

**THE GENUS *LEPTOGRAPHIUM*:
A CRITICAL TAXONOMIC ANALYSIS**

by

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Dedicated to my husband and best friend

Rudi

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PREFACE

The genus *Leptographium* Lagerb. & Melin dates back to early in the twentieth Century, when it was established for species of fungi that cause blue-stain in the sapwood of pine and spruce. The taxonomic history of *Leptographium* has been characterized by considerable confusion, with several species having been transferred between various genera. *Leptographium* includes many species that are morphologically similar. This fact makes the accurate identification of species extremely difficult, even for experienced mycologists. This is an especially relevant impediment where correct identification of potential pathogens is important. As a result of the morphological similarity of species of *Leptographium*, misidentification of important taxa can have serious economic implications. The lack of a comprehensive key to species of *Leptographium* has exacerbated this problem. Correct identification of species in this genus could, in the past, be achieved only through comparison with herbarium type specimens and original descriptions. This was not always possible due to a lack of well-preserved herbarium specimens or cultures. These problems have emphasized the need for a key to both the asexual forms, as well as the sexual states of *Leptographium* spp.

Some species of *Leptographium* are economically important and are well known as agents of blue stain of timber. Other species are regarded as saprophytes. The staining of sapwood, although it does not affect the integrity of the wood, can have major economic implications for commercial wood production. The stain fungi rarely kill trees, but they reduce the value of the timber, which makes this an undesirable trait.

Apart from the species of *Leptographium* that cause blue stain, there are a few species that are pathogens. The best known of these are the three varieties of *Leptographium wagneri*, that are responsible for a serious root disease of conifers in the Western United States. Another suspected pathogen is *L. procerum*, which has

been associated with pine root disease and decline chiefly in Eastern North America. The role of this fungus in the disease complex, is however, still a matter of debate.

This thesis is divided into two parts. The first part is a monograph that deals with all species described in *Leptographium*. The aim of this part of the thesis is to aid in the identification of *Leptographium* spp. The second part of the thesis represents a variety of studies that, over the course of four years, has made it possible to develop the monograph.

Part I

The monograph aims to provide a comprehensive review of all the known species of *Leptographium*, including complete descriptions and illustrations of each species. *Leptographium* anamorphs, known only by their teleomorph states, are provided with names. We believe that this is necessary, since most species have no known teleomorph. Where teleomorph structures are known, they are in most cases represented by poor herbarium material (Shaw & Hubert, 1952; Samuels & Seifert, 1995). This makes comparisons difficult, or even impossible. Most species of *Leptographium* are typically found only in the anamorph state and this is also the state which is typically used in identification.

Part II

The second part of the thesis consists of eight chapters. Chapter one attempts to determine the phylogenetic relationships amongst species of *Leptographium*, as well as to verify placement of the genus at an ordinal level. With the exception of three species, all *Leptographium* spp. are compared based on ribosomal DNA sequence analysis. Considerable difficulty has been experienced in amplifying DNA of this region, and thus this study is restricted to the ITS 2 (internal transcribed spacer region) and part of the large subunit of the rDNA. A sub-set of *Leptographium* spp.

are also used in a comparison with other Ascomycete genera to confirm their ordinal placement. Morphological data generated in the monograph were coded and analyzed in the same manner as the molecular data. Taxonomically important characters were preferentially weighted and analyzed. The resulting dendrograms were then compared with those generated from the molecular analysis, in an attempt to assess the phylogenetic value of morphological characteristics.

The second chapter deals with *L. abietinum* that is found in association with various bark beetles on conifers in North America and is characterized by distinctly curved conidia. A second species, *L. engelmannii*, is also characterized by curved conidia and is indistinguishable from *L. abietinum*. These species are compared morphologically and *L. engelmannii* is formally synonymised with *L. abietinum*. Kendrick (1962) examined several isolates identified as *L. abietinum*. Two of these isolates were, however, isolated from unusual hosts, unlike other isolates of this species. These isolates originated from Borneo and a similar isolate from Vietnam was made available to us. These isolates are carefully compared with authenticated isolates of *L. abietinum*. Consequently, a new species, *L. hughesii* sp. nov., is described in this chapter.

