (2007). Some degree of relatedness between

these species is indicated in this study, although *P. italicum* appears to be more closely related to *P. paneum* than *P. expansum*. *Penicillium paneum* forms an independent subclade (I-D) closely related to *P. expansum*, and is classified in section *Roquefortii*, ser. *Roquefortii*. A characteristic unique to this section is the ability to survive high concentrations of various acids; however a relationship between *P. paneum* and *P. expansum* is indicated by both species having the ability to produce patulin (Frisvad and Samson, 2004). *Penicillium chrysogenum* is classified in section *Chrysogena,* ser. *Chrysogena* and is the only species in subgenus *Penicillium* capable of growth at 37°C (Frisvad and Samson, 2004). Growth of other species in subgenus *Penicillium* at 37°C is usually negative (Pitt, 1991). In this study, *P. chrysogenum* groups were divided into two subclades (I-F and I-G) supported by a strong bootstrap value of 93%. Groups in subclade I-F differ from I-G by four base pair substitutions (Appendix II). This variability is validated by *Lwe*I digest of the β-tubulin gene region. This may represent two different strains of *P. chrysogenum*. All species in subclades I-B to I-G could be differentiated from one another using various enzymes through PCR-RFLP. The genetic diversity within subgenus *Penicillium* however, did not allow for differentiation between the species using only a single enzyme (data not shown).

Subclade II-A was identified as *P. biourgeianum/bialowiezense* and subclade II-A as *P. brevicompactum*. *Penicillium biourgeianum/bialowiezense* groups clustered with 100% bootstrap support, while *P. brevicompactum* formed a strongly supported (100%), distinct, yet closely related subclade. Samson *et al.* (2004) had a similar finding, with *P. brevicompactum* being distantly removed, yet included in subgenus *Penicillium*. This phenomenon may be due to several species yet to be discovered or long-branch attraction - when lineages evolve rapidly, several species remain indirectly yet closely related regardless of their actual evolutionary relationship (Samson *et al.,* 2004; Bergsten, 2005). *Penicillium brevicompactum* and *P. bialowiezense* are mass producers of mycophenolic acid and asperphenamate (Bird and Campbell, 1982; Samson *et al.,* 2004). Mass production of these secondary metabolites is desired as they are widely used in the medical sector for their antibacterial, antifungal and antiviral properties (Larsen *et al.,* 2005).

By analysing the ITS-LSU rDNA, partial calmodulin and partial translation elongation factor 1-α regions, Peterson (2004) indicated that *P. biourgeianum* is a close relative of *P. brevicompactum* while *P. bialowiezense* is more closely related to *P. polonicum.* In this study, no relatedness of these groups to *P. polonicum* was found. In contrast, phylogenetic data for only the β-tubulin gene region of terverticillate penicillia grouped *P. bialowiezense* with *P. brevicompactum* (Samson *et al.,* 2004). Beta-tubulin BLAST results for these groups yielded a similar identification however, BLAST analysis of the ITS gene region cannot be dismissed. These groups could not be differentiated from one another through digestion of the ITS gene region (data not shown) however, digestion of the β-tubulin gene region with *Hpa*II, enabled *P. brevicompactum* to be differentiated from *P. biourgeianum/bialowiezense.* Therefore, it is essential to sequence more than one gene region in order to differentiate between closely related species (Seifert and Louis-Seize, 2000; Peterson, 2004).

Clade III consists of all groups identified as *P. glabrum* with subclades III-A, III-B and III-C corresponding to banding patterns A, B and C in the *Bfa*I digest of the β-tubulin gene region. Subclades III-A and III-B differ from one another by five nucleotide substitutions and form two unique banding patterns, yet only one subclade resulted. Potential bootstrap values for these two subclades may have been below the set confidence level of 70%, after which individual clades would have collapsed. These groups may represent two strains of *P. glabrum.* Subclade III-C is unique from subclades III-A and III-B, with another five nucleotide substitutions. Sequence alignment analysis of groups forming the individual subclades however, showed some similarities between them. Several instances were found where subclade III-C was unique to III-A and III-B while in other cases, subclade III-B and III-C shared similarities in the β-tubulin sequence alignment. It was also noted that in some cases subclade III-A and III-C shared similarities, with subclade III-B being unique (data not shown). This indicates relatedness between all groups in the three subclades, potentially as different strains or sub-species. According to Pitt (1991), *P. glabrum* is incapable of growth at 37°C. This is consistent with the groups in subclades III-A and III-B but not with subclade III-C.

Groups 12 and 16 were repeatedly capable of germination to microcolony formation at 37°C (data not shown). The taxonomic position of these groups remains to be resolved.

Clade IV consists of *P. citrinum*, *P. steckii* and *P. sumatrense*. *Penicillium steckii* is considered to be a variant of *P. citrinum* (Pitt, 1979; Pitt, 1991). This is verified by strong bootstrap support of 94% for these species. Malmstrøm *et al.* (2000), confirmed this by analysis of secondary metabolites produced by *P. citrinum* and related species (*P. steckii*), while secondary metabolite production by *P. sumatrense* differs from both of these species. *Penicillium sumatrense* appears to share a phylogenetic relationship with *P. steckii* and ultimately with *P. citrinum* supported by a strong bootstrap value of 99%. Peterson (2000) performed a phylogenetic study where *P. citrinum* and *P. sumatrense* formed a similar grouping. Pitt (1979) previously indicated *P. sumatrense* to be a synonym of *P. corylophilum*. A later publication (Pitt, 1991), suggested a relationship between *P. citrinum* and *P. corylophilum* however, neither of these relationships are indicated in in this study as *P. corylophilum* clusters in Clade V. This has previously been demonstrated by Wang and Zhuang (2007). Genetic similarity between these three species is indicated by no cleavage of the amplification product when the β-tubulin gene region is digested with *Bfa*I. This was unique to these three species however; they were differentiated from one another by digestion of the β-tubulin gene region with *Tai*I. This indicates that these species are defined, individual species that are closely related.

Clade V consists of *P. citreonigrum* and *P. corylophilum*. As discussed previously, it has been proposed that *P. corylophilum* shares genetic relationships with *P. sumatrense* as well as *P. citrinum* (Pitt, 1979; Pitt, 1991). None of these proposals are substantiated in this study. In contrast, Wang and Zhuang (2007) suggested *P. corylophilum* to be a solitary taxon with no close relatives. Although *P. corylophilum* is a biverticillate isolate, it has been shown previously to cluster with a monoverticillate isolate, namely *P. restrictum* [Gilman and Abbott] (Wang and Zhuang, 2007). A similar situation was found in this study, as *P. corylophilum* clusters with *P. citreonigrum*, also a monoverticillate isolate. Support for this clade is strong with 100% bootstrap values. Conversely, *P. citreonigrum* has been shown to be most closely related to *P. miczynskii*

[Zaleski], a biverticillate species (Pitt, 1991). Genetic similarity between *P. citreonigrum* and *P. corylophilum* was shown when the β-tubulin gene region was digested with *Bfa*I. Differentiation could be made between these two species groups by *Apo*I digest of the β-tubulin gene region.

Group 36 (identified as a *Penicillium* species) had conflicting identifications, both morphologically and molecularly. This group could not be identified to species level morphologically (data not shown), while ITS sequence identification was *P. rolfsii* [Thom] and β-tubulin resulted in *P. piscarium* [Westling]. The banding pattern for this group, generated by *Bfa*I digest of the β-tubulin gene region, is unique within this study. Phylogenetic analysis placed this group in a solitary position from all other *Penicillium* species in this study. An independent clade was formed with this group, close to the outgroup *Fusarium oxysporum.* The taxonomy and identification of this group requires further investigation.

Terverticillate species in this study were too diverse to develop restriction maps according to species and taxonomy (data not shown). Peterson (2000) stated that it would be advantageous to determine whether the other subgenera are as rich in species diversity as *Penicillium.* However, this cannot be validated in this study, as species diversity within subgenera *Aspergilloides* [Dierckx], *Furcatum* and *Biverticillium* was low. *Penicillium thomii* (T*) and *P. citreonigrum* (C*) isolated from related studies, were included to increase the diversity of the monoverticillate isolates. Although the ITS gene region is highly conserved between closely related species of *Penicillium* (Glass *et al.,* 1995; Skouboe *et al.,* 2000; Samson *et al.,* 2004; Wang and Zhuang, 2007), *Hae*III digestion of this gene region for identification purposes was effective for the monoverticillate isolates (*P. glabrum, P. thomii* and *P. citreonigrum*). Further investigations with monoverticillate isolates of greater diversity are required to validate this.

Although higher diversity was found with the biverticillate groups in comparison to those that are monoverticillate, with five *Penicillium* species (*P. citrinum, P. sumatrense, P.*

steckii, P. corylophilum and group 36 – unidentified *Penicillium* species), all these groups belong to subgenus *Furcatum.* *Penicillium minioluteum* (PM) belonging to subgenus *Biverticillium* was incorporated into this analysis to increase diversity. Based on banding patterns of *Hae*III digest of the ITS gene region, subgenus *Biverticillium* (represented by PM) is clearly distinct from the *Furcatum* species of this study. However, this will need to be investigated further with other *Biverticillium* species. *Furcatum* species display three banding patterns, one for *P. citrinum*, one for group 36 and another for the remaining species (*P. sumatrense*, *P. steckii* and *P. corylophilum*). Such an analysis without phylogenetic substantiation may lead to the conclusion that *P. sumatrense* and *P. steckii* are genetically related to *P. corylophilum* as previously discussed, but it has been established that the ITS gene region is highly conserved and thus ineffective in resolving relationships between closely related species.

Clade I and II represent subgenus *Penicillium* (terverticillate) while Clades III, IV and V (including group 36) are a combination of *Aspergilloides* (monoverticillate) and *Furcatum* (biverticillate). These two groupings form distinct branches within the combined phylogenetic tree. Related phylogenetic studies have indicated similar findings (Peterson, 2000; Wang and Zhuang, 2007). A previous study evaluating the ITS gene region showed that *Penicillium* is not monophyletic with other Ascomycetes (Berbee *et al.,* 1995) which may indicate that *Penicillium* species have the ability to evolve within their own genus.

Presently, fourteen species of *Penicillium* can be differentiated from one another through PCR-RFLP of the β-tubulin gene region with four restriction enzymes namely *Bfa*I, *Apo*I, *Hpa*II and *Tai*I. Potential strains of *P. chrysogenum* and *P. glabrum* were differentiated by using *Lwe*I and *Bfa*I respectively. The use of PCR-RFLP is a repeatable, reliable and cost effective alternative to *Penicillium* identification and differentiation.

5. CONCLUSION

Morphology is an essential component in *Penicillium* identification however, due to variability within this genus; it may not provide the required accuracy or specificity. Alternative identification methods such as DNA sequencing are costly and time-consuming. This study focussed on identifying *Penicillium* species throughout the South African litchi export chain and developing a rapid, cost-effective identification method. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used, as it is reliable, repeatable, cost-effective and quick to execute.

The South African litchi export industry has suffered a decline in fruit quality, predominantly due to postharvest decay by *Penicillium* species. Due to the dominance of *Penicillium* species throughout the fruit export chain and high rate of decay caused by this saprophytic organism, rapid identification to species and strain level serves to benefit the industry. It is essential to develop a rapid test method that will enable accurate identification of contamination sources to enable the industry to manage the control of *Penicillium* species more effectively. This method may be applied in disputed cases between producers and exporters, when consignments are rejected due to decay.

This study may serve as a precursor in the development of a PCR-RFLP restriction map database for routine screening of *Penicillium* species, ultimately reducing the necessity for DNA sequencing and morphological identification. This method may be applied to *Penicillium* species isolated from a number of different environments. As the method is easy to perform, scientists with little knowledge of molecular biology or *Penicillium* may be able to identify species with confidence under basic laboratory conditions.

Future research should focus on the identification of additional *Penicillium* species through PCR-RFLP. Analysis of additional gene regions may provide clarity in terms of identifying species, as was found in this study with *P. biourgeianum/bialowiezense* as well as the unidentified *Penicillium* species. Taxonomic issues surrounding several

Penicillium species in this study should be resolved and descriptions of potential strains or subspecies should be clarified.

