Chapter 1

Literature Review

Terminalia spp in Africa with special reference to its health status

ABSTRACT

The genus *Terminalia* is the second largest genus in the Combretaceae. The family is distributed throughout the tropical and sub-tropical regions of the world and approximately fifty species of *Terminalia* are naturally distributed throughout western, eastern and southern Africa. *Terminalia* spp. range from small shrubs or trees to large deciduous forest trees. Some species, such as *T. ivorensis* and *T. superba* develop as elements of the canopy or sub-canopy layer in evergreen, semi-deciduous to deciduous, primary and secondary forests, whereas species such as *T. sericea*, thrive well in open woodlands and mixed deciduous forests. *Terminalia* spp. can be propagated naturally by seeds or through vegetative methods with wildings, seedlings, stump plants or striplings. *Terminalia* spp. provide economical, medical, spiritual and social benefits. Limited information on the pests and diseases affecting *Terminalia* spp. exists. Many insect species are associated with *Terminalia* spp. but no widespread pest problems have been recorded. Nevertheless, some locally common species are potentially dangerous, mostly affecting the early stages of trees. Very few pathogens have been reported from *Terminalia* spp. The majority of reports include limited detail, often representing no more than a brief mention. Often the causal agents were identified based only on morphology and were not classified to species level. Scanty information regarding the pathogens associated with introduced and native *Terminalia* is a limitation that might be detrimental for the survival and the successful exploitation of these trees.

1. INTRODUCTION

Terminalia (Combretaceae, Myrtales) is a pantropical genus accommodating about 200 species (McGaw *et al.* 2001). About fifty of these are native to Africa and distributed throughout the sub-saharan region (Lebrun and Stork 1991). Based on both their functional uses and distribution in Africa, the most important are *Terminalia ivorensis* A. Chev. and *T. superba* Engl. and Diels. in West and Central Africa and *T. prunioides* M.A. Lawson and *T. sericea* Burch : DC in Southern Africa (Irvine 1961; Lamb and Ntima 1971; Coastes-Palgrave 1977; Groulez and Wood 1985; Schmidt *et al.* 2002; Lawes *et al.* 2004).

Terminalia trees are planted in several countries in the tropics as a source of high quality solid timber for fine carpentry, joinery, building, flooring and plywood manufacture (Schmidt *et al*. 2002; Smith *et al.* 2004). *Terminalia ivorensis* and *T. superba*, especially, form an important component of the forestry industries in many countries (Anonymous 1997). *Terminalia* spp. are also commonly planted in mixed crop systems to establish a "taungya" agri-sylvicultural system in which they provide shade and play a major role in increasing soil fertility (Nichols *et al*. 2001; Norgrove and Hauser 2002a). Furthermore, members of the genus *Terminalia* are among some of the plants most widely used for medicinal purposes in Africa (Masoko *et al*. 2005; Kamtchouing *et al.* 2006).

Despite the importance of *Terminalia* spp., very little research has been done regarding the fungal diseases affecting these trees. Evidence of die-back, leaf spot and canker has been reported from *Terminalia* spp. (Lamb and Ntima 1971; Ofosu Siedu and Cannon 1976; Hodges and Fereira 1981). Gryzenhout *et al.* (2005), recently reported a serious disease problem that emerged on non-native *T. ivorensis* in Ecuador, while in South Africa, two *Ceratocystis* spp. have been reported from *T. sericea* (Roux *et al*. 2004; Kamgan *et al*. 2008).

The last or the 20th Century was marked by an increasing requirement for timber, fuel and medicine from trees. This has resulted in unsustainable logging of native trees in Africa. To supplement this requirement, plantations of non-native trees, including *Eucalyptus* spp., *Pinus* spp., *Acacia* spp. and *Cupressus* spp. are been established in many parts of the tropics and the southern hemisphere (Turnbull 1991; Wingfield *et al*. 2002; Anonymous 2007). In Africa, as in most other countries, these non-native trees are established in close proximity to native trees. This close association may in the long run, expose trees to new pests and diseases. One

might thus see the movement of native pests and pathogens onto introduced tree species. This is of great concern since this could provide pathogens with an elevated opportunity to spread to the country of origin of its new host, through reciprocal international trade of wood and wood products, causing large-scale mortality of trees in their native ecosystems (Wingfield 2003; Slippers *et al.* 2005; Wingfield *et al*. 2008). On the other hand, the non-native tree might be the source of non-native pathogens and pests, which may spread to the native trees in its new country, resulting in disease epidemics. An increasing number of examples for both case scenarios exist. For example, it has been shown that *Chr. austroafricana* Gryzenh. & M. J. Wingf., the cause of canker and death of plantation grown *Eucalyptus* spp. in South Africa, also occurs on native Myrtales in Africa (Heath *et al*. 2006; Nakabonge *et al*. 2006). This pathogen is thought to have originated from Africa (Heath *et al.* 2006). On the other hand, in California, native Monterey pine (*Pinus radiata* D. Don) are seriously affected by the pitch canker pathogen, *Fusarium circinatum* Niremberg & O'Donnell, following its introduction with Mexican pines (Gordon *et al.* 2001). In this respect, knowledge of indigenous tree diseases would be useful to establish firm risk assessment programs.

In Africa, some species of *Terminalia* generally occur as elements of the canopy or subcanopy layer in evergreen, semi-deciduous to deciduous primary and secondary forests. Other species thrive in open woodlands and littoral areas. Within this natural habitat of *Terminalia* spp., several non-native tree species are frequently encountered. There is, therefore, a good chance of introduced pathogens spreading onto native *Terminalia* trees, or innocuous fungi on *Terminalia* spp., moving onto the non-native trees. The objective of this review is to present a summary of knowledge pertaining to *Terminalia* spp. in Africa. A specific focus is given to their origin and distribution, botanic description, ecology**,** propagation, management, functional uses and international trade. Also, the limited knowledge regarding pests and diseases on these trees is reviewed, providing a background for the contents of the dissertation that follows this review and that focuses on fungi associated with native and introduced *Terminalia* spp. on the African continent.