Ophiostoma europhioides is a well-known associate of the bark beetle, *Ips typographus*, and is known to cause blue-stain of conifers. *Ophiostoma piceaperdum* is also an associate of bark beetles in North America and is known to cause blue-stain. These species are indistinguishable based on morphology and previous studies have suggested that they may be the same. In chapter three, these species are compared morphologically. *Ophiostoma europhioides* is synonymised with *O. piceaperdum*. The taxonomic placement of *Ceratocystis pseudoeurophioides* is also considered in this chapter.

Chapter four provides a critical re-evaluation of *Phialocephala phycomyces*. This species is characterized by reddish-brown conidiophores, which is unlike other species of *Phialocephala*. In addition, the phialides are not as deep-seated as those of the type of *Phialocephala*, i.e. *P. dimorphospora*. The taxonomic placement of *P.*

phycomyces is re-evaluated based on light microscopy, scanning and transmission electron microscopy and molecular comparisons. As a result, a new genus, *Kendrickiella* gen. nov. is proposed for *P. phycomyces*.

In chapter five, a new species of *Leptographium*, *L. eucalyptophilum* sp. nov., is described. This species is unique in that it has been isolated from the Congo in Central Africa. In addition, this species is found on *Eucalyptus*, which is an unusual habitat for members of this genus. This is the first report of a *Leptographium* sp. from *Eucalyptus*. Some comments are also made regarding the pathogenicity and ecology of this species.

Leptographium procerum has been associated with a root decline of white pine (*Pinus strobus*) particularly in the Eastern USA. However, controversy still surrounds the pathogenicity of this fungus, with several studies indicating that it is, at best, a weak pathogen. During the past two decades, a large collection of isolates, tentatively identified as *L. procerum*, has been assembled. Examination of these isolates has revealed four different morphological groups, including *L. procerum* s.str. In chapter six, three new species of *Leptographium*, similar to *L. procerum* are described.

Leptographium spp. have mostly been described from conifers in the Northern hemisphere. In recent years, several new species have been described from other hosts and geographical areas. Chapter seven includes the description of three new *Leptographium* spp. The first of these originates from Indonesia. The other two species are described from red spruce (*Picea rubra*) and balsam fir (*Abies balsamea*) growing on high elevation sites in Eastern North America.

Despite the fact that large parts of Russia and especially Siberia are occupied by coniferous forests, little is known regarding the occurrence of blue-stain fungi in these areas. A survey of conifer diseases in Russia has led to the consistent isolation of an unknown *Leptographium* sp. Chapter eight provides a description of this species.

The overall aim of this thesis is to aid in the identification of known *Leptographium* spp., as well as previously undescribed species. It is also my hope that this dissertation will renew scientific interest in *Leptographium* and that it will lead to the description of many more species, especially from regions where this group of fungi has not been previously considered.

Summary

Leptographium have been known since the early part of the 20th Century and include of many species causing blue stain of timber. Among these species are several species known or believed to be involved in causing diseases of trees. *Leptographium* spp. occur mainly on conifers and many species are recognized as anamorphs of *Ophiostoma*. Similar to *Ophiostoma*, *Leptographium* spp. are closely associated with insects. Their morphology thus reflects this association, and they thus have upright conidiophores with slimy masses that are produced in beetle galleries.

Leptographium spp. are morphologically very similar to each other and this makes their accurate identification difficult. The first part of this thesis, presents dichotomous, as well as synoptic keys for the identification of these species. These keys are supported by comprehensive descriptions accompanied by both photographs and line drawings.

The second part of this thesis deals with several key taxonomic questions pertaining to *Leptographium*. Chapter one represents a phylogenetic study of the majority of species in *Leptographium*. Morphological characters were coded and analyzed. The results of the molecular and the morphological analyses are compared to determine whether any morphological characters might be used to infer phylogeny. The results indicate that morphology does not infer phylogenetic relatedness.

Chapter two represents a comparison between *Leptographium abietinum* and *L. engelmannii*. These species are morphologically similar, and various authors have suggested that they are synonyms. Based on morphology, *L. engelmannii* was synonymised with *L. abietinum*. Furthermore, examination of various atypical isolates led to the description of the new species, *L. hughesii*.

In chapter three, *Ophiostoma europhioides*, *O. piceaperdum* and *Ceratocystis pseudoeurophioides* are compared. These species have *Leptographium* anamorphs and are morphologically identical. Both *O. europhioides* and *C. pseudoeurophioides* are synonymised with *O. piceaperdum*, and a name is provided for the anamorph of *O. piceaperdum*.