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7. APPENDIX I - ITS SEQUENCE ALIGNMENT

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                          ....|....|  ....|....|  ....|....|  ....|....|  ....|....|
                               5          15         25         35         45
47-P. biourgeianum        ----------  ----------  ----------  ----------  ----------
1- P. biourgeianum        ----------  ------CTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
3- P. biourgeianum        ----------  ---GACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
28-P. biourgeianum        ----------  ----------  ----------  --------TG  AGG-GCCCTC
54-P. brevicompactum      ------GTAG  GTGAACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
52-P. brevicompactum      ---TCCGTAG  GTGAACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
56-P. corylophilum        ----------  -------TGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
40-P. chrysogenum         ----------  ----------  ----------  ----------  -----CCCTC
2- P. chrysogenum         ----------  -----CCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
9- P. chrysogenum         ----------  -GGGACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
29-P. chrysogenum         ----------  ----------  -------TCA  TTACCGAGTG  AGG-GCCCTC
31-P. chrysogenum         -CTTCCGTAG  GTGAACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
35-P. chrysogenum         ----------  ----------  ----------  ----CGAGTG  AGG-GCCCTC
51-P. citreonigrum        ----------  ------CTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
49-P. citreonigrum        ----------  ----------  ----------  ----------  ----------
50-P. citrinum            ----------  ----------  ----------  ----------  ----------
27-P. citrinum            ----------  ----------  ----------  ----------  ----------
44-P. citrinum            ----------  ----------  ----------  ----------  ----------
34-P. commune             ----------  ----------  ----------  ----------  -GG-GCCCTC
20-P. commune             ----------  ----------  ----------  TTACCGAGTG  AGG-GCCCTC
21-P. commune             ----------  -----CCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
18-P. crustosum           ----------  ------CTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
4- P. crustosum           ----------  ------CTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
14-P. crustosum           ----------  ----------  ----------  ------AGTG  AGG-GCCCTC
17-P. crustosum           ----------  ----------  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
6- P. echinulatum         -TTTCCGTAG  GTGAACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
48-P. expansum            ----------  ----------  -----GATCA  TTACCGAGTG  AGG-GCCCTT
37-P. expansum            CTTCCGTAGG  TGGAACCTGC  GGAAGGATCA  TTACCGAGTG  AGGAGCCCTT
45-P. expansum            ----------  -------TGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTT
55-P. glabrum             ----------  ------CTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
5- P. glabrum             ----------  ------CTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
8- P. glabrum             -CTTCCGTAG  GTGAACCTGC  GGAAGGATCA  TTA-TGAGTG  AGG-GCCCTC
11-P. glabrum             ----------  ----------  -----GATCA  TTACTGAGTG  AGG-GCCCTC
12-P. glabrum             ----------  ------CTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
13-P. glabrum             ----------  ----------  ----------  ----TGAGTG  AGG-GCCCTC
15-P. glabrum             ----------  ----------  -------TCA  TTACTGAGTG  AGG-GCCCTC
16-P. glabrum             ----------  ----------  -------TCA  TTACTGAGTG  AGG-GCCCTC
19-P. glabrum             ----------  -----CCTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
24-P. glabrum             ----------  ----------  ----------  ----TGAGTG  AGG-GCCCTC
26-P. glabrum             ----------  ------CTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
30-P. glabrum             ----------  ----------  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
33-P. glabrum             ----------  -----CCTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
41-P. glabrum             ----------  ------CTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
53-P. glabrum             ----------  ---GACCTGC  GGAAGGATCA  TTACTGAGTG  AGG-GCCCTC
57-P. italicum            ----------  -------TGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
38-P. paneum              ----------  --------GC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
22-P. paneum              ----------  ----------  -----GATCA  TTACCGAGTG  AGG-GCCCTC
23-P. paneum              CCTTCCGTAG  GTGGACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTC
39-P. polonicum           ----------  ----------  ----------  ----------  -----CCCTT
7- P. polonicum           ----------  --GAACCTGC  GGAAGGATCA  TTACCGAGTG  AGG-GCCCTT
25-P. polonicum           ----------  ----------  ----------  ---CCGAGTG  AGG-GCCCTT
32-P. polonicum           ----------  ----------  ----------  ----------  ----GCCCTT
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36-P. rolfsii          ---------- ---------- ---------- ---------- ---GGCCCTC
46-P. solitum          ---------- ---------- -------TCA TTACCGAGTG AGG-GCCCTC
10-P. solitum          ---------- --------GC GGAAGGATCA TTACCGAGTG AGG-GCCCTC
43-P. steckii          ---------- ---------- ---------- ---CCGAGTG AGG-GCCCTC
42-P. sumatrense       ---------- ---------- ---------- ---------- ----------

                       ....|....| ....|....| ....|....| ....|....| ....|....|
                            55         65         75         85         95
47-P. biourgeianum     TGGGTCCAAC CTTCCCCACC CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
1- P. biourgeianum     TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
3- P. biourgeianum     TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
28-P. biourgeianum     TGGGTCCAAC CTCCCAC--- CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
54-P. brevicompactum   TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
52-P. brevicompactum   TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
56-P. corylophilum     TGGGTCCAAC CTCCCAC--C CATGTTTATT GTACCT-TGT TGCT-TCGGC
40-P. chrysogenum      TGGGTCCAAC -TCCCAC--C CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
2- P. chrysogenum      TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
9- P. chrysogenum      TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
29-P. chrysogenum      TGGGTCCAAC CTCCCAC--C -GTGTTTATT TTACCT-TGT TGCT-TCGGC
31-P. chrysogenum      TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
35-P. chrysogenum      TGGGTCCAAC TCCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
51-P. citreonigrum     TGGGTCCAAC CTCCCAC--C CGTGTTTATC GTACCT-TGT TGCT-TCGGC
49-P. citreonigrum     ---------- ---------- ---------- --ACCT-TGT TGCT-TCGGC
50-P. citrinum         ---------- ---------- ---------- ---------- --GGGCCCAA
27-P. citrinum         ---------- ---------- ---------- ---------- ----------
44-P. citrinum         ---------- ---------- ---------- ---------- ----------
34-P. commune          TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TGT TGCT-TCGGC
20-P. commune          TGGGTCCAAC CTCCCAC--C -GTGTTTATT TTACCT-TGT TGCT-TCGGC
21-P. commune          TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
18-P. crustosum        TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
4- P. crustosum        TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
14-P. crustosum        TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
17-P. crustosum        TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
6- P. echinulatum      TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
48-P. expansum         TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-CGT TGCT-TCGGC
37-P. expansum         TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-CGT TGCT-TCGGC
45-P. expansum         TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-CGT TGCT-TCGGC
55-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
5- P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
8- P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
11-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
12-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
13-P. glabrum          TGGGTCCAAC CTCCCAC--C -GTGTTTATT GTACCT-TGT TGCT-TCGGT
15-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
16-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
19-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
24-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
26-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
30-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCTCTCGGT
33-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
41-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
53-P. glabrum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT GTACCT-TGT TGCT-TCGGT
57-P. italicum         TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCA-CGT TGCT-TCGGC
38-P. paneum           TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TAT TGCT-TCGGC
22-P. paneum           TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TAT TGCT-TCGGC
23-P. paneum           TGGGTCCAAC CTCCCAC--C CGTGTTTATT T-ACCT-TAT TGCT-TCGGC
39-P. polonicum        TGGGTCCAAC -TCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
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7- P. polonicum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
25-P. polonicum          TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
32-P. poloicum           TGGGTCCACC -TCCCAC--C -GTGTTTATT T-ACCT-TGT TGCT-TCGGC
36-P. rolfsii            TGGGTCCACC TCCCCCC--- -GTGTTT-TC GATCCT-TGT TGCT-TCGGC
46-P. solitum            TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
10-P. solitum            TGGGTCCAAC CTCCCAC--C CGTGTTTATT TTACCT-TGT TGCT-TCGGC
43-P. steckii            TGGGTCCAAC CTCCCTC--C CGTGTTGCAC GAACCTGTGT TGCT-TCGGC
42-P. sumatrense         ---------- ---------- ---------- ---------- ----------

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                             105        115        125        135        145
47-P. biourgeianum       GAGCCTGCCT T--TTGGCTG CCGGGGGACG TCAGTCCCCG GGTCCGTGCT
1- P. biourgeianum       GAGCCTGCCT T--TTGGCTG CCGGGGGACG TCAGTCCCCG GGTCCGTGCT
3- P. biourgeianum       GAGCCTGCCT T--TTGGCTG CCGGGGGACG TCAGTCCCCG GGTCCGTGCT
28-P. biourgeianum       GAGCCTGCCT T--TTGGCTG CCGGGGGACG TCAGTCCCCG GGTCCGTGCT
54-P. brevicompactum     GAGCCTGCCT T--TGGGCTG CCGGGGGACG TCTGTCCCCG GGTCCGCGCT
52-P. brevicompactum     GAGCCTGCCT T--TTGGCTG CCGGGGGACA TCTGTCCCCG GGTCCGCGCT
56-P. corylophilum       GGGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCTCT GGCCCGCGCC
40-P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
2- P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
9- P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
29-P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
31-P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
35-P. chrysogenum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
51-P. citreonigrum       GGGCCCGCCG CA--AGGCCG CCGGGGGGCA TCTGCCCTCT GGCCCGCGCC
49-P. citreonigrum       GGGCCCGCCG CA--AGGCCG CCGGGGGGC- TCTGCCCTCT GGCCCGCGCC
50-P. citrinum           CCTCCCACCC GTGTTGCCCG AACCTATGTT GCCTCGGCGG GCCCCGCGCC
27-P. citrinum           ---------- ---------- ---------- ----CGGCGG GCCCCGCGCC
44-P. citrinum           ---------- ---------- --CCTATGTT GCCTCGGCGG GCCCCGCGCC
34-P. commune            GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
20-P. commune            GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
21-P. commune            GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
18-P. crustosum          GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
4- P. crustosum          GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
14-P. crustosum          GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
17-P. crustosum          GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
6- P. echinulatum        GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
48-P. expansum           GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
37-P. expansum           GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
45-P. expansum           GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
55-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
5- P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
8- P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
11-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
12-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
13-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
15-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
16-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
19-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
24-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
26-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
30-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
33-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
41-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
53-P. glabrum            GCGCCCGCCT CA--CGGCCG CCGGGGGGCT TCTGCCCCCG GGTCCGCGCG
57-P. italicum           GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
38-P. paneum             GGGCCCGCCT TCACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
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22-P. paneum          GGGCCCGCCT TCACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
23-P. paneum          GGGCCCGCCT TCACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
39-P. polonicum       GGGCCCGCCT TTACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
7- P. polonicum       GGGCCCGCCT TTACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
25-P. polonicum       GGGCCCGCCT TTACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
32-P. polonicum       GGGCCCGCCT TTACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
36-P. rolfsii         GAGCCCGCCT CA--CGGCCG CCGGGGGGCA TCCGCCCCCG GGCCCGCGCT
46-P. solitum         GGGCCCGCCT TAACTGGCCG CCGGGGGGCT CACGCCCCCG GGCCCGCGCC
10-P. solitum         GGGCCCGCCT TAACTGGCCG CCGGGGGGCT TACGCCCCCG GGCCCGCGCC
43-P. steckii         GGGCCCGCCG CC-TAGGCCG CCGGGGGGCA TCCGCCCCCG GGCCCGCGCC
42-P. sumatrense      ---------- ---------- ---GGGGGC- TCCGCCCCCG GGCCCGCGCC