2. THE GENUS *TERMINALIA*

2.1. Origin and distribution

The family Combretaceae is comprised of 20 genera and about 475 species (Thiombiano *et al.* 2006). Of these about 200 belong to the genus *Terminalia,* making it the second largest genus of the family after *Combretum* (McGaw *et al.* 2001). The family is distributed throughout the tropical and sub-tropical regions of the world (Lamb and Ntima 1971). Approximately 54 species of *Terminalia* are naturally distributed throughout western, eastern and southern Africa (Lebrun and Stork 1991; Smith *et al.* 2004). *Terminalia ivorensis* and *T. superba* are the most important species found in West and Central Africa (Norgrove and Hauser 2002b), but are also established in plantations within and outside their natural range, e.g. in South and Central America, east Africa, Hawaii, Fiji and the Solomon Islands (Jones 1969). Within the Malaysian region, further trials exist in Sabah, Kalimatan and the Philippines (Lamprecht 1989). On the other hand, *T. prunioides*, *T. brachystemma* Welw. ex Hiern, *T. sericea*, *T. gazensis* Bak., *T. mollis* Laws. and *T. sambesiaca* Engl. & Diels. are the most common species in eastern and southern Africa (Coates-Palgrave 1988; Masoko *et al.* 2005).

2.2. Botanical description and ecology

The genus *Terminalia* derives its Latin name (terminalis = end) from the position of the leaves, which are crowded at the ends of the shoots (Lamb and Ntima 1971; Rogers and Verotta 1996). The taxonomy of the genus has not been without problems, with the assignment of sub-genera, especially presenting differing views (Stace 1965). However, the different species have now been grouped into three sections according to characteristics of the fruits. These sections include the Section *Abbreviatae* Excell with shorter fruits, Section *Psidioides* Excell with longer fruits and the Section *Platycarpae* Engels & Diels emend. Excell, with fruits that are broader in the centre (Carr 1988).

Terminalia spp. range from small and medium sized shrubs or trees to large deciduous forest trees, ranging in height from 1.5 to 75 m tall (Lebrun and Stork 1991; Schmidt *et al.* 2002). Some species, such as *T. ivorensis, T. superba* and *T. trichipoda* Diels have cylindrical boles that are very straight and long (Figure 1a) with small to large, flat buttresses (six meters above the soil surface) and are sometimes branchless for up to 30 m and 2-5 m in girth (Lemmens *et*

al. 1995). Mature trees are extensively flat topped, with a wide horizontal canopy of evenly distributed foliage arising from the apex of the straight bole (Dupuy and Mille 1993). It is these characteristics, and their relatively fast growth, that make *T. ivorensis* and *T. superba* popular timber species. Other species, such as *T. sericea* and *T. prunioides* are common as small shrubs (Figure 1b) to bushy trees that may be single or multi-stemmed with a girth of up to 1.5 m (Coates-Palgrave 1977).

The bark of *Terminalia* trees is smooth and light grey to dark brown when young and on branchlets. The inner bark and contact zone with the cambium is frequently yellow. In mature trees, the bark surface cracks and flakes off in long thin strips or small patches, often becoming blackish and developing deep longitudinal fissures as the trees grow (Keay 1989; Lemmens *et al.* 1995).

The root systems of *Terminalia* trees are frequently fairly shallow. As the trees age, the tap roots disappear. In the tall species such as *T. ivorensis*, buttresses from which descending roots arise at some distance from the trunk, develop to support the trees (Keay 1989; Lamprecht 1989).

The leaves are frequently simple and obovate, clustered spirally at the ends of the dwarfed lateral branchlets, or crowded near the ends of the branches. Some species, like *T. brassii* Exell., have prominent glands at the leaf bases (Lamb and Ntima 1971). In mature trees the crown is usually flat or very slightly domed, giving *Terminalia* trees a distinctive shape.

Terminalia trees are bi-sexual or hermaphroditic with male and female flowers carried on the same plants. These flowers are apetalous, small, and cream to pale, bright yellow or greenish-white, in spicate inflorescences. The stalked male flowers tend to be grouped towards the apex and the bisexual flowers towards the base of the inflorescences (Coates-Palgrave 1977). *Terminalia* spp. have an effective system of self-incompatibility. Although male and female flowers are in the same plant, self-pollination cannot produce viable zygotes (Newbegin *et al.* 1994). The flowers are pollinated by various insects (*Coleoptera, Diptera, Hemiptera, Hymenoptera* and *Lepidoptera*) (Uzoechina 1978). The flowering-to-fruiting period may last about 4 months, depending on the species and the locality where it is grown (Coates- Palgrave 1977; Keay 1989).

Terminalia fruits, in combination with leaf characteristics, are of great diagnostic value and absolutely essential to distinguish between species (Coode 1969; Excell and Stace 1972). The fruits are hard, flattened, two-winged and to some extent inconsistent in size, especially the length and width of the wings. The general seed shape is consistent however, specific characters such as fruit colour when ripe (yellow, red, purple green, brown or pink depending on the species) and fruit morphology (elongate, broad, narrow, ovoid, oblong or elliptic) (Figure 2) vary and are helpful in species differentiation (Dale and Greenway 1961; Coates Palgrave 1977; Dale and Keay 1989). The fruits of some *Terminalia* trees, together with the bark, are important sources of tannin (Lemmens and Wulijarni-Soetjipto 1991; Ellery and Ellery 1997; Mabberley 1997). *Terminalia* fruits differ from those of closely related *Combretum* spp. in having a sclerenchymatous endocarp (Lamb and Ntima 1971). Most of the African *Terminalia* spp. produce fruits from January to September, with the exception of species such as *T. ivorensis* and *T. glaucescens* Planch.: Benth. that produce fruits from July onwards (Coates Palgrave 1977; Keay 1989).

The African species of *Terminalia* generally occur in various environments. Some species, such as *T. ivorensis* and *T. superba* develop as elements of the canopy or sub-canopy layer in evergreen, semi-deciduous to deciduous, primary and secondary forests (Keay 1989). Species such as *T. sericea* on the other hand, thrive well in open woodlands to wooded savannahs and mixed deciduous forests (Dale and Greenway 1961; Lebrun and Stork 1991; Carr 1994). *Terminalia* trees can tolerate light to moderate shade, when young (Jones 1969). Thereafter, they should receive full overhead light for optimal growth (Veenendaal *et al.* 1996). Few individuals of this genus are able to grow at high altitude; most species perform well at altitudes less than 2000 m a.s.l. The climates in which *Terminalia* trees grow varies from areas with year round rain for species occurring in the forest areas (> 2000 mm per annum) to seasonal with moderate rainfall (< 1200 mm per annum) for those occurring in savannah zones (White 1983).