Chapter four represents a re-evaluation of *Phialocephala phycomyces*. The inconspicuous collarettes, characteristic of this fungus, are unlike the deep-seated collarettes of the type species of *Phialocephala* (*P. dimorphospora*). Scanning and transmission electron microscopy revealed that conidiogenesis in *P. phycomyces* is phialidic, placing this species among other *Phialocephala* spp. However, *P. phycomyces* is able to tolerate high concentrations of cycloheximide, characteristic of *Leptographium* spp. DNA analysis indicates that this species does not belong in either *Phialocephala* or *Leptographium*. A new genus *Kendrickiella* is described to accommodate this species.

In chapter five, a new species of *Leptographium*, *L. eucalyptophilum*, is described. This species is unique in that it occurs on *Eucalyptus*, which is an unusual host for this species. In addition, this species is one of several described from tropical regions and it is apparently adapted to this habitat.

Chapter six represents a critical re-evaluation of isolates identified as *L. procerum*. Morphological comparison of these isolates revealed that *L. procerum sensu lato*, represents more than one taxon. From this study, three new species of *Leptographium* were described. These are *L. alethinum*, *L. pityophilum* and *L. euphyes*. These species can easily be distinguished from *L. procerum s. str.* and their incorrect identification is probably as a result of their shared habitat.

In chapter seven, I describe an additional three species of *Leptographium*. Like most other *Leptographium* spp., these were isolated from conifers. The first of these, *L. pineti*, originates from Indonesia. The other two species is found in

high elevation sites in Eastern North America. These are *L. abicolens* and *L. peucophilum*. These species are unique in that they are associated with the conifer swift moth, which is an unusual insect associate of *Leptographium*.

Chapter eighth presents a description of a new species of *Leptographium* from Russia. This species, *L. sibiricum*, is associated with staining and mortality in siberian fir (*Abies sibirica*). The role of the fungus in the disease complex is still unknown, and awaits further study.

This thesis represents a comprehensive review of all known, as well as newly described species. It should greatly facilitate plant pathologists and mycologists in the identification of *Leptographium* spp. This should lead to extensive pathogenicity tests, to determine the economic impact of species in this genus as blue-stain fungi and pathogens. It is my sincere wish that it will renew interest in this group of fungi, and will lead to the description of many more species in this genus.

Opsomming

Leptographium is bekend sedert vroeg in the 20ste eeu en bevat verskeie spesie wat verkleuring van hout veroorsaak. Onder hierdie is ook verskeie spesies wat siektes veroorsaak of moontlik veroorsaak. *Leptographium* spp. kom meestal voor of konifere en verskeie spesies is bekend as anamorf stadia van *Ophiostoma*. Soortgelyk aan *Ophiostoma*, is *Leptographium* spp. nou geassosieer met insekte. Dit word gereflekteer in die morfologie van hierdie fungi, met hul regop konidiofore met spoordruppels wat in the baskewer tonnels gevorm word.

Leptographium spp. is morfologies soortgelyk, wat die identifikasie van spesies moeilik maak. Die eerste deel van die tesis verskaf digotome, sowel as sinoptiese sleutes vir die identifikasie van spesies. Die sleutels word verder ondersteun deur foto's en lynsketse.

Die tweede deel van die tesis behandel verskeie sleutel vrae oor die taksonomie van *Leptographium*. Hoofstuk een verteenwoordig 'n filogenetiese studie van die meerderheid spesies in *Leptographium*. Morfologiese karakters is gekodeer en geanaliseer. Die resultate van die molekulêre en die morfologiese analises is vergelyk om te bepaal of sekere morfologiese karakters filogenie bepaal. Die resultate van hierdie studie bevestig dat geen enkele morfologiese karakter filogenie bepaal nie.

Hoofstuk twee verteenwoordig 'n vergelyking tussen *Leptographium abietinum* en *L. engelmannii*. Hierdie spesies is morfologies soortgelyk en verskeie werkers het voorgestel dat hulle sinonieme is. *Leptographium engelmannii* is sinoniem gemaak met *L. abietinum*, gebaseer op morfologie. Verdere bestudering van atipiese isolate het gelei tot die beskrywing van 'n nuwe spesie, *L. hughesii*.