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                          155        165        175        185        195
47-P. biourgeianum    CGCCGGAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
1- P. biourgeianum    CGCCGGAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
3- P. biourgeianum    CGCCGGAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
28-P. biourgeianum    CGCCGGAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
54-P. brevicompactum  CGCCGAAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
52-P. brevicompactum  CGCCGAAGAC ACCTTA--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-AT
56-P. corylophilum    CGCCGAAGAC ACCATT--GA ACACTGTCT- GAAGATTGCA GTCTGAG-CA
40-P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
2- P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
9- P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
29-P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
31-P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
35-P. chrysogenum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGTA GTCTGAG-TG
51-P. citreonigrum    CGCCGAAGAC ACCATT--GA ACGCTGTCT- GAAGATTGCA GTCTGAG-CA
49-P. citreonigrum    CGCCGAAGAC ACCATT--GA ACGCTGTCT- GAAGATTGCA GTCTGAG-CA
50-P. citrinum        CGCCGACGGC CCCCCT--GA ACGCTGTCT- -GAAGTTGCA GTCTGAGACC
27-P. citrinum        CGCCGACGGC CCCCCT--GA ACGCTGTCT- -GAAGTTGCA GTCTGAGACC
44-P. citrinum        CGCCGACGGC CCCCCT--GA ACGCTGTCT- -GAAGTTGCA GTCTGAGACC
34-P. commune         CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
20-P. commune         CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
21-P. commune         CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
18-P. crustosum       CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
4- P. crustosum       CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
14-P. crustosum       CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
17-P. crustosum       CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
6- P. echinulatum     CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
48-P. expansum        CGCCGAAGAC ACCCCC--GA ACTCTGCCT- GAAGATTGTC GTCTGAG-TG
37-P. expansum        CGCCGAAGAC ACCCCC--GA ACTCTGCCT- GAAGATTGTC GTCTGAG-TG
45-P. expansum        CGCCGAAGAC ACCCCC--GA ACTCTGCCT- GAAGATTGTC GTCTGAG-TG
55-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
5- P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
8- P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
11-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
12-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
13-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
15-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
16-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
19-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
24-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
26-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
30-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
33-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
41-P. glabrum         CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
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53-P. glabrum          CACCGGAGAC ACTATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-CA
57-P. italicum         CGCCGAAGAC ACCCCC--GA ACTCTGCCT- GAAGATTGTC GTCTGAG-TG
38-P. paneum           CGCCGAAGAC ACCC-C--GA ACTCTGTCT- GAAGAATGAA GTCTGAG-TG
22-P. paneum           CGCCGAAGAC ACCC-C--GA ACTCTGTCT- GAAGAATGAA GTCTGAG-TG
23-P. paneum           CGCCGAAGAC ACCC-C--GA ACTCTGTCT- GAAGAATGAA GTCTGAG-TG
39-P. polonicum        CGCCGAAGAC ACCCCC--GA ACTCTGTCT- GAAGAT-GAA GTCTGAG-TG
7- P. polonicum        CGCCGAAGAC ACCCCC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
25-P. polonicum        CGCCGAAGAC ACCCCC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
32-P. polonicum        CGCCGAAGAC ACCCCC--GA ACTCTGTCT- GAAGATTGAA GACTGAG-TG
36-P. rolfsii          CGCCGAAAAC ACCATT--GA ACTCTGTCT- GAAGATTGCA GTCTGAG-TG
46-P. solitum          CGCCGAAGAC ACCCTC--GA ACTCTGTCTT GAAGATTGAA GTCTGAG-TG
10-P. solitum          CGCCGAAGAC ACCCTC--GA ACTCTGTCT- GAAGATTGAA GTCTGAG-TG
43-P. steckii          CGCCGAAGCC CCCCTCT-GA ACGCTGTCT- GAAGTT-GCA GTCTGAG-AA
42-P. sumatrense       CGCCGAAGCC CCCCCCTTGA ACGCTGTCT- GAAGTTTGCA GTCTGAG-AA

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                          205        215        225        235        245
47-P. biourgeianum     TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
1- P. biourgeianum     TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
3- P. biourgeianum     TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
28-P. biourgeianum     TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
54-P. brevicompactum   TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
52-P. brevicompactum   TAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
56-P. corylophilum     ATTAGCTAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
40-P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
2- P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
9- P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
29-P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
31-P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
35-P. chrysogenum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
51-P. citreonigrum     ATTAGTTAAA TAACTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
49-P. citreonigrum     ATTAGTTAAA TAACTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
50-P. citrinum         TATAACGAAA TTAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
27-P. citrinum         TATAACGAAA TTAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
44-P. citrinum         TATA-CGAAA TTAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
34-P. commune          AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
20-P. commune          AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
21-P. commune          AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
18-P. crustosum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
4- P. crustosum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
14-P. crustosum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
17-P. crustosum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
6- P. echinulatum      AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
48-P. expansum         AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
37-P. expansum         AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
45-P. expansum         AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
55-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
5- P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
8- P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
11-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
12-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
13-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
15-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
16-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
19-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
24-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
26-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
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30-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
33-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
41-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
53-P. glabrum          TAAAC-TAAA TAAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
57-P. italicum         AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
38-P. paneum           AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
22-P. paneum           AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
23-P. paneum           AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
39-P. polonicum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
7- P. polonicum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
25-P. polonicum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
32-P. polonicum        AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
36-P. rolfsii          ATTAACTAAA TCAGTTAAAA CTTTCAACAA CGGATCT-TT GGTTCCGGCA
46-P. solitum          AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
10-P. solitum          AAAATATAAA TTATTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
43-P. steckii          ACTAGCTAAA TTAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA
42-P. sumatrense       ACTAGCTAAA TTAGTTAAAA CTTTCAACAA CGGATCTCTT GGTTCCGGCA

                       ....|....| ....|....| ....|....| ....|....| ....|....|
                          255        265        275        285        295
47-P. biourgeianum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
1- P. biourgeianum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
3- P. biourgeianum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
28-P. biourgeianum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
54-P. brevicompactum   TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
52-P. brevicompactum   TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
56-P. corylophilum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
40-P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
2- P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
9- P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
29-P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
31-P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
35-P. chrysogenum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
51-P. citreonigrum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
49-P. citreonigrum     TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CAGAAT
50-P. citrinum         TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
27-P. citrinum         TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
44-P. citrinum         TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
34-P. commune          TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
20-P. commune          TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
21-P. commune          TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
18-P. crustosum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
4- P. crustosum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
14-P. crustosum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
17-P. crustosum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
6- P. echinulatum      TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
48-P. expansum         TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
37-P. expansum         TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
45-P. expansum         TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
55-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
5- P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
8- P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
11-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
12-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
13-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
15-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
16-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
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19-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
24-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
26-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
30-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
33-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
41-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
53-P. glabrum          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
57-P. italicum         TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
38-P. paneum           TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
22-P. paneum           TCGATGAAGA ACGCAGCGAA ATCGCGATAC GTAATGTGAA TTG-CA-AAT
23-P. paneum           TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
39-P. polonicum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
7- P. polonicum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
25-P. polonicum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
32-P. polonicum        TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
36-P. rolfsii          TCGATGAACA ACGCA-CGAA AT-GCGATAA GTAATGTGAA TTGTCAGAAT
46-P. solitum          TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
10-P. solitum          TCGATGAAGA ACGCAGCGAA AT-GCGATAC GTAATGTGAA TTG-CA-AAT
43-P. steckii          TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT
42-P. sumatrense       TCGATGAAGA ACGCAGCGAA AT-GCGATAA CTAATGTGAA TTG-CAGAAT

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                          305        315        325        335        345
47-P. biourgeianum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
1- P. biourgeianum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
3- P. biourgeianum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
28-P. biourgeianum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
54-P. brevicompactum   TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
52-P. brevicompactum   TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
56-P. corylophilum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC TTGGTATTCC
40-P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
2- P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
9- P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
29-P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
31-P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
35-P. chrysogenum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
51-P. citreonigrum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
49-P. citreonigrum     TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
50-P. citrinum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
27-P. citrinum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
44-P. citrinum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
34-P. commune          TCAGTGAATC ATCGA-TCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
20-P. commune          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
21-P. commune          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
18-P. crustosum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
4- P. crustosum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
14-P. crustosum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
17-P. crustosum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
6- P. echinulatum      TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
48-P. expansum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
37-P. expansum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
45-P. expansum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
55-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
5- P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
8- P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
11-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
12-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
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13-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
15-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
16-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
19-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
24-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
26-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
30-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
33-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
41-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
53-P. glabrum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
57-P. italicum         TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
38-P. paneum           TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
22-P. paneum           TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
23-P. paneum           TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
39-P. polonicum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
7- P. polonicum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
25-P. polonicum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
32-P. polonicum        TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
36-P. rolfsii          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTAT-CC
46-P. solitum          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCC CTGGTATTCC
10-P. solitum          TCAGTGAATC ATCGAGTCTT TTGAACGCAC ATTGCGCCCC CTGGTATTCC
43-P. steckii          TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC
42-P. sumatrense       TCAGTGAATC ATCGAGTCTT T-GAACGCAC ATTGCGCCCT CTGGTATTCC

                       ....|....| ....|....| ....|....| ....|....| ....|....|
                          355        365        375        385        395
47-P. biourgeianum     GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
1- P. biourgeianum     GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
3- P. biourgeianum     GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
28-P. biourgeianum     GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
54-P. brevicompactum   GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
52-P. brevicompactum   GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
56-P. corylophilum     GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
40-P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTTCT GCCCTCAAGC ACGGCTTGTG
2- P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
9- P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
29-P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTTCT GCCCTCAAGC ACGGCTTGTG
31-P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
35-P. chrysogenum      GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
51-P. citreonigrum     GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
49-P. citreonigrum     GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
50-P. citrinum         GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
27-P. citrinum         GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
44-P. citrinum         GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
34-P. commune          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
20-P. commune          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
21-P. commune          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
18-P. crustosum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
4- P. crustosum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
14-P. crustosum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
17-P. crustosum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
6- P. echinulatum      GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
48-P. expansum         GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
37-P. expansum         GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
45-P. expansum         GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
55-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
5- P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
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8- P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
11-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
12-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
13-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
15-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
16-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
19-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
24-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
26-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
30-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
33-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
41-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
53-P. glabrum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
57-P. italicum         GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
38-P. paneum           GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
22-P. paneum           GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
23-P. paneum           GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
39-P. polonicum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
7- P. polonicum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
25-P. polonicum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
32-P. polonicum        GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
36-P. rolfsii          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
46-P. solitum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
10-P. solitum          GGGGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC CCGGCTTGTG
43-P. steckii          GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG
42-P. sumatrense       GGAGGGCATG CCTGTCCGAG CGTCATTGCT GCCCTCAAGC ACGGCTTGTG

                          ....|....| ....|....| ....|....| ....|....| ....|....|
                             405        415        425        435        445
47-P. biourgeianum     TGTTGGGCCC C-GTCCTCC- --T--TCCGG GGGACGGGTC CGAAA-GGCA
1- P. biourgeianum     TGTTGGGCCC C-GTCCTCC- --T--TCCGG GGGACGGGTC CGAAA-GGCA
3- P. biourgeianum     TGTTGGGCCC C-GTCCTCC- --T--TCCGG GGGACGGGTC CGAAA-GGCA
28-P. biourgeianum     TGTTGGGCCC C-GTCCTCC- --T--TCCGG GGGACGGGTC CGAAA-GGCA
54-P. brevicompactum   TGTTGGGCTC C-GTCCTCC- --T--TCCGG GGGACGGGCC CGAAA-GGCA
52-P. brevicompactum   TGTTGGGCTC C-GTCCTCC- --T--TCCGG GGGACGGGCC CGAAA-GGCA
56-P. corylophilum     TGTTGGGCCC C-GTCCTCC- -TT--CCCGG GGGACGGGCC CGAAA-GGCA
40-P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
2- P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
9- P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
29-P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
31-P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
35-P. chrysogenum      TGTTGGGCCC C-GTCCTCCG ATC---CCGG GGGACGGGCC CGAAA-GGCA
51-P. citreonigrum     TGTTGGGCTC C-GTCCTCC- -T---CCCGG GGGACGGGCC CGAAA-GGCA
49-P. citreonigrum     TGTTGGGCTC C-GTCCTCC- -T---CCCGG GGGACGGGCC CGAAA-GGCA
50-P. citrinum         TGTTGGGCCC C-GTCCCCCC C---GCCGGG GGGACGGGCC CGAAA-GGCA
27-P. citrinum         TGTTGGGCCC C-GTCCCCCC CC--GCCGGG GGGACGGGCC CGAAA-GGCA
44-P. citrinum         TGTTGGGCCC C-GTCCCCCC C---GCCGGG GGGACGGGCC CGAAA-GGCA
34-P. commune          TGTTGGGCCC C-GTCCTCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
20-P. commune          TGTTGGGCCC C-GTCCTCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
21-P. commune          TGTTGGGCCC C-GTCCCCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
18-P. crustosum        TGTTGGGCCC C-GTCCCCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
4- P. crustosum        TGTTGGGCCC C-GTCCCCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
14-P. crustosum        TGTTGGGCCC C-GTCCTCCG ATT--TCCGG GGGACGGGCC CGAAA-GGCA
17-P. crustosum        TGTTGGGCCC C-GTCCCCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
6- P. echinulatum      TGTTGGGCCC C-GTCCTCCG ATT--TCCGG GGGACGGGCC CGAAA-GGCA
48-P. expansum         TGTTGGGCCC C-GTCCTCCG AT---TCCGG GGGACGGGCC CGAAA-GGCA
37-P. expansum         TGTTGGGCCC C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
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45-P. expansum          TGTTGGGCCC C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
55-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
5- P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
8- P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
11-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
12-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
13-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
15-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
16-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
19-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
24-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
26-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
30-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAAAGGCA
33-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
41-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
53-P. glabrum           TGTTGGGCTC C-GTCCCCC- --------CG GGGACGGGTC CGAAA-GGCA
57-P. italicum          TGTTGGGCCC C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
38-P. paneum            TGTTGGGCCT C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
22-P. paneum            TGTTGGGCCT C-GTCCTCCG ATT---CCGG GG-ACGGGCC CGAAA-GGCA
23-P. paneum            TGTTGGGCCT C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
39-P. polonicum         TGTTGGGCCC C-GTCCTCCG AT---TCCGG GGGACGGGCC CGAAA-GGCA
7- P. polonicum         TGTTGGGCCC C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
25-P. polonicum         TGTTGGGCCC C-GTCCTCCG ATT---CCGG GGGACGGGCC CGAAA-GGCA
32-P. polonicum         TGTTGGGCCC C-GTCCTCCG AT---TCCGG GGGACGGGCC CGAAA-GGCA
36-P. rolfsii           TGTTGGGCCC C-GCCCCCCG GTT---CCGG GGGGCGGACC CGAAA-GGCA
46-P. solitum           TGTTGGGCCC C-GTCCTCCG ATT--TCCGG GGGACGGGCC CGAAA-GGCA
10-P. solitum           TGTTGGGCCC C-GTCCCCCG ATC--TCCGG GGGACGGGCC CGAAA-GGCA
43-P. steckii           TGTTGGGCCC C-GTCCCCCC CGC-GCCGGG GGGACGGGCC CGAAA-GGCA
42-P. sumatrense        TGTTGGGCCC CCGTCCCCCC CTCTGCCGGG GGGACGGGCC CGAAA-GGCA