2.3. Propagation and management

Terminalia spp. can be propagated naturally by seeds or through vegetative methods with wildings, seedlings, stump plants or striplings (Lemmens *et al.* 1995). However, obtaining plants is difficult and with most *Terminalia* spp., propagation is not easy (Carr 1994).

2.3.1. Seed propagation

Freshly fallen seed should be collected from the ground, as seed still on the tree may not be fully mature. Sometimes the seeds are collected from the trees by cutting off the branches, because fallen seed are often parasitized by insects, leading to low viability. As far as possible, seeds should be collected from healthy mother trees with a vigorous stem and crown (Browse 1979; Hartman and Kester 1983). The number of fruits and seeds (per kilogram) vary greatly between *Terminalia* spp. For example, *T. ivorensis* can produce 5500 - 7300 seeds per kg (Lamb and Ntima 1971), *T. superba* can produce 8000 - 10000 seeds/kg (Groulez and Wood 1985) and *T. prunioides* 8200 seeds/kg (Palmer and Pitman 1972). Seeds are extracted from the fruits by placing them in a heap, spraying them with water and then covering them with grass or leaves. After a day or so the fruit wing is stripped off and the seeds are extracted manually (Carr 1994).

In general, seeds vary in the length of time that they remain viable. The viability of seed varies between species. The viability of most species diminishes rapidly, with the exception of *T. superba*, of which the seeds can be stored in sealed containers at 2-4 °C for one year, adding to its suitability for commercial exploitation (Groulez and Wood 1985).

Seeds of *Terminalia* spp. must undergo a period of dormancy before germination occurs. There are two types of dormancy in plants, namely physical and physiological dormancy (Weber and Stoney 1986). Several methods of pre-treatment can be used to overcome the two types of dormancy in seeds of *Terminalia* spp. As seeds of *Terminalia* spp. are covered by a hard protective coat, the physical dormancy ends when the seed coat is opened through different processes such as mechanical abrasion, nicking or soaking in water (Browse 1979; Weber and Stoney 1986). Most often, for some species of *Terminalia*, seeds are pre-treated by soaking in water for 12-48 hours, by manual scarification, or, in the case of *T. ivorensis*, by alternate soaking and drying for one week (Lamb and Ntima 1971). For *T. ivorensis* the germination rate is 10-50 %, but up to 93 % under experimental temperature fluctuations, while for *T. superba* it is 60-80 % (Lemmens *et al.* 1995), 15-35 % for *T. prunioides* and 1-2 % for *T. sericea* (Carr 1994). However, as the viability in seeds is not assured, covering seed or fruit in the seedbed is important for increasing the germination percentage. Light shade is generally applied during germination, but it should be removed after one to two months. The seedbed should be watered frequently to provide adequate moisture during germination

(Browse 1979). The sowing medium should be sand with low levels of "fines" or a light soil, half mixed with sifted compost to promote good drainage and avoid water logging during heavy rain periods (Carr 1994).

The physiological dormancy of *Terminalia* seed ends within two weeks after sowing and is followed by epigeous germination which lasts two to five weeks. Pricking out should be done early enough to avoid disturbing the rapidly developing taproot (Lemmens *et al.* 1995). For *T. superba,* pricking out is recommended six weeks after sowing when two leaves have developed (Groulez and Wood 1985), whereas for *T. ivorensis*, it should be as soon as the two cotyledons unfold and the seedlings are 20-30 cm tall (Lamb and Ntima 1971).

2.3.2. Vegetative propagation

As an alternative to overcome seed viability problems, long growth periods and inconsistency of seed germination (Carr 1994), vegetative propagation through cuttings and stump plants can be used (Lemmens *et al*. 1995). However, vegetative propagation has in Africa been used early for species such as *T. ivorensis* and *T. superba* in Africa (Fisher 1976).

The planting stock for vegetative propagation should be taken from young, vigorous shoots or suckers from healthy, mature trees, during the dormant season, to encourage fast-growing stems (Browse 1979). Short cuttings are taken from different parts of the stem (e.g. from the rejuvenated stump plants and from branches). These stems (25-35 cm long) are cut just above the proposed top bud and horizontally at the base, if possible dipping in a rooting hormone before planting. Depending on the species, the cuttings can be placed either in pots filled with water, or directly into a trench which is kept moist (Hartman and Kester 1983). After a period of time shoots will produce roots, and they can then be transplanted to a permanent site. Stumps should have a diameter of at least 1.3 cm. Cuttings of *T. superba* and *T. ivorensis* produce roots within two weeks, with a rooting percentage of 11-100 % for *T. superba* according to the degree of rejuvenation (Lemmens *et al.* 1995).

2.3.3. Tending of trees

Planting of *Terminalia* trees should be done early in the rainy season to ensure an adequate water supply, and thus to avoid loss through drought (Lamb and Ntima 1971). Where necessary, hand watering should be used to supplement insufficient rainfall. Weeding is

necessary during the first 3-4 years to give the seedlings adequate light and air circulation, and to prevent competition for nutrients from weeds (Carr 1994).

Most *Terminalia* spp. have a good to extremely good self-pruning capacity (Lamb and Ntima 1971; Swaine and Hall 1983), adding to their popularity as plantation trees. The tall *T. superba* tree is occasionally branchless up to 90 % of the total tree height (Lemmens *et al.* 1995). Pruning by hand is, therefore, not required in commercial plantations. However, because of the wide spreading branches, the tree needs considerable space between stems (Groulez and Wood 1985).

Terminalia spp. are planted in Africa on a wide variety of soil types ranging from alluvial, sandy, salty and coral soils to heavy cracking clays (Coates-Palgrave 1988). The coppicing ability is good for a number of *Terminalia* spp. planted in Africa and India. *Terminalia chebula* Retz. and *T. albida* Scott-Elliot are known to withstand fire well, but *T. superba* and *T. ivorensis* are very vulnerable in this respect. The average rotation age for *T. superba* and *T. ivorensis* in Africa is 40 years, with trees reaching heights of 50-60 m and diameters of five meters in this time (Keay 1989; Lemmens *et al.* 1995).