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                             455        465        475        485        495
47-P. biourgeianum      GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCACTCGC
1- P. biourgeianum      GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCACTCGC
3- P. biourgeianum      GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCACTCGC
28-P. biourgeianum      GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCACTCGC
54-P. brevicompactum    GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCTCCCGC
52-P. brevicompactum    GCGGCGGCAC CGCGTCCGGT CCTCAAGCG- TATGGGGCTT TGTCACCCGC
56-P. corylophilum      GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
40-P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
2- P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
9- P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
29-P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
31-P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
35-P. chrysogenum       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
51-P. citreonigrum      GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
49-P. citreonigrum      GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
50-P. citrinum          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
27-P. citrinum          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
44-P. citrinum          GCGGCGGCAC CGCGTCCGGT CCTCGAGCGG TATGGGGCTT CGTCACCCGC
34-P. commune           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
20-P. commune           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- T-TGGGGCTT TGTCACCCGC
21-P. commune           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
18-P. crustosum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
4- P. crustosum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGT-------
14-P. crustosum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
17-P. crustosum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
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6- P. echinulatum      GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
48-P. expansum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
37-P. expansum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
45-P. expansum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
55-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
5- P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
8- P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
11-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
12-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
13-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
15-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
16-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
19-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
24-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
26-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
30-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCGG TATGGGGCTT TGTCACCCGC
33-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
41-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
53-P. glabrum          GCGGCGGCAC CGAGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
57-P. italicum         GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
38-P. paneum           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTC TGTCACCCGC
22-P. paneum           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- T-TGGGGCTC TGTCACCCGC
23-P. paneum           GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTC TGTCACCCGC
39-P. polonicum        GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
7- P. polonicum        GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
25-P. polonicum        GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
32-P. polonicum        GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
36-P. rolfsii          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
46-P. solitum          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
10-P. solitum          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT TGTCACCCGC
43-P. steckii          GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC
42-P. sumatrense       GCGGCGGCAC CGCGTCCGGT CCTCGAGCG- TATGGGGCTT CGTCACCCGC

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                          505        515        525        535        545
47-P. biourgeianum     TTT-GTAGG- CCTGGCCGGC GCTTG-CCGA ----TCAACC AAACTTTTT-
1- P. biourgeianum     TTT-GTAGG- CCTGGCCGGC GCTTG-CCGA ----TCAACC AAACTTTTT-
3- P. biourgeianum     TTT-GTAGG- CCTGGCCGGC GCTTG-CCGA ----TCAACC AAACTTTTT-
28-P. biourgeianum     TTT-GTAGG- CCTGGCCGGC GCTTG-CCGA ----TCAACC AAACTTTTT-
54-P. brevicompactum   TTT-GTAGG- ACTGGCCGGC GCCTG-CCGA ----TCACCG AAACTTTTT-
52-P. brevicompactum   TTT-GTAGG- ACTGGCCGGC GCCTG-CCGA ----TCAACC AAACTTTTT-
56-P. corylophilum     TCTTGTAGG- CCCGGCCGGC GCTTG-CCGA ----CAACCA TCAATCTTTT
40-P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
2- P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
9- P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
29-P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
31-P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
35-P. chrysogenum      TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
51-P. citreonigrum     TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----CA--CA TCAATCTTTT
49-P. citreonigrum     TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----CA--CA TCAATCTTTT
50-P. citrinum         TCTAGTAGG- CCCGGCCGGC GCCAG-CCGA CCCCCAACCT TTAATTATC-
27-P. citrinum         TCTAGTAGG- CCCGGCCGGC GCCAG-CCGA CCCCCAACCT TTAATTATC-
44-P. citrinum         TCTAGTAGG- CCCGGCCGGC GCCAG-CCGA CCCCCAACCT TTAATTATC-
34-P. commune          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
20-P. commune          TCT-G-AGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
21-P. commune          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
18-P. crustosum        TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
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4- P. crustosum          ---------- ---------- ---------- ---------- ----------
14-P. crustosum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
17-P. crustosum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
6- P. echinulatum        TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTTT
48-P. expansum           TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
37-P. expansum           TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
45-P. expansum           TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
55-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
5- P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
8- P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
11-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
12-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
13-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
15-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
16-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
19-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
24-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
26-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
30-P. glabrum            TCT-GTAGGT CCCGGCCGGC CCCAGACCGA ----CAACCA ATCATCCTTT
33-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
41-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
53-P. glabrum            TCT-GTAGG- CCCGGCCGGC GCCAG-CCGA ----CAACCA ATCATCCTTT
57-P. italicum           TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTTT
38-P. paneum             TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
22-P. paneum             TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
23-P. paneum             TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
39-P. polonicum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
7- P. polonicum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
25-P. polonicum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
32-P. polonicum          TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
36-P. rolfsii            TCT-GAAGG- CCCGGCCGGC GCCCG-CCGG ----CGACCC CAATCAATAC
46-P. solitum            TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
10-P. solitum            TCT-GTAGG- CCCGGCCGGC GCTTG-CCGA ----TCAACC CAAATTTTT-
43-P. steckii            TCTTGTAGG- CCCGGCCGGC GCCAG-CCGG ACCCCCAACC TTTATATTT-
42-P. sumatrense         TCTTGTAGG- CCCGGCCGGC GCCAG-CCGA --CCCCAACC CTAAATTTT-

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                            555        565        575        585        595
47-P. biourgeianum       -ATCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
1- P. biourgeianum       -ATCAGGTTG ACCTCGGTAC GAG-TAGGGA T-ACCCG--- ----------
3- P. biourgeianum       -ATCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
28-P. biourgeianum       -ATCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
54-P. brevicompactum     -TA------- ---------- ---------- ---------- ----------
52-P. brevicompactum     -TCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
56-P. corylophilum       TTC-AGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
40-P. chrysogenum        ATCCAGGT-G ACCTCGGATC AGG-TAGGGA T-ACCCG-TG AACTTAAGCA
2- P. chrysogenum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
9- P. chrysogenum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA TCACCCGCTG AACTTAAGCA
29-P. chrysogenum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
31-P. chrysogenum        ATCCAG-TTG ACCTCGGATC A--------- ---------- ----------
35-P. chrysogenum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
51-P. citreonigrum       TTCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
49-P. citreonigrum       TTCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
50-P. citrinum           --TCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
27-P. citrinum           --TCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
44-P. citrinum           --TCAGGTTG ACCTCGT-TC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
34-P. commune            ATCCAG-TTG ACCTCGGATC AG--TAGGGA T-ACCCG-TG AACTTAAGCT
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20-P. commune          ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
21-P. commune          ATCCAGGTTG ACCTCGGATC AG-------- ---------- ----------
18-P. crustosum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
4- P. crustosum        ---------- ---------- ---------- ---------- ----------
14-P. crustosum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
17-P. crustosum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
6- P. echinulatum      ATCCAGGTTG ACCTCGGATC AGGATAGGGA T-ACCCGCTG AACTTAAGCA
48-P. expansum         ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
37-P. expansum         ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
45-P. expansum         ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
55-P. glabrum          TTT------- ---------- ---------- ---------- ----------
5- P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
8- P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
11-P. glabrum          TTTCAGGTTG ACCTCGGATG AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
12-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
13-P. glabrum          TTTCAG-TTG ACCTCG-ATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
15-P. glabrum          TTTCAGGTTG ACCTCGG--C AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
16-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
19-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
24-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
26-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
30-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCC---- ----------
33-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
41-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
53-P. glabrum          TTTCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
57-P. italicum         ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
38-P. paneum           ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
22-P. paneum           ATCCAGGTTG ACCTC----- ---------- ---------- ----------
23-P. paneum           ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
39-P. polonicum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
7- P. polonicum        ATC------- ---------- ---------- ---------- ----------
25-P. polonicum        ATCCAGGTTG ACCTCGGAT- AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
32-P. polonicum        ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
36-P. rolfsii          TTACCAGTTG ACCACGGATC ATG--GAGGA T-ACCCGCTG AACTTAACCA
46-P. solitum          ATCCAGGTTG ACCTCGGATC AGG-TAGGGA T-ACCCGCTG AACTTAAGCA
10-P. solitum          ATCCAGGTTG ACCTCGGATC A--------- ---------- ----------
43-P. steckii          TCTCAGGTTG ACCTCG---- ---------- ---------- ----------
42-P. sumatrense       TTTCAGGT-G ACCTCGGATC AGG------- ---------- ----------

                       ....|....| ....|....
                          605        615
47-P. biourgeianum     TATCA----- ---------
1- P. biourgeianum     ---------- ---------
3- P. biourgeianum     TATC------ ---------
28-P. biourgeianum     TATCAATAAG ---------
54-P. brevicompactum   ---------- ---------
52-P. brevicompactum   TATCAATAA- ---------
56-P. corylophilum     TATCAA---- ---------
40-P. chrysogenum      TAT-AT--AA GCGGAGGA-
2- P. chrysogenum      TATCAATAA- ---------
9- P. chrysogenum      TATCATTAAA GCGGAGGAA
29-P. chrysogenum      TATCAATAAG ---------
31-P. chrysogenum      ---------- ---------
35-P. chrysogenum      TATCAAT-AA GCGG-----
51-P. citreonigrum     TATCAATAAG CCGGA----
49-P. citreonigrum     TATCAATAAG CGGG-----
50-P. citrinum         TATCAATAAG CGGAGGA--
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27-P. citrinum          TATCAATAAG CGGG-----
44-P. citrinum          TATCAATAAG CGGAGGA--
34-P. commune           ---------- ---------
20-P. commune           TATCAATAAG CGGGAGGA-
21-P. commune           ---------- ---------
18-P. crustosum         TATCAATA-- ---------
4- P. crustosum         ---------- ---------
14-P. crustosum         TATCAATAAG CGGG-----
17-P. crustosum         TATCATAA-- ---------
6- P. echinulatum       TATCAATAAG CGGAGGAA-
48-P. expansum          TATCAATAAG CGGGAGGA-
37-P. expansum          TATCATTAAG CGGAGGAA-
45-P. expansum          TATCAATAA- ---------
55-P. glabrum           ---------- ---------
5- P. glabrum           TATCAATAA- ---------
8- P. glabrum           TATCATTAAA GCGGAGGAA
11-P. glabrum           TATCAATAAG CGGGAGGA-
12-P. glabrum           TATCAATAAG CGGGAGGA-
13-P. glabrum           TATCAATAAG CGGGAGG--
15-P. glabrum           TATCAATAA- ---------
16-P. glabrum           TATCAATAAG CGGGA----
19-P. glabrum           TATCAATAAG CGGAGGA--
24-P. glabrum           TATCA----- ---------
26-P. glabrum           TATCAATAA- ---------
30-P. glabrum           ---------- ---------
33-P. glabrum           TATCAATAAG CGGAGGAA-
41-P. glabrum           TATCATAA-- ---------
53-P. glabrum           TATCAATAAG CGGAGGAA-
57-P. italicum          TATC------ ---------
38-P. paneum            TATCA----- ---------
22-P. paneum            ---------- ---------
23-P. paneum            TATCATTAAG CGGAGGAA-
39-P. polonicum         TATCAATAAG CGGGAGGA-
7- P. polonicum         ---------- ---------
25-P. polonicum         TATCA----- ---------
32-P. polonicum         TATCAATAAG CGGAGGA--
36-P. rolfsii           TATCAATAAG CGGAGGA--
46-P. solitum           TATCAATAAG CGGAGGA--
10-P. solitum           ---------- ---------
43-P. steckii           ---------- ---------
42-P. sumatrense        ---------- ---------
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8. APPENDIX II - BETA TUBULIN SEQUENCE ALIGNMENT

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                          ....|....| ....|....| ....|....| ....|....| ....|....|
                              5          15         25         35         45
47-P. bialowiezense       ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCGGCACCG
1- P. bialowiezense       ---------- ---------G TGACCCTTGG CCCAGTTGTT ACCGGCACCG
3- P. bialowiezense       ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCGGCACCG
28-P. bialowiezense       -----TACCC TCAGGTGTAG TGACCCTTGG CCCAGTTGTT ACCGGCACCG
54-P. brevicompactum      -------ACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
52-P. brevicompactum      ---------- ------GTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
56-P. corylophilum        -----GTACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCGGCACCA
40-P. chrysogenum         ---------- ---------G TGACCCTTGG CCCAGTTGTT ACCAGCACCG
2- P. chrysogenum         ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCACCG
9- P. chrysogenum         --------CC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCNCCG
29-P. chrysogenum         ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
31-P. chrysogenum         ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCNCCG
35-P. chrysogenum         -----TACCC TCCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
51-P. citreonigrum        ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCGGCACCG
49-P. citreonigrum        ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCGGCACCG
50-P. citrinum            ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
27-P. citrinum            ---------- ---------- --ACCCTTGG CCCAGTTGTT ACCAGCACCG
44-P. citrinum            ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
34-P. commune             ---------- ---------- ---------- ---------- ----------
20-P. commune             ---------- ---------- ---------- ---------- ----------
18-P. crustosum           ---------- ---------- ---CCCTTGG CCCAGTTGTT ACCAGCNCCG
4- P. crustosum           ---TATCCCT CANCTGTAGC TGACCCTTGG CCCNGTTGTT ACCAGCNCCG
14-P. crustosum           ---------- ------TA-G TGACCCTTGG CCCAGTTGTT ACCAGCACCG
17-P. crustosum           ---------- ---------- -GACCCTTGG CCCAGTTGTT ACCAGCACCG
6- P. echinulatum         -----TACCC TCAGNGTATG TGACCCTTGG CCCAGTTGTT ACCAGCNCCG
48-P. expansum            -------ACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
37-P. expansum            ------TACC CTCAGNTTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
45-P. expansum            ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
55-P. glabrum             -------ACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
5- P. glabrum             ---------- ---------- ---------- ---------- ----------
8- P. glabrum             ---------- ---------G TGACCCTTGG CCCAGTTGTT ACCAGCACCG
11-P. glabrum             ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCACCG
12-P. glabrum             ---------- --------AG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
13-P. glabrum             ---------- ---------G TGACCCTTGG CCCAGTTGTT ACCAGCACCG
15-P. glabrum             ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCACCG
16-P. glabrum             ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
19-P. glabrum             ---------- ------TTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
24-P. glabrum             ---------- --------AG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
26-P. glabrum             ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCACCG
30-P. glabrum             ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
33-P. glabrum             ---------- --------AG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
41-P. glabrum             ---------- ---------- -GACCCTTGG CCCAGTTGTT ACCAGCACCG
53-P. glabrum             ---------- --------AG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
57-P. italicum            ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
38-P. paneum              ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
22-P. paneum              ---------- ---------A TGACCCTTGG CCCAGTTGTT ACCAGCACCG
23-P. paneum              ---------- -------TAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
36-P. piscarium           ---------- ---------- TGACCCTTGG CCCAGTTGTT ACCAGCACCA
39-P. polonicum           ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
7- P. polonicum           --------CC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
25-P. polonicum           CCCNGGTACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
32-P. polonicum           -------ACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCAGCACCG
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46-P. solitum          ---------- ---------G TGACCCTTGG CCCAGTTGTT ACCAGCACCG
10-P. solitum          ---------- ---------- ---CCCTTGG CCCAGTTGTT ACCAGCACCG
21-P. solitum          ------TACC CTCAGTGTAG TGACCCTTGG CCCAGTTGTT ACCANCACCG
43-P. steckii          ---------- ---------- ---------- ---------- -CCAGCCCCA
42-P. sumatrense       ---------- ---------- --ACCCTTGG CCCAGTTGTT ACCAGCACCA

                       ....|....| ....|....| ....|....| ....|....| ....|....|
                            55         65         75         85         95
47-P. bialowiezense    GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
1- P. bialowiezense    GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
3- P. bialowiezense    GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
28-P. bialowiezense    GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
54-P. brevicompactum   GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
52-P. brevicompactum   GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
56-P. corylophilum     GACTG-TCCG AAGACGANAG TTGTC-GGGA CGGAAGAGCT TGCCGAAGGG
40-P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
2- P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
9- P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
29-P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
31-P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
35-P. chrysogenum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
51-P. citreonigrum     GACTG-GCCG AAGATGAA-G TTGTC-GGGA CGGAAGAGCT TGCCGAAGGG
49-P. citreonigrum     GACTG-GCCG AAGATGAA-G TTGTC-GGGA CGGAAGAGCT TGCCGAAGGG
50-P. citrinum         GATTG-ACCG AAAACGAA-G TTGTC-GGGA CGGAAAAGCT TGCCGAAGGG
27-P. citrinum         GATTG-ACCG AAAACGAA-G TTGTC-GGGA CGGAAAAGCT TGCCGAAGGG
44-P. citrinum         GATTG-ACCG AAAACGAA-G TTGTC-GGGA CGGAAAAGCT TGCCGAAAGG
34-P. commune          ---------- ---------G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
20-P. commune          ---------- ---------- ---------- ---------- TGCCGAAAGG
18-P. crustosum        GACTG-GCCG AAGANGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
4- P. crustosum        GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
14-P. crustosum        GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
17-P. crustosum        GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
6- P. echinulatum      GACTG-ACCG AAGACGAA-G TTGTC-GGGG NGGAAAAGCT TGCCGAAGGG
48-P. expansum         GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
37-P. expansum         GACTG-ACCG AAGACGAA-G TTGTCAGGGG CGGAAAAGCT TGCCGAAGGG
45-P. expansum         GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
55-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAATAGAC CACCGAAGGG
5- P. glabrum          --CTG-ACCG AAA-CGAA-G TTGTC-GGGG CGGAATAGAC CACCGAAGGG
8- P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAATAGAC CACCGAAGGG
11-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAATAGAC CACCGAAGGG
12-P. glabrum          GACTG-GCCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
13-P. glabrum          GACTG-GCCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
15-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
16-P. glabrum          GACTG-GCCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
19-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
24-P. glabrum          GACTG-GCCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
26-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
30-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
33-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
41-P. glabrum          GACTG-GCCG AAAACGAA-G TTGTC-GGGG CGGAAGAGAC CACCGAAGGG
53-P. glabrum          GACTG-ACCG AAAACGAA-G TTGTC-GGGG CGGAATAGAC CACCGAAGGG
57-P. italicum         GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
38-P. paneum           GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
22-P. paneum           GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
23-P. paneum           GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
36-P. piscarium        GACTG-GCCG AAGACGAA-G TTGTC-GGGA CGGAAGAGCT TGCCAAAGGG
39-P. polonicum        GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
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7- P. polonicum          GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
25-P. polonicum          GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
32-P. polonicum          GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAAGG
46-P. solitum            GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
10-P. solitum            GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
21-P. solitum            GACTG-GCCG AAGANGAA-G TTGTC-GGGG CGGAAAAGCT TGCCGAAGGG
43-P. steckii            GATTGCACCG CAAACGAA-G TTGTC-GGGA CGGAAAAGCT TGCCGAAGGG
42-P. sumatrense         GACTG-ACCG AAGACGAA-G TTGTC-GGGG CGGAAGAGCT TGCCGAAGGG

                         ....|....| ....|....| ....|....| ....|....| ....|....|
                            105        115        125        135        145
47-P. bialowiezense      ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
1- P. bialowiezense      ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
3- P. bialowiezense      ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
28-P. bialowiezense      ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
54-P. brevicompactum     ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
52-P. brevicompactum     ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACGAGGACGG
56-P. corylophilum       ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACCAGAACGG
40-P. chrysogenum        ACCGGAGCGG ACAGCATCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
2- P. chrysogenum        ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
9- P. chrysogenum        ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
29-P. chrysogenum        ACCGGAGCGG ACAGCATCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
31-P. chrysogenum        ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
35-P. chrysogenum        ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
51-P. citreonigrum       ACCGGCACGG ACAGCATCCA TGGTACCGGG CTCCAAGTCG ACCAGGACGG
49-P. citreonigrum       ACCGGCACGG ACAGCATCCA TGGTACCGGG CTCCAAGTCG ACCAGGACGG
50-P. citrinum           ACCAGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACCAGGACGG
27-P. citrinum           ACCAGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACCAGGACGG
44-P. citrinum           ACCAGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACCAGGACGG
34-P. commune            ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
20-P. commune            ACCGGANCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
18-P. crustosum          ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
4- P. crustosum          ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
14-P. crustosum          ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
17-P. crustosum          ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
6- P. echinulatum        ACCGGAGCGG ACAGNGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
48-P. expansum           ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACGAGAACGG
37-P. expansum           ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACGAGAACGG
45-P. expansum           ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACGAGAACGG
55-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
5- P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
8- P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
11-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
12-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
13-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
15-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
16-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
19-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
24-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
26-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
30-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
33-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
41-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
53-P. glabrum            ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACAAGGACGG
57-P. italicum           ACCGGAGCGG ACAGCGTCCA TGGTACCGGG CTCCAAATCG ACCAGAACGG
38-P. paneum             ACCAGAGCGG ACGGCGTCCA TGGTGCCGGG CTCCAAATCG ACCAGAACGG
22-P. paneum             ACCAGAGCGG ACGGCGTCCA TGGTGCCGGG CTCCAAATCG ACCAGAACGG
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23-P. paneum            ACCAGAGCGG ACGGCGTCCA TGGTGCCGGG CTCCAAATCG ACCAGAACGG
36-P. piscarium         ACCGGCACGG ACGGCATCCA TGGTACCGGG CTCCAGATCG ACCAGAACGG
39-P. polonicum         ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
7- P. polonicum         ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
25-P. polonicum         ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
32-P. polonicum         ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
46-P. solitum           ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
10-P. solitum           ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
21-P. solitum           ACCGGAGCGG ACAGCGTCCA TGGTACCAGG CTCCAAATCG ACGAGAACGG
43-P. steckii           GCCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAAGTCG ACCAGAACGG
42-P. sumatrense        ACCGGCACGG ACAGCGTCCA TGGTACCGGG CTCCAGATCA ACCAGAACGG