2.4. Functional uses of *Terminalia* trees

Terminalia spp. provide economical, medicinal, spiritual and social benefits. The wood of *Terminalia* spp. is highly appreciated as constructional timber. It is currently used for light construction, door and window frames, coffin boards, mouldings, beams, rafters, joists, flooring, furniture, carts, tool handles, spindles, shuttles, picker sticks, walking sticks, bowls, boat building, masts, mine props, foundation piles, veneer and plywood (Irvine 1961; Lemmens *et al.* 1995; Schmidt *et al.* 2002; Smith *et al.* 2004). The fruits and bark of *T. sericea* and *T. catappa* L. are important sources of tannin, as well as gum and resins for glazing pottery (Irvine 1961; Lemmens and Wulijarni-Soetjipto 1991; Ellery and Ellery 1997). Dyes of various colours (black, red, orange, yellow, brown) are extracted from the leaves, fruits, bark and roots of species such as *T. mollis* Lawson, *T. ivorensis, T. laxiflora* Engl. & Diels., *T. catappa* L. and *T. superba* and used for decorating the walls of houses and buildings with murals, for dyeing clothes, mattings, rattan, spoons and walking sticks (Dalziel 1937; Errington and Chisumpa 1987). The seed of some species is edible and considered one of the best flavoured tropical nuts. Furthermore, consumable oil can be extracted from the

seed of *T. catappa* and used as a substitute for groundnut (*Arachis hypogea* L.), cotton seed (*Gossypium* spp.) and silk cotton seed (*Ceiba* spp.) oils (Irvine 1961).

In Africa, forests are sometimes use for ritual and spiritual purposes. Certain trees can serve to link the living with their ancestors, as this is often symbolized in the relationship between the sky and the earth. In Southern Africa, Tswana people believe that good crops are ensured at harvest and planting times by thrusting a stick of *T. sericea* into the floor of a shrine in homage to ancestral spirits, and that cutting down an entire tree will result in hail-storms (Coates-Palgrave 1977).

The importance of traditional medicines, derived from plants, is of great importance in most parts of both Africa and Asia (Lawes *et al.* 2004; Steenkamp *et al.* 2004; Moshi and Mbwambo 2005). Many *Terminalia* spp. have been identified as sources of medicines, for use in pharmaceuticals and cosmetic production (Dalziel 1937; Irvine 1961). Extracts of the flowers, fruits, bark, leaves, stems and roots from species such as *T. glaucescens*, *T. macroptera* Gill.& Perr., *T. laxiflora*, *T. superba*, *T. prunioides*, *T. brachystemma*, *T. sericea, T. gazensis, T. catappa, T. mollis* and *T. sambesiaca* are used in traditional medicine to treat diseases such as malaria, eczema, candidosis, asthenia, gonorrhoea, diabetics, dermatitis, scurfy affection, leprosy and tuberculosis (Batawila *et al.* 2005; Masoko *et al.* 2005; Fyhrquist *et al.* 2006; Kamtchouing *et al.* 2006). The bark of *T. macroptera* is burned and used by Sudanese women as a perfume (Irvine 1961).

Terminalia spp. have a number of agricultural uses. They are important sources of fodder for animals. *T. sericea* is one of the palatable woody plants found in South African savannahs (Owen-Smith and Cooper 1987; Lawes *et al.* 2004). Its leaves are browsed by domestic cattle, goats and game during the hot, dry season (Ellery and Ellery 1997; Mabberley 1997; Katjiua and Ward 2006).

Terminalia spp. are commonly established in the "taungya system" (Lamb and Ntima 1971), where they are combined in the early stages with the growing of agricultural crops like banana and cocoa (Nichols *et al.* 2001; Norgrove and Hauser 2002b). *T. ivorensis* and *T. superba* have been successfully used in this respect throughout West Africa, particularly in Cameroon (Diaw *et al.* 1999; Norgrove and Hauser 2002a, 2002b). In this part of the continent, cacao farms are managed in harmony with several tree species by adjustment of forest cover to

provide shade for cacao trees. One of the advantages of the system is that it reduces the initial costs of establishment by alleviating the cost of forest clearing and weeding in the first two or three years of the agricultural crop. In addition, many of these shade trees provide additional services and incomes through the provision of medicinal products and timber (Sonwa *et al.* 2000). The cultivation of the crops greatly stimulates the early growth of the trees, providing fertilisation through the leaf litter (Singh *et al.* 2002; Goma-Tchimbakala and Bernhard-Reversat 2006). This form of tree management through enhancement of carbon sequestration confers environmental sustainability to the cocoa plantations and thus justifies its use as a model of promotion within the framework of developing agroforestry systems with perennial crops in Africa.

In West and Central Africa, native *Terminalia* spp. are of great economic importance as they are among the most important export timbers. In 1995, Cameroon exported 62000 m^3 of *Terminalia* logs, together with 15000 m^3 of sawn wood, 10000 m^3 of veneer and an unrecorded amount of plywood. This amount of exported *Terminalia* ranked it third in national export for the country (Anonymous 1999). During the same period, the Democratic Republic of Congo exported 3000 m^3 of *Terminalia* logs, 1000 m^3 of sawn wood and small quantities of veneer, while the other Congo Republic exported 10000 m^3 of logs (Anonymous 1997). Côte d'Ivoire exported 7000 m^3 of logs and a small amount of veneer, while Ghana exported 18000 m^3 of *Terminalia* logs, 3000 m^3 of sawn wood and 1000 m^3 of veneer during 1995 (Anonymous 1997). The wood of African *Terminalia* spp. is used particularly in Egypt, France, Belgium, Germany, Nederland, Switzerland, USA, Philippines, Brazil, Thailand, and Japan (Anonymous 1997).

2.5. Pests and diseases

Relatively few studies of the pests and diseases affecting *Terminalia* spp. have been made. There are many insects species associated with *Terminalia* spp. but, as yet, no widespread pest problems have emerged. Nevertheless, some locally common species are potentially dangerous, mostly affecting the early stages of the trees.

2.5.1. Insects

2.5.1.1. Fruit Borers

A 3-mm-long weevil (*Nanophyes* sp.), is considered the most serious pest of *T. ivorensis* and *T. superba* in Nigeria and Ghana. The weevil deposits its eggs in the ripening seed on the tree and can reduce germination by up to 40 % (Lamb and Ntima 1971). Attacked seed can be recognised by the presence of a dark brown spot, consisting of excrement, on the seed surface.