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                           155        165        175        185        195
47-P. bialowiezense     CACGGGGAAC ATACTTGTCA CCACTAGCCT GGGAGGTCAA AAAAT----C
1- P. bialowiezense     CACGGGGAAC ATACTTGTCA CCACTAGCCT GGGAGGTCAA AAAAT----C
3- P. bialowiezense     CACGGGGAAC ATACTTGTCA CCACTAGCCT GGGAGGTCAA AAAAT----C
28-P. bialowiezense     CACGGGGAAC ATACTTGTCA CCACTAGCCT GGGAGGTCAA AAAAT----C
54-P. brevicompactum    CACGGGGAAC GTACTTGTCA CCACTAGCCT GGGCGGTCAA GAATA----T
52-P. brevicompactum    CACGGGGAAC GTACTTGTCA CCACTAGCCT GGGCGGTCAA GAATA----T
56-P. corylophilum      CACGGGGAAC GTACTTGTCG TTGCTAGCCT G----CAGGG AAACAA----
40-P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGT
2- P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGC
9- P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGC
29-P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGT
31-P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGC
35-P. chrysogenum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTGTCAA AGAAAAACGC
51-P. citreonigrum      CACGGGGAAC GTACTTGTCG TTGCTGGCCT ATTGATAAAG AGAGAA----
49-P. citreonigrum      CACGGGGAAC GTACTTGTCG TTGCTGGCCT ATTGATAAAG AGAGAA----
50-P. citrinum          CACGGGGAAC ATACTTGTCA CCGGAAGCCT ATTGATAAAA -CAAACAATA
27-P. citrinum          CACGGGGAAC ATACTTGTCA CCGGAAGCCT ATTGATAAAA -CAAACAATA
44-P. citrinum          CACGGGGAAC ATACTTGTCA CCGGAAGCCT ATTGATAAAA -CAAACAATA
34-P. commune           CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAA-CAT
20-P. commune           CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAA-CAT
18-P. crustosum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAA-CAT
4- P. crustosum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA GGAAAA-CAT
14-P. crustosum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAG GGAAAA-CAT
17-P. crustosum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA GGAAAA-CAT
6- P. echinulatum       CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATTAG GGAAAA-CAT
48-P. expansum          CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAAAGAT
37-P. expansum          CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAAAGAT
45-P. expansum          CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAAAGAT
55-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
5- P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
8- P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
11-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
12-P. glabrum           CACGGGGAAC GTAACGGTTG CTGCTGGCCT A-----TCAA GATCAA----
13-P. glabrum           CACGGGGAAC GTAACGGTTG CTGCTGGCCT A-----TCAA GATCAA----
15-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
16-P. glabrum           CACGGGGAAC GTAACGGTTG CTGCTGGCCT A-----TCAA GATCAA----
19-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
24-P. glabrum           CACGGGGAAC GTAACGGTTG CTGCTGGCCT A-----TCAA GATCAA----
26-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
30-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
33-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
41-P. glabrum           CACGGGGAAC GTAACGGTTG CTGCTGGCCT A-----TCAA GATCAA----
53-P. glabrum           CACGGGGAAC GAAACGGTTG CTGCTGGCCT A-----TCAA GATAAA----
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57-P. italicum          CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGAGTTTCAA AGAAAA-GAT
38-P. paneum            CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATT---AA AGAAAAACAT
22-P. paneum            CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATT---AA AGAAAAACAT
23-P. paneum            CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATT---AA ATAAAAACAT
36-P. piscarium         CACGGGGAAC GTACTTGTCA CCGCTGGCCT GGA---AAAC AAAACATCCA
39-P. polonicum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATATCAAA GAAAAA-CAT
7- P. polonicum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATATCAAA GAAAAA-CAT
25-P. polonicum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATATCAAA GAAAAA-CAT
32-P. polonicum         CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATATCAAA GAAAAA-CAT
46-P. solitum           CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAG GGAAAA-CAT
10-P. solitum           CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAG GGAAAA-CAT
21-P. solitum           CACGGGGAAC GTACTTGTCA CCGCTGGCCT AGATTATCAA AGAAAA-CAT
43-P. steckii           CACGGGGAAC ATACTTGTCA CCGGAAGCCT ATTCAGAAAA ACAAACAATC
42-P. sumatrense        CACGGGGAAC ATACTTGTCA CCGGAAGCCT ATTGTAAACA -GAAAAACAA

                        ....|....| ....|....| ....|....| ....|....| ....|....|
                            205        215        225        235        245
47-P. bialowiezense     CGGGTGAGCA AACACACAAC AAGATTTTTC CA---AGGCA TTGT-ACTCA
1- P. bialowiezense     CGGGTGAGCA AACACACAAC AAGATTTTTC CA---AGGCA TTGT-ACTCA
3- P. bialowiezense     CGGGTGAGCA AACACACAAC AAGATTTTTC CA---AGGCA TTGT-ACTCA
28-P. bialowiezense     CGGGTGAGCA AACACACAAC AAGATTTTTC CA---AGGCA TTGT-ACTCA
54-P. brevicompactum    GAGGTGAGAA AATGCACAAC CAGAGTTCTT CA---CATCA TTGT-ACTCA
52-P. brevicompactum    GAGGTGAGAA AATGCACAAC AAGAGTTCTT CA---CATCA TTGT-ACTCA
56-P. corylophilum      --ATTGAGAT TAGATTAGAT CGGTCGAGGC AT---TA-AT GTGACACATA
40-P. chrysogenum       CCGATCAGAT GATGCACAAT CAATCGATTC CC---AGTCA TTGT-ACTCA
2- P. chrysogenum       CCGATCAGAT GATGCACAAT TAATCGATTC CC---AGCCA TTGT-ACTCA
9- P. chrysogenum       CCGATCAGAT GATGCACAAT TAATCGATTC CC---AGTCA TTGT-ACTCA
29-P. chrysogenum       CCGATCAGAT GATGCACAAT CAATCGATTC CC---AGTCA TTGT-ACTCA
31-P. chrysogenum       CCGATCAGAT GATGCACAAT TAATCGATTC CC---AGTCA TTGT-ACTCA
35-P. chrysogenum       CCGATCAGAT GATGCACAAT TAA--GATTC CC---AGTCA TTGT-ACTCA
51-P. citreonigrum      --ATCATACT TAGATAAGAT CAATCGAAGT GG---TACGG ATGTCACTTA
49-P. citreonigrum      --ATCATACT TAGATAAGAT CAATCGAAGT GG---TACGG ATGTCACTTA
50-P. citrinum          GTTGGTTAGA TAATGATTCC AATGGCATTG G----GGTCA GTATCACTTA
27-P. citrinum          GTTGGTTAGA TAATGATTCC AATGGCATTG G----GGTCA GTATCACTTA
44-P. citrinum          GTTGGTTAGA TAATGATTCC AATGGCATTG G----GGTCA GTATCACTTA
34-P. commune           CCGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTCG TTGG-ACTCA
20-P. commune           CCGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTCG TTGG-ACTCA
18-P. crustosum         CCGATTAGAT GATGCACGAT TATTCGGTTT CC---CGTCG TTGA-ACTCA
4- P. crustosum         CCGATCAGAT GATGCACTAT TATTCGGTTT CC---TGTCG TTGG-ACTCA
14-P. crustosum         CCGATCAGAT GATGCACTAT TATTCGGTTT CC---TGTCG TTGG-ACTCA
17-P. crustosum         CCGATCAGAT GATGCACTAT TATTCGGTTT CC---TGTCG TTGG-ACTCA
6- P. echinulatum       CCGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTCG TTGG-ACTCA
48-P. expansum          CCGATCAGAT GATGCACGAT TATTCGGTAA AC---AGTCG GTGT-ACTCA
37-P. expansum          CCGATCAGAT GATGCACGAT TATTCGGTAA AC---AGTCG GTGT-ACTCA
45-P. expansum          CCGATCAGAT GATGCACGAT TATTCGGTAA AC---AGTCG GTGT-ACTCA
55-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---AATTC C-AC-ACATA
5- P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---AATTN CCAC-ACATA
8- P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---AATTC C-AC-ACATA
11-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---AATTC C-AC-ACATA
12-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
13-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
15-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
16-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
19-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
24-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
26-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
30-P. glabrum           --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---GATTC C-AC-ACATA
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33-P. glabrum          --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
41-P. glabrum          --CATTAGAG AAGCCTTTAT ACTTCTAGCT TC---AATTC C-AC-ACATA
53-P. glabrum          --CATTAGAG AAGCCTTTAT ACTTCTAACT TC---AATTC C-AC-ACATA
57-P. italicum         CCGATCAGAT TATGCACAAA TATTCGGTTC CA---AGTCG CTGT-ACTCA
38-P. paneum           CCGATCAGAT TGTGCACGAT TAATCAATGT CC---AGTTG TTGT-ACTCA
22-P. paneum           CCGATCAGAT TGTGCACGAT TAATCAATGT CC---AGTTG TTGT-ACTCA
23-P. paneum           CCGATCAGAT TGTGCACGAT TAATCAATGT CC---AGTTG TTGT-ACTCA
36-P. piscarium        CTGATTAGCG CCCACGTTGA TATTGAGGTA TTGATAAGAC ACGCAACTTA
39-P. polonicum        CTGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTGA TTGG-ACTCA
7- P. polonicum        CTGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTGA TTGG-ACTCA
25-P. polonicum        CTGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTGA TTGG-ACTCA
32-P. polonicum        CTGATCAGAT GATGCACGAT TATTCGGTTT CC---AGTGG TTGG-ACTCA
46-P. solitum          CCGATCAGAT GATGCACGAT TATTCGGTTT CC---GGTCG TTGG-ACTCA
10-P. solitum          CCGATCAGAT GATGCACGAT TATTCGGTTT CC---GGTCG TTGG-ACTCA
21-P. solitum          CCGATTAGAT GATGCACGAT TATTCGGTTT CC---CGTCG TTGA-ACTCA
43-P. steckii          GTTAGTTGGA TAAT-ATTTC AATTGCATTG A----GGTCA GCATCACTTA
42-P. sumatrense       GCCATTAGAA TCTCAAGACT AAGTGTATTG ATGG-GATTG TTGTCGCTTA

                       ....|....| ....|....| ....|....| ....|....| ....|....|
                          255        265        275        285        295
47-P. bialowiezense    CATGGTTGAA GTAGACGTTC ATACGCTCCA GCTGGAGGTC GGAGGTACCG
1- P. bialowiezense    CATGGTTGAA GTAGACGTTC ATACGCTCCA GCTGGAGGTC GGAGGTACCG
3- P. bialowiezense    CATGGTTGAA GTAGACGTTC ATACGCTCCA GCTGGAGGTC GGAGGTACCG
28-P. bialowiezense    CATGGTTGAA GTAGACGTTC ATACGCTCCA GCTGGAGGTC GGAGGTACCG
54-P. brevicompactum   CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTGCCA
52-P. brevicompactum   CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTGCCA
56-P. corylophilum     CCTCGTTGAA GTAGACGTTC ATGCGCTCGC GCTGGAGATC GGAAACACCA
40-P. chrysogenum      CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
2- P. chrysogenum      CATGGTTGAA GTAGACGTTC ATGCGTTCGA GCTGGAGGTC GGAGGTACCA
9- P. chrysogenum      CATGGTTGAA ATAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
29-P. chrysogenum      CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
31-P. chrysogenum      CATGGTTGAA ATAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
35-P. chrysogenum      CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
51-P. citreonigrum     CCTCGTTGAA GTAGACGTTC ATGCGCTCGC GCTGGAGGTC GGAGACACCA
49-P. citreonigrum     CCTCGTTGAA GTAGACGTTC ATGCGCTCGC GCTGGAGGTC GGAGACACCA
50-P. citrinum         CGTGGGTGAA GTAGACGTTC ATGCGCTCCA GCTGGAGATC GGAGGTTCCG
27-P. citrinum         CGTGGGTGAA GTAGACGTTC ATGCGCTCCA GCTGGAGATC GGAGGTTCCG
44-P. citrinum         CGTGGGTGAA GTAGACGTTC ATGCGCTCCA GCTGGAGATC GGAGGTTCCG
34-P. commune          CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
20-P. commune          CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
18-P. crustosum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
4- P. crustosum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
14-P. crustosum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
17-P. crustosum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
6- P. echinulatum      CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
48-P. expansum         CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
37-P. expansum         CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
45-P. expansum         CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
55-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
5- P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
8- P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
11-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
12-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
13-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
15-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
16-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
19-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
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24-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
26-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
30-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
33-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
41-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
53-P. glabrum          CCTCGTTGAA GTAGACGTTC ATGCGCTCCA ACTGGAGGTC GGAAGCCTCG
57-P. italicum         CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
38-P. paneum           CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC AGAGGTTCCA
22-P. paneum           CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC AGAGGTTCCA
23-P. paneum           CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC AGAGGTTCCA
36-P. piscarium        CGTGGGTGAA GTAGACATTC AAGCGCTCGA GCTGTTGATC GGAGGTACCA
39-P. polonicum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
7- P. polonicum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
25-P. polonicum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
32-P. polonicum        CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
46-P. solitum          CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
10-P. solitum          CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
21-P. solitum          CATGGTTGAA GTAGACGTTC ATACGCTCGA GCTGGAGGTC GGAGGTACCA
43-P. steckii          CGTGGGTGAA GTAGACGTTC ATGCGCTCCA GCTGGAGGTC GGAGGTTCCG
42-P. sumatrense       CATGGGTGAA GTAAACGTTC ATGCGCTCCA GCTGGAGGTC AGAGGTACCG

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                          305        315        325        335        345
47-P. bialowiezense    TTGTACCTAA C---AATATA TCAGAA-CC- -AATCCACAT AGGATCC-CA
1- P. bialowiezense    TTGTACCTAA C---AATATA TCAGAA-CC- -AATCCACAT AGGATCC-CA
3- P. bialowiezense    TTGTACCTAA C---AATATA TCAGAA-CC- -AATCCACAT AGGATCC-CA
28-P. bialowiezense    TTGTACCTAA C---AATATA TCAGAA-CC- -AATCCACAT AGGATCC-CA
54-P. brevicompactum   TTGTACCTAA C---AAGATC TCAGAC-CC- -AATCCACGC GTAATTC-GA
52-P. brevicompactum   TTGTACCTAA C---AAGATC TCAGAC-CC- -CATCCACGC ATAATTC-GA
56-P. corylophilum     GCGTACCTAT ATC-AAAACA TCAGAC--CG CTATTTCCTG TCAGGTCGGA
40-P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA GAA-TCCCTA
2- P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA AAA-TCCCCA
9- P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA GAA-TCCCCA
29-P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA GAA-TCCCTA
31-P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA GAA-TCCCCA
35-P. chrysogenum      TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCCACCA GAA-TCCCCA
51-P. citreonigrum     GCGTACCTAT ACC-AAAACA TCAGAC--CG CTAGTTCGTG TTGACGGGTG
49-P. citreonigrum     GCGTACCTAT ACC-AAAACA TCAGAC--CG CTAGTTCGTG TTGACGGGTG
50-P. citrinum         TTGTAGCTGC CCA-AAAATA TCAGAC--CG CCATTCTCGA AAAAACGTAA
27-P. citrinum         TTGTAGCTGC CCA-AAAATA TCAGAC--CG CCATTCTCGA AAAAACGTAA
44-P. citrinum         TTGTAGCTGC CCA-AAAATA TCAGAC--CG CCATTCTCGA AAAAACGTAA
34-P. commune          TTGTACCTAG G---AAGATA TCAGAT-GTA TGATCTACCG GAACCCCCCA
20-P. commune          TTGTACCTAG G---AAGATA TCAGAT-GTA TGATCTACCG GAACCCCCCA
18-P. crustosum        TTGTATCTAG G---AATATA TCAGAT-GTG TAATCCACCA GAACCCCCTG
4- P. crustosum        TTGTACCTAG G---AAGATA TCAGAT-GTG TAATCCACCG GAAACCCCTA
14-P. crustosum        TTGTACCTAG G---AAGATA TCAGAT-GTG TGATCCACCG GAAACCCCCA
17-P. crustosum        TTGTACCTAG G---AAGATA TCAGAT-GTG TAATCCACCG GAAACCCCTA
6- P. echinulatum      TTGTACCTAG G---AAGATG TCAGAT-GTG TGATCCACCA GAAACCCCCA
48-P. expansum         TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACTA GAAACCC--A
37-P. expansum         TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACTA GAAACCC--A
45-P. expansum         TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACTA GAAACCC--A
55-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
5- P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
8- P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
11-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
12-P. glabrum          TTGACGCTAA ----AAATTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
13-P. glabrum          TTGACGCTAA ----AAATTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
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15-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCTACCT CGCAATCTCA
16-P. glabrum          TTGACGCTAA ----AAATTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
19-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCTACCT CGCAATCTCA
24-P. glabrum          TTGACGCTAA ----AAATTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
26-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCTACCT CGCAATCTCA
30-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
33-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCTACCT CGCAATCTCA
41-P. glabrum          TTGACGCTAA ----AAATTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
53-P. glabrum          TTGACGCTAA ----AATTTA TCAGACCGCC ATTTCCACCT CGCAATCTCA
57-P. italicum         TTGTACCTAG C---AAGATA TCAGTT-GTG TGATCAACCG GAAGCCC--A
38-P. paneum           TTGTACCTAG C---AAAATA TCAGAC-GTG TGATCCACCG GAAACCC-CA
22-P. paneum           TTGTACCTAG C---AAAATA TCAGAC-GTG TGATCCACCG GAAACCC-CA
23-P. paneum           TTGTACCTAG C---AAAATA TCAGAC-GTG TGATCCACCG GAAACCC-CA
36-P. piscarium        TTGTAGCTAG CCAAAAAATA TCAGACCGCC ATTCCGCGTC CGATGATATA
39-P. polonicum        TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACCG GAAACCCACA
7- P. polonicum        TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACCG GAAACCCACA
25-P. polonicum        TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACCG GAAACCCACA
32-P. polonicum        TTGTACCTAG C---AAGATA TCAGAC-GTG TGATCTACCG GAAACCCACA
46-P. solitum          TTGTACCTAG G---AAGATG TCAGAT-GTA TGATCCACCG GAAACCCCCA
10-P. solitum          TTGTACCTAG G---AAGATG TCAGAT-GTG TGATCCACCG GAAACCCCCA
21-P. solitum          TTGTATCTAG G---AATATA TCAGAT-GTG TAATCCACCA GAACCCCCTG
43-P. steckii          TTGTAGCTGC CCA-AAAATA TCAGAC--CG CCATTCTCCA AAAAACATAA
42-P. sumatrense       TTGTAGCTGC CCA-AAAATA TCAGAC--CG CCATTCTC-G AAAATCAAAA

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                          355        365        375        385        395
47-P. bialowiezense    -----GTACG CTC-----CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
1- P. bialowiezense    -----GTACG CTC-----CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
3- P. bialowiezense    -----GTACG CTC-----CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
28-P. bialowiezense    -----GTACG CTC-----CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
54-P. brevicompactum   -----ACACA GTCGTC--CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
52-P. brevicompactum   -----ACACA GTCGTC--CA CTTACTGTCC ATCGCCATCG AGACCGTGCT
56-P. corylophilum     -----TTTTG GGCG-----C CTTACTGGCC ATCGCCGTCA AGGCCGTGCT
40-P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
2- P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
9- P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
29-P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
31-P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
35-P. chrysogenum      -----TCACT GTTA----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
51-P. citreonigrum     -----TTATG GGCG-----A CTTACTGGCC ATCGCCGTCA AGGCCATGCT
49-P. citreonigrum     -----TTATG GGCG-----A CTTACTGGCC ATCGCCGTCA AGGCCATGCT
50-P. citrinum         ACACTTCTTT GTCGAAAGAA CTTACTGTCC ATCGCCATCA AGGCCGTGCT
27-P. citrinum         ACACTTCTTT GTCGAAAGAA CTTACTGTCC ATCGCCATCA AGGCCGTGCT
44-P. citrinum         ACACTTCTTT GTCGAAAGAA CTTACTGTCC ATCGCCATCA AGGCCGTGCT
34-P. commune          -----TCACA GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
20-P. commune          -----TCACA GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
18-P. crustosum        -----TTACT GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
4- P. crustosum        -----TCACT GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
14-P. crustosum        -----TCACA GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
17-P. crustosum        -----TCACT GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
6- P. echinulatum      -----TCACA GTTAG---AA CTTACTGTCC ATCACCATCG AGACCGTGCT
48-P. expansum         -----TCACC GTTG----AA CTTACTGTCC ATCACCATCG AGACCGTGCT
37-P. expansum         -----TCACC GTTG----AA CTTACTGTCC ATCACCATCG AGACCGTGCT
45-P. expansum         -----TCACC NTTG----AA CTTACTGTCC AT-------- ----------
55-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
5- P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
8- P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
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11-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
12-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
13-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
15-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
16-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
19-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
24-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
26-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
30-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
33-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
41-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
53-P. glabrum          -----TCGAT GTTGA---AA CTCACTGTCC ATCACCATCA AGTCCGTGCT
57-P. italicum         -----CCACT GCTG----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
38-P. paneum           -----TCATC GTTG----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
22-P. paneum           -----TCATC GTTG----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
23-P. paneum           -----TCATC GTTG----AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
36-P. piscarium        GTCGAGGAAT ATCGGACAAA CTTACTGGCC ATCGCCGTCA AGGCCGTGCT
39-P. polonicum        -----TCACC ATTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
7- P. polonicum        -----TCACC ATTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
25-P. polonicum        -----TCACC ATTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
32-P. polonicum        -----TCACC ATTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
46-P. solitum          -----TCACA GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
10-P. solitum          -----TCACA GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
21-P. solitum          -----TTACT GTTAA---AA CTTACTGTCC ATCGCCATCG AGACCGTGCT
43-P. steckii          ACACTTCTTT GTCGAAAGAA CTTACTGGCC ATCGCCATCA AGACCGTGCT
42-P. sumatrense       ------CCTG GTCGAAATCA CTCACTGTCC ATCGCCATCA AGGCCGTGCT

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                          405        415        425        435        445
47-P. bialowiezense    CGCCGGAGAT AGTTTGCCTT T-ATGTCAGT TAGCA--AGA TG--TCAATT
1- P. bialowiezense    CGCCGGAGAT AGTTTGCCTT T-ATGTCAGT TAGCA--AGA TG--TCAATT
3- P. bialowiezense    CGCCGGAGAT AGTTTGCCTT T-ATGTCAGT TAGCA--AGA TG--TCAATT
28-P. bialowiezense    CGCCGGAGAT AGTTTGCCTT T-ATGTCAGT TAGCA--AGA TG--TCAATT
54-P. brevicompactum   CGCCGGAGAT AGTTTGCCTT T-GAGTCAAT TAGCA--AAA TG--TCAATT
52-P. brevicompactum   CGCCGGAGAT AGTTTGCCTT T-AAGCCAGT TAGCA--AAT TG--TCAATT
56-P. corylophilum     CGCCAGCAAT GGTTTGCCTG G-AATTAAGT CAGTA--AAT CG-TTTCTCG
40-P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
2- P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
9- P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
29-P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
31-P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
35-P. chrysogenum      CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGCA--ACT TG--TCAATT
51-P. citreonigrum     CACCAGCAAT GGTTTGCCTG G-AATTCAGT CAGTA--TAA TG-TCTCTCG
49-P. citreonigrum     CACCAGCAAT GGTTTGCCTG G-AATTCAGT CAGTA--TAA TG-TCTCTCG
50-P. citrinum         CGCCAGCAAT GGTTTGCCTA TAGAATTGGT CAGTA--TAT TG---CTCTT
27-P. citrinum         CGCCAGCAAT GGTTTGCCTA TAGAATTGGT CAGTA--TAT TG---CTCTT
44-P. citrinum         CGCCAGCAAT GGTTTGCCTA TAGAATTGGT CAGTA--TAT TG---CTCTT
34-P. commune          CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGTA--ACC TG--TCAATT
20-P. commune          CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGTA--ACC TG--TCAATT
18-P. crustosum        CGCCAGAGAT GGTTTGCCTG T-AATCCAGT CAGGA--ACC CG--TCAATT
4- P. crustosum        CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGGA--ACC TG--TCAATT
14-P. crustosum        CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGTA--ACC TG--TCAATT
17-P. crustosum        CGCCAGAGAT GGTTTGCCTG T-AATCCAGT TAGGA--ACC TG--TCAATT
6- P. echinulatum      CGCCAGAGAT GGTTTGCCTG T-AAACCAGT TAGTA--ACC TG--TCAATT
48-P. expansum         CGCCAGAGAT GGTTTGCCTG C-AATCCAGT TAGTA--AAT TG--TCAATT
37-P. expansum         CGCCAGAGAT GGTTTGCCTG C-AATCCAGT TAGTA--AAT TG--TCAATT
45-P. expansum         ---------- ---------- ---------- ---------- ----------
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55-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
5- P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
8- P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
11-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
12-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT TAGTG--ATT CGGTCCAACA
13-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT TAGTG--ATT CGGTCCAACA
15-P. glabrum          CGCCAGCAAT GGTTTGCCTA C-AAATCAGT CAGTG--ATT CGGTCCAATA
16-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT TAGTG--ATT CGGTCCAACA
19-P. glabrum          CGCCAGCAAT GGTTTGCCTA C-AAATCAGT CAGTG--ATT CGGTCCAATA
24-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT TAGTG--ATT CGGTCCAACA
26-P. glabrum          CGCCAGCAAT GGTTTGCCTA C-AAATCAGT CAGTG--ATT CGGTCCAATA
30-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
33-P. glabrum          CGCCAGCAAT GGTTTGCCTA C-AAATCAGT CAGTG--ATT CGGTCCAATA
41-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT TAGTG--ATT CGGTCCAACA
53-P. glabrum          CGCCAGCAAT GGTTTGCCTA G-AAATCAGT CAGTG--ATT CGGTCCAATA
57-P. italicum         CGCCAGAGAT GGTTTGCCTG C-AATCCAAT TAGTA--AAT TG--TCAATT
38-P. paneum           CGCCAGAAAT GGTTTGCCTG G-AATTCGGT TAGTA--ATT TG--TCAATT
22-P. paneum           CGCCAGAAAT GGTTTGCCTG G-AATTCGGT TAGTA--ATT TG--TCAATT
23-P. paneum           CGCCAGAAAT GGTTTGCCTG G-AATTCGGT TAGTA--ATT TG--TCAATT
36-P. piscarium        CACCAGCAAT GGTCTGCCTG TAGGTTGAGT CAGTACAATC TGCTCATTAA
39-P. polonicum        CGCCAGAGAT GGTTTGCCTG T-AATCGAGT TAGTA--ACC TG--TCAATT
7- P. polonicum        CGCCAGAGAT GGTTTGCCTG T-AATCGAGT TAGTA--ACC TG--TCAATT
25-P. polonicum        CGCCAGAGAT GGTTTGCCTG T-AATCGAGT TAGTA--ACC TG--TCAATT
32-P. polonicum        CGCCAGAGAT GGTTTGCCTG T-AATCGAGT TAGTA--ACC TG--TCAATT
46-P. solitum          CGCCAGAGAT GGTTTGCCTG T-AAACCAGT TAGTA--ACC TG--TCAATT
10-P. solitum          CGCCAGAGAT GGTTTGCCTG T-AAACCAGT TAGTA--ACC TG--TCAATT
21-P. solitum          CGCCAGAGAT GGTTTGCCTG T-AATCCAGT CAGGA--ACC CG--TCAATT
43-P. steckii          CGCCAGCAAT GGTTTGCCTA TAGAATTGGT CAGTA--ATT TG---CCCTC
42-P. sumatrense       CGCCAGCAAT GGTTTGCCTG TTGAATTGAT TAGTT--TAT TG---CTTCA