2.5.1.2. Stem Borers

Stem damage, caused by a thyridid moth, *Tridesmodes ramiculata* Warren, and ambrosia beetles (*Dolipygus* spp.) have been reported from higher altitude zones in Nigeria and Ghana (Lamb and Ntima 1971). The larvae of the moth occur as shoot borers on *Terminalia* seedlings in nurseries as well as in plantations (Lamb and Ntima 1971). The death of infested shoots results in the production of multiple stems. This moth is considered to be of potential importance (Groulez and Wood 1985). The ambrosia beetle prefers to attack newly felled trees and those which are injured or sickly, leading to very serious economical damage as they reduce the tree quality and consequently reduce the financial returns (Robert 1987).

Zeuzera coffeae Nietner is a branch boring caterpillar, primarily infesting branches of *Terminalia* spp. in Africa and Asia (Lamb and Ntima 1971). However, it can also attack saplings where it may be found in the main stems (Bigger 1998). It has a wide host range in the South Pacific Region, including several important plantation species such as *Paraserianthes falcate* Becker, *Casuarina equisetifolia* L., *Eucalyptus deglupta* Blume, *Swietenia* macrophylla King, *Tectona* grandis L., *Terminalia* brassii Excell and *T. ivorensis* (Bigger 1998). Elsewhere, in Panama, multiple xylem borer attacks by a *Cossula* sp. was observed on *T. ivorensis*, affecting wood quality where the incidence is high (Kapp *et al.* 1997).

2.5.1.3. Defoliators

Many insects feed on the leaves of *Terminalia* spp. *Zononocerus variegatus* L., the variegated grasshopper, is widely distributed throughout tropical Africa and feeds on the

leaves of young trees in nurseries and plantations (Akabi and Ashiru 1991, Messi *et al.* 2006). A number of coleopterans (*Trochalus* sp., *Maladera* sp., *Pseudotrochalus* sp.) (Browne 1968) and lepidopterans (*Maurilia phaea* Hamps., *Negeta luminosa* Wkr., *Westermania cuprea* Hamps., *Tortrix dinota* Meyrick, *Trabala lambaurni* Beth-Baker) also feed on the leaves of *Terminalia* spp. in nurseries and plantations (Browne 1968). In Côte d'Ivoire and Nigeria, several species of caterpillars infest plantations of *T. ivorensis* and *T. superba*, but the most serious defoliator is *Epicerura pergrisea* Hampson, which can cause severe damage to its hosts (Akanbi and Ashiru 1991, Kanga and Fediere 1991). Sucking pests, such as *Cryptoflata* spp., as well as *Otionotus* sp. and *Tricoceps albescens* Funkh. have been reported to occur commonly in Ghana where their adults and colonies of nymphs affect the growth of *T. ivorensis* shoots, mainly in nurseries (Browne 1968). In Papua New Guinea, *Roeselia lignifera* Walker causes substantial harm to *T. brassii* plantations. The larva of this moth was associated with defoliation of young entire plantations in that country (Lemmens *et al.* 1995), while leaf cutting ants (*Atta* sp.) have damaged young plantations of *T. ivorensis* in Panama (Kapp *et al.* 1997).

2.5.1.4. Termites

When *Terminalia* trees are split, many insects, notably termites, usually infest them (Lamb and Ntima 1971). However, these insects occur only sporadically and are not a major threat.

2.5.2. Wildlife

In Eastern Nigeria, the foliage of *T. ivorensis* are prone to browsing by small antelopes (C*ephalopus maxwelli* Hamilton-Smith, Maxwell's duiker), which may give rise to injuries. Other serious damage is caused by elephants (Lamb and Ntima 1971). These wounds, from wildlife, could open trees for infection by pathogens, such as those residing in the genus *Ceratocystis*, as recently shown in South Africa for *T. sericea* (Kamgan *et al*. 2008).

2.5.3. Diseases

Terminalia spp. are potential hosts for many fungal pathogens in Africa and elsewhere. It is, however, evident that the incidence of pathogens on *Terminalia* spp. has not been studied thoroughly. Very few pathogens have been reported from *Terminalia* spp. The majority of

the reports that have been made include limited detail, often representing no more than a brief mention. Often the causal agents were identified based only on morphology, and not classified to the species level. This is problematic for the establishment of quarantine guidelines.

2.5.3.1. Root diseases

Howes (cit. Piening, 1962), reported *Armillaria mellea* (Vahl ex Fr.) Kummer, the honey fungus, associated with a *Terminalia* sp. in Ghana. However, no particulars were given. Certainly, modern taxonomic treatments of the genus *Armillaria* in Africa has shown that it represents numerous species (Coetzee *et al.* 2000; Mwenje *et al.* 2006) suggest that the fungus on *Terminalia* was identified only in the broad sense. More recently, it was observed that root rot caused by species of *Rosellinia* and *Phytophthora*, leads to die-back of *T. ivorensis* in Panama and Costa Rica (Kapp *et al.* 1997).

2.5.3.1. Stem diseases

In nurseries and forest plantations in Nigeria, Parker (1964) reported die-back, leaf spot and canker due to a *Sphaeronaema* sp. on *T. ivorensis*. The main symptoms were a cessation of growth, accompanied by die-back of the main shoots, but wilting and yellowing of the foliage occurred occasionally. Otherwise, observations of black stem cankers, frequently associated with reddening of leaves, causing mortality and stagnation in nursery plants were reported in this country (Lamb and Ntima 1971). An *Endothiella* sp. has also been found on cankers on *T. ivorensis* in Ghana (Ofosu Siedu and Cannon 1976), while pink disease, caused by *Erythricium salmonicolor* Berk, causes stem canker on tropical almond (*T. catappa*) in India (Thomson and Evans 2006). Recently, Gryzenhout *et al.* (2005) described of a new pathogen, *Rostraureum tropicale* Gryzenh. & M. J. Wingf. in association with dying *T. ivorensis* in Ecuador.

2.5.3.3. Leaf diseases

Some foliage diseases caused by unidentified species of *Cercospora*, *Ramularia*, *Irenina* and *Spaceloma* have been reported from *T. superba* in Africa (Groulez and Wood 1985). In Brazil, *Korinomyces terminaliae* Hodge & Ferreira causes leaf spots on seedlings and young

T. ivorensis plants (Hodges and Fereira 1981), and *Auerswaldiella parvispora* causes black blotches on leaves (Farr 1989).

2.5.3.4. Stain diseases

When freshly felled, logs are liable to attack by fungi whose effect is mainly aesthetic, but which is nevertheless serious in a wood renowned for its agreeable colour. In this respect, blue stain of logs caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl was described on *T. superba* by Fougerousse (1958). The fungus is able to completely spoil the lustrous creamy-white colour of the log even if only the sheen of the wood is altered.

3. CONCLUSIONS

Terminalia spp. are dominant trees in many African ecosystems and are planted extensively in timber plantations and in intercropping systems. Furthermore, selected species such as *T. sericea* that regenerate easily, are relatively fast growing and are favoured by local communities and grown in protected stands as part of a process to rehabilitate degraded woodlands. *Terminalia* spp. provides medicinal, spiritual and social benefits. However, it appears from the present review that despite the fact that species of *Terminalia* are not immune from disease infections, scanty information regarding the diseases associated with introduced and native *Terminalia* trees are available. This limitation might be detrimental for the survival and the successful exploitation of these trees, but also presents potential threats to surrounding vegetation and crops.

Fungal pathogens and pests present a serious threat to the future of plants and trees on the African continent. This is especially important because of the rate and ease of spread of fungal pathogens between continents that is increasingly more common. Our native African biodiversity is, therefore, under threat from numerous fungal pathogens, non-native to the continent. These pathogens may enter the continent on a number of different hosts, especially those related to our native trees.

The purpose of the studies presented in this dissertation will be to address the above mentioned issues by studying the fungal flora and possible diseases of *Terminalia* spp. in Africa. Surveys will be conducted in Southern and Western Africa in order to identify fungal

pathogens on these trees, as well as their means of infection and spread. Hopefully, results of this study will serve as valuable tools in forestry management in Africa.

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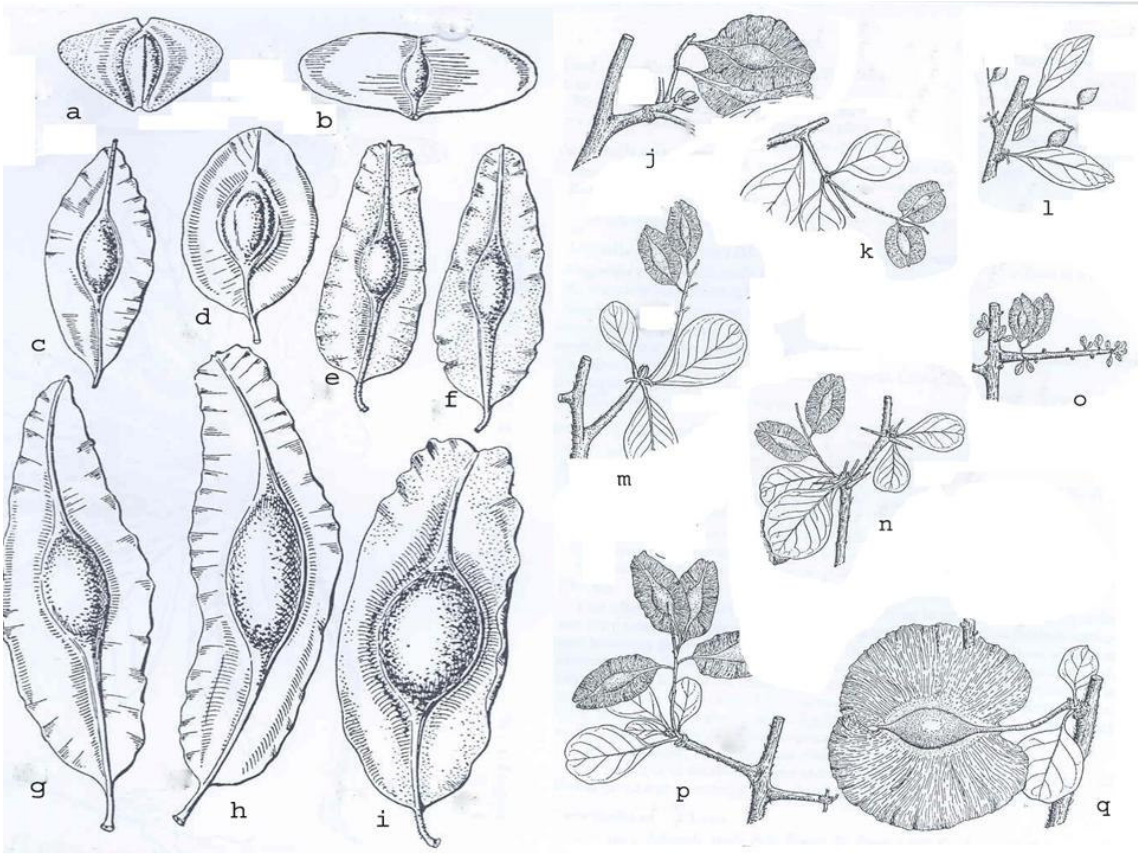
Figure 1: Growth forms of *T. ivorensis* (a) and *T. sericea* (b) in Africa.


a
b

Figure 2: Fruits of some African *Terminalia* spp. (a) *T. scutifera* Planch. ex Lawson, (b) *T. superba,* (c) *T. laxiflora*, (d) *T. brownii,* (e) *T. glaucescens,* (f) *T. avicennioides,* (g,h) *T. macroptera,* (i) *T. mollis,* (j) *T. kilimandscharica* Engl., (k,m) *T. brevipes* Pampan, (l) *T. fatraea* (Poir.) DC., (n) *T. spinosa* Engl., (o) *T. parvula* Engl. & Diels, (p) *T. prunioides,* (q) *T. orbicularis* Engl. & Diels (Dale and Greenway 1961; Keay 1969).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Chapter 2