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                          455        465        475        485        495
47-P. bialowiezense    GATACCCANC CATT------ ----GCGGGA GGAAAAAAA- --GANCGTGA
1- P. bialowiezense    GATACCCAGC CATT------ ----GCGGGA GGAAAAAAA- --GACCGTGA
3- P. bialowiezense    GATACCCAGC CATT------ ----GCGGGA GGAAAAAAAA --GACCGTGA
28-P. bialowiezense    GATACCCAGC CATT------ ----GCGGGA GGAAAAAAA- --GACCGTGA
54-P. brevicompactum   GATACCCAGC CATA------ ----GCGGGA GAAGAAAAA- ---GACATGG
52-P. brevicompactum   GATACCCAGC CATA------ ----GCGGGA AAAGAAAAA- ---GACATGG
56-P. corylophilum     TAGTCTCAAT ---------- -----TGAT- ---GAAAAT- ----GTCTGG
40-P. chrysogenum      GATACCCAAC ---------- ----GCGAA- --AAAAAAA- --------GC
2- P. chrysogenum      GAAACCCAAC ---------- ----GCGAA- --AAAAAAA- --------GC
9- P. chrysogenum      GATACCCAAC ---------- ----GCGAA- --AAAAAAA- ---------A
29-P. chrysogenum      GATACCCAAC ---------- ----GCGAA- --AAAAAAA- --------GC
31-P. chrysogenum      GATACCCAAC ---------- ----GCGAA- --AAAAAAA- -------AGC
35-P. chrysogenum      GATACCCAAC ---------- ----GCGAA- --AAAAAAG- ---------C
51-P. citreonigrum     GAGCCTCAAT ---------- -----TGAT- ---GGATCTT ----GTCCAG
49-P. citreonigrum     GAGCCTCAAT ---------- -----TGAT- ---GGATCTT ----GTCCAG
50-P. citrinum         TGTATCCAAC AAT------- -----TGATC TTTCAGGAT- ---------T
27-P. citrinum         TGTATCCAAC AAT------- -----TGATC TTTCAGGAT- ---------T
44-P. citrinum         TGTATCCAAC AAT------- -----TGATC TTTCAGGAT- ---------T
34-P. commune          GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- -------AA-
20-P. commune          GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- -------AA-
18-P. crustosum        GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- -------GNT
4- P. crustosum        GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- -------GCT
14-P. crustosum        GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- ----------
17-P. crustosum        GATACCCAAC ---------- ----GCGAA- -AAAAAAAA- -------GCT
6- P. echinulatum      GATACCCAAC ---------- ----GCGAG- -AAAAAAAA- -------AG-
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48-P. expansum          GATACCCANC ---------- ----GCGAA- ---AAAAAA- -------GCT
37-P. expansum          GATACCCAAC ---------- ----GCGAA- ---AAAAAA- -------GCT
45-P. expansum          ---------- ---------- ---------- ---------- ----------
55-P. glabrum           GGCTATCNAT ---------- -----TGGC- ---GATCGT- -------GGT
5- P. glabrum           GGCTATCAAT ---------- -----TGGC- ---GATCGT- -------GGT
8- P. glabrum           GGCTATCAAT ---------- -----TGGC- ---GATCGT- -------GGT
11-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---TATCGT- -------GGA
12-P. glabrum           GGCTATCAAT ---------- -----TGGC- ---GATCGT- -------GGT
13-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---TATCGT- -------GGA
15-P. glabrum           GGTTATCAAT ---------- -----TGGT- ---GATCGT- -------GGT
16-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---TATCGT- -------GGA
19-P. glabrum           GGTTATCAAT ---------- -----TGGT- ---GATCGT- -------GGT
24-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---TATCGT- -------GGA
26-P. glabrum           GGTTATCAAT ---------- -----TGGT- ---GATCGT- -------GGT
30-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---GATCGT- -------GGT
33-P. glabrum           GGTTATCAAT ---------- -----TGGT- ---GATCGT- -------GGT
41-P. glabrum           GGCTATCAAT ---------- -----TGGT- ---TATCGT- -------GGA
53-P. glabrum           GGCTATCAAT ---------- -----TGGC- ---GATCGT- -------GGT
57-P. italicum          GATACCCAAC ---------- ----GCGAA- ---AAATAA- -------GCT
38-P. paneum            GATACCCAAC ---------- ----GCGAA- ---AAAAAG- -------GCT
22-P. paneum            GATACCCAAC ---------- ----GCGAA- ---AAAAAG- -------GCT
23-P. paneum            GATACCCAAC ---------- ----GCGAA- ---AAAAAG- -------GCT
36-P. piscarium         GACACGCAAT TGAGGGGCTG CTATGTTGTG CTGGGCAGTT TTTTGGTGGG
39-P. polonicum         GATACCCAAC ---------- ----GCGAAG AAGAAAAAA- -------AAG
7- P. polonicum         GATACCCAAC ---------- ----GCGAAG AAGAAAAAA- -------AAG
25-P. polonicum         GATACCCAAC ---------- ----GCGAAG AAGAAAAAA- -------AAG
32-P. polonicum         GATNCCCAAC ---------- ----GCGCAT AANAAAAAA- -------AAG
46-P. solitum           GATACCCAAC ---------- ----GCGAG- -TAAAAAAA- -------GCT
10-P. solitum           GATACCCAAC ---------- ----GCGAG- -TAAAAAAA- -------GCT
21-P. solitum           GATACCCAAC ---------- ----GCGAA- -AAAAAAA-- -------GCT
43-P. steckii           TGTATCCAAC AAT------- -----TGATC TTTCAGGAT- ---------T
42-P. sumatrense        TGCATTCAAC AAT------- -----TGATT GTTTTGGGA- ---------T

                        ....|....| ....|....| ....|....| ....|....| .
                           505        515        525        535
47-P. bialowiezense     TGCGGTACAT -ANCAGAAAG CA-------- ---------- -
1- P. bialowiezense     TGCGGTACAT -ACCAGAAAG CAGCACC--- ---------- -
3- P. bialowiezense     TGCGGTACAT -ACCANAAAG CAGCACC--- ---------- -
28-P. bialowiezense     TGCGGTACAT -ACCAGAAAG CAGCACCGAT NTGGTTACCA -
54-P. brevicompactum    TGCGATACAT -ACCAGAAAG CAGNACCGAT TTGGTTACCA -
52-P. brevicompactum    TGCGATACAT -ACCAGAAAG CAGCACCGT- ---------- -
56-P. corylophilum      TTCTGCACGT -ACCAGAAAG CAGCACCGA- ---------- -
40-P. chrysogenum       TCCGAGACTT -ACCAGAAAG CAGCACC--- ---------- -
2- P. chrysogenum       TCCGAGACTT -ACCAGAAAG CAGCACC--- ---------- -
9- P. chrysogenum       G--------- ---------- ---------- ---------- -
29-P. chrysogenum       TCCGAGACTT -ACCAGAAAG CAGCACC--- ---------- -
31-P. chrysogenum       TCCGAGACTT -ACCANAAAG CAGC------ ---------- -
35-P. chrysogenum       TCCGAGACTT -ACCAGAAAG CAGC------ ---------- -
51-P. citreonigrum      GTCTGCACGT -ACCAGAAAG CAGCACC--- ---------- -
49-P. citreonigrum      GTCTGCACGT -ACCAGAAAG CAGCACCGAT TTGGTTACCA -
50-P. citrinum          TGCAGCACGT -ACCAGAAAG CAGCACC--- ---------- -
27-P. citrinum          TGCAGCACGT -ACCAGAAAG CAGCACC--- ---------- -
44-P. citrinum          TGCAGCACGT -ACCAGAAAG CAGCACCGAT TTNGGTTACC A
34-P. commune           ---------- ---------- ---------- ---------- -
20-P. commune           ---------- ---------- ---------- ---------- -
18-P. crustosum         --CGGCACTT -ACCANAAAG CAGCACCGAT TTGGTT---- -
4- P. crustosum         TCCGGCACTT TACCANAAAG CAGCNCCNAT TTGNTTNC-- -
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14-P. crustosum      ---------- ---------- ---------- ---------- -
17-P. crustosum      --CGGCACTT -ACCANAAAG CAGCACCGAT TTGGTT---- -
6- P. echinulatum    ---------- ---------- ---------- ---------- -
48-P. expansum       --CGGCACTT -ACCAGAAAG CANCACCGAT TTGG-TTACC A
37-P. expansum       --CGGCACTT -ACCAGAAAG CAGCACCGAT TTGGGTTACC A
45-P. expansum       ---------- ---------- ---------- ---------- -
55-P. glabrum        TGCNACACNT -ACCAGAAAG CAGCACCGAT TTGGT----- -
5- P. glabrum        TGCAACACGT -ACCAGAAAG CAGCAC---- ---------- -
8- P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCGAT TTNGGTTACC -
11-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACC--- ---------- -
12-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCAC---- ---------- -
13-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCAC---- ---------- -
15-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCA----- ---------- -
16-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCGAT T--------- -
19-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCGAT TTGGTTACCA -
24-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACC--- ---------- -
26-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCG-- ---------- -
30-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCG-- ---------- -
33-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCG-- ---------- -
41-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACC--- ---------- -
53-P. glabrum        TGCAACACGT -ACCAGAAAG CAGCACCGAT TTTGGTTACC C
57-P. italicum       --CGGGACTT -ACCAGAAAG CAGCACCG-- ---------- -
38-P. paneum         --CGGCACTT -ACCAGAAAG CAGCACCGAT TTGGTTACCA -
22-P. paneum         --CGGCACTT -ACCAGAAAG CAGCAC---- ---------- -
23-P. paneum         --CGGCACTT -ACCAGAAAG CAGCACCGAT TTGGTTACCC -
36-P. piscarium      CGTGGCACGT -ACCAGAAAG CAGC------ ---------- -
39-P. polonicum      CTCGGCACTT -ACCAGAAAG CAGCACCG-- ---------- -
7- P. polonicum      CTCGGCACTT -ACCAGAAAG CAGCAC---- ---------- -
25-P. polonicum      CTCGGCACTT -ACCAGAAAG CAGCACCGAT TTTGGTTAC- -
32-P. polonicum      CTCGGNACTT -ACCA----- ---------- ---------- -
46-P. solitum        --CGGCACTT -ACCAGAAAG CAGCACCG-- ---------- -
10-P. solitum        --CGGCACTT -ACCAGAAAG CAGCACCGAT TTGGTTACCA -
21-P. solitum        --CGGCACTT -ACCANAAAG CAGCACCGAT TTGGTTACAC -
43-P. steckii        TGCAGCACGT -ACCAGAAAG CAGCACCGAT TNGGTTACCA -
42-P. sumatrense     TGCAGCACGT -ACCAGAAAG CAGC------ ---------- -
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