Botryosphaeriaceae associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar

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ABSTRACT

Species in the Botryosphaeriaceae represent some of the most important fungal pathogens of woody plants. Although these fungi have been relatively well studied on economically important crops, hardly anything is known regarding their taxonomy or ecology on native or non-commercial tree species. The aim of this study was to compare the diversity and distribution of the Botryosphaeriaceae on *Terminalia catappa*, a tropical tree of Asian origin planted as an ornamental in Cameroon, Madagascar and South Africa. A total of 83 trees were sampled, yielding 79 Botryosphaeriaceae isolates. Isolates were initially grouped based on morphology of cultures and conidia. Representatives of the different morphological groups were then further characterized using sequence data for the ITS, *tef 1-α*, *rpb2*, BOTF15 and *β-tub* gene regions. Five species of the Botryosphaeriaceae were identified, including *Neofusicoccum parvum*, *N. batangarum sp. nov.*, *Lasiodiplodia pseudotheobromae*, *L. theobromae* and *L. mahajangana sp. nov*. *Lasiodiplodia pseudotheobromae* and *L. theobromae,* were the most commonly isolated species (62%), and were found at all the sites. *Neofusicoccum parvum* and *N. batangarum* were found in South Africa and Cameroon respectively, whereas *L. mahajangana* was found only in Madagascar. Greenhouse inoculation trials performed on young *T. catappa* trees showed variation among isolates tested, with *L. pseudotheobromae* being the most pathogenic. The Botryosphaeriaceae infecting *T. catappa* appear to be dominated by generalist species that also occur on various other hosts in tropical and sub-tropical climates.

1. INTRODUCTION

The Botryosphaeriaceae is a diverse group of fungi that accommodates numerous species spread over many anamorph genera, the best known of which are *Diplodia*, *Lasiodiplodia*, *Neofusicoccum*, *Pseudofusicoccum, Dothiorella* and *Sphaeropsis* (Crous *et al.* 2006). Members of the Botryosphaeriaceae have a worldwide distribution and occur on a large variety of plant hosts including monocotyledons, dicotyledons, gymnosperms and angiosperms, on which they are found as saprophytes, parasites and endophytes (Slippers and Wingfield 2007; von Arx 1987).

It has long been recognized that species of the Botryosphaeriaceae are important pathogens of several plants (Von Arx 1987). Infected plants can exhibit a multiplicity of symptoms such as die-back, canker, blight and rot on all above ground plant organs (Punithalingham 1980; Slippers *et al.* 2007). A particularly dangerous feature of these fungi is that they can live as endophytes in plant organs, in a latent phase, without producing clear symptoms, and diseases only emerge following the onset of unfavourable conditions to the tree (Smith *et al.* 1996). This implies that they can easily, and unobtrusively, be moved around the world with seeds, cuttings and even fruit.

Extensive studies have been conducted on diseases of economically important species of fruit (e.g. Lazzizera *et al.* 2008; Phillips 1998; Slippers *et al.* 2007; van Niekerk *et al.* 2004) and timber trees (e.g. Mohali *et al.* 2007; Sanchez *et al.* 2003) caused by fungi in the Botryosphaeriaceae. Much less is known about the Botryosphaeriaceae on plants with no large-scale international commercial value (Denman *et al.* 2003; Pavlic *et al.* 2008), such as *Terminalia catappa*, but which have social and environmental significance (Gure *et al.* 2005). Without knowledge of the Botryosphaeriaceae on hosts with limited or no commercial value, and hosts in their native environments, the impact and biology of the important pathogens in this group will never be fully understood.

Terminalia catappa, frequently referred to as “tropical almond”, belongs to the Combretaceae and originates from Southern India to coastal South-East Asia (Smith 1971). These trees are widely cultivated in tropical and subtropical coastal areas and utilised by local communities for a number of household uses. The multitude of non-wood products and services pertaining

to this tree species make it an important component, especially for coastal communities. The tree is planted for shade and ornamental purposes in urban environments, the timber is converted into decorative tools, furniture and many other applications, leaves and bark are commonly used in traditional medicine and its fruits contain edible kernels from which high energy oil is extracted and which can also be admixed into diesel fuel (Chen *et al.* 2000; Hayward 1990; Kinoshita *et al.* 2007).

The diversity and spatial distribution of the Botryosphaeriaceae, associated with a specific host, is important. Whether it accommodates similar or different fungal assemblages depending on the environment, is useful in understanding the ecology and host-pathogen relationships of these fungi. This knowledge in turn can be applied where recommendations for disease management strategies are required. Several studies have compared assemblages of fungal endophytes in different geographic regions (Fisher *et al.* 1994; Gallery *et al.* 2007; Gilbert *et al.* 2007; Taylor *et al.* 1999). However, such studies dealing with a specific endophytic group of fungi are limited. Similarly, very few studies have compared the assemblages of Botryosphaeriaceae from a specific host at a regional level (Taylor *et al.* 2005; Urbez-Torrez *et al.* 2006).

Among all the species of *Terminalia* present on the African continent, *T. catappa* is one of the few species planted widely in West, Central, East and Southern Africa. As part of a larger project in which we explore diseases of *Terminalia* spp. in Africa, the broad distribution of this species over the continent made it an ideal candidate to characterise endophytic species of the Botryosphaeriaceae under variable geographic and climatic conditions. The aims of this study were, therefore, to investigate the diversity of the Botryosphaeriaceae occurring on introduced *T. catappa* and to analyse the patterns of their distribution in three African countries. Pathogenicity trials were also undertaken to assess the ecological significance of the Botryosphaeriaceae collected from *T. catappa*.

2. MATERIALS AND METHODS

2.1. Isolates

Collections were made from *T. catappa* trees in Cameroon, Madagascar and South Africa. In Cameroon, samples were collected along the beach front of Kribi, a seaside town within the tropical forest and bordering the Atlantic Ocean (N2 58.064, E9 54.904, 7 m asl). The climate in this area is characterized by high humidity, precipitation up to 4000 mm per annum and relatively high temperatures, averaging 26 ˚C. In South Africa, sampling was done in Richardsbay (S28 46.886 S, E32 03.816, 0 m asl), a harbour city on the Indian Ocean where *T. catappa* trees are planted to provide shade in open spaces and in parking areas. Climatic conditions in this area are typically subtropical to tropical. The average temperature in summer is 28 °C and 22 °C in the winter. The humidity levels tend to be very high in summer and the annual rainfall is ~1200 mm. In Madagascar, samples were collected from the towns of Morondava (S20 17.923, E44 17.926, 3 m asl) and Mahajanga (S15 43.084, E46 19.073, 0 m asl), both located on the west coast of the country. In these areas, the climate is between semi-arid and tropical humid with mean annual temperatures of 23.5 °C and average rainfall between 400 and 1200 mm per annum.

Samples were collected from 83 *T. catappa* trees in all three countries in 2007. Forty trees were randomly sampled in Kribi, 15 in Richardsbay, 20 and eight in Morondava and Mahajanga, respectively. Except for the trees in Richardsbay, that were showing symptoms of die-back at the time of collection, those at all the other sites were healthy. One branch (~0.5 - 1 cm diameter) per tree was cut and all the samples placed in paper bags and taken to the laboratory where they were processed after one day.

From each branch, two segments (1 cm in length each) were cut and split vertically into four halves. Samples were surface sterilized by dipping the wood pieces in 96 % ethanol for 1 min, followed by 1 min in undiluted 3.5 % sodium hypochlorite and 1 min in 70 % ethanol, before rinsing in sterile distilled water and allowing them to dry under sterile conditions. The four disinfected branch pieces from each tree were plated on 2 % malt extract agar (MEA) (2 % malt extract, 1.5 % agar; Biolab, Midrand, Johannesburg, S.A.) supplemented with 1 mg ml^{-1} streptomycin (Sigma, St Louis, MO, USA) to suppress bacterial growth. The Petri dishes

were sealed with Parafilm and incubated at 20 °C under continuous near-Ultra Violet (UV) light. One week later, filamentous fungi growing out from the plant tissues and resembling the Botryosphaeriaceae were transferred to new Petri dishes containing fresh MEA.

All cultures are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Representatives of all species have also been deposited at the Centaalbureau voor Schimmelcultures (CBS, Utrecht, Netherlands). Herbarium materials for previously underscribed species have been deposited at the National Fungal Collection (PREM), Pretoria, South Africa.

2.2. Morphology and cultural characteristics

Fungal isolates were grown on plates containing 1.5 % water agar (Biolab, S.A.) overlaid with three double-sterilized pine needles and incubated at 25 °C under near UV-light for two to six weeks to induce the formation of fruiting bodies (pycnydia and/or pseudothecia). Morphological features of the resultant fruiting bodies were observed using a HRc Axiocam and accompanying Axiovision 3.1 camera (Carl Zeiss Ltd., München, Germany). For previously undescribed species, sections of fruiting bodies were made with a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa) and mounted on microscope slides in 85 % lactic acid. For the undescribed species 50 measurements of all relevant morphological characters were made for the isolate selected as the holotype and 30 measurements were made for the remaining isolates. These measurements are presented as the extremes in brackets and the range calculated as the mean of the overall measurements plus or minus the standard deviation.

The morphology of fungal colonies growing on 2 % MEA at 25 °C under near UV-light for two weeks was described and colony colours (upper and reverse surfaces) of the isolates were recorded using the colour notations of Rayner (1970). Growth rates of cultures on 2 % MEA in the dark was determined at 5 °C intervals from 10 to 35 °C. For growth rates, evaluations of five plates were used for each isolate at each temperature. Two measurements, perpendicular to each other, were made after three days for each plate resulting in 10 measurements for each isolate at each temperature. The experiment was repeated once.

2.3. DNA extraction

Mycelium was scraped from 10-day-old cultures representing different morphological groups, using a sterile scalpel and transferred to 1.5 µl Eppendorf tubes for freeze-drying. The freeze-dried mycelium was mechanically ground to a fine powder by shaking for 2 min at 30.0 $1s^{-1}$ frequency in a Retsch cell disrupter (Retsch Gmbh, Germany) using 2 mm-diameter metal beads. Total genomic DNA was extracted using the method described by Möller *et al.* (1992). The concentration of the resulting DNA was determined using a ND-1000 uv/Vis Spectrometer (NanoDrop Technologies, Wilmington, DE USA) version 3.1.0.

2.4. PCR amplification

The oligonucleotide primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5'TCCTCCGCTTATTGA TATGC 3') (White *et al.* 1990), EF1 (5' TGCGGTGGTATCGACAAGCG T 3') and EF2 (5' AGCATGTTGTC GCCGTTGAAG 3') (Jacobs *et al.* 2004), BT2A (5'GGTAACCAAATCGGTGCTGCTTTC3') and BT2B (5'ACCCTCAGTGTAGTGACCCTTGGC 3') (Glass and Donaldson 1995), RPB2bot6F (5'GGTAGCGACGT CACTCCC3') and RPB2bot7R (5'GGATGGATCTCGCAATGCG3') (Sakaladis 2004), BOT15 (5' CTGACTT GTGACGCCGGCTC3') and BOT16 (5' CAACCTGCTCAGCAAGCGAC3') (Slippers *et al.* 2004c) were respectively used to amplify and sequence the internal transcribed spacer regions (ITS), including the complete 5.8S gene, the translation elongation factor 1-α gene (*tef 1-α*), partial sequence of the β-tubulin gene (*β-tub*), part of the second largest subunit of RNA polymerase II gene (*rbp2*) and an unknown locus (BotF15) containing microsatellite repeats. A "hot start" polymerase chain reaction (PCR) protocol was used on an Icycler thermal cycler (BIO-RAD, Hercules, CA, USA). The 25 µl PCR reaction mixtures for the ITS, BT and RPB2 regions contained 0.5 µl of each primer (10 mM) (Integrated DNA Technology, Leuven, Belgium), 2.5 µl DNTPs (10 mM), 4 µl of a 10 mM $MgCl_2$ (Roche Diagnostics GmbH, Mannheim, Germany), 2.5 µl of 10 mM reaction buffer (25 mM) (Roche Diagnostics GmbH, Mannheim, Germany), 1 U of *Taq* polymerase (Roche Diagnostics GmbH, Mannheim, Germany), between 60-100 ng/µl of DNA and 13.5 µl of sterile distilled water (SABAX water, Adcock Ingram Ltd, Bryanston, S.A.). The amplification conditions were as follows: an initial denaturation step at 96 °C for 1 min, followed by 35 cycles of 30 seconds at 94 °C, annealing for 1min at 54 °C, extension for

90 seconds at 72 °C and a final elongation step of 10 min at 72 °C. To amplify the *tef 1-α* gene region, the 25 µl PCR reaction mixture contained 0.5 µl of each primer (10 mM), 2.5 µl DNTPs (10 mM), 2.5 µl of 10 mM reaction buffer with $MgCl_2$ (25 mM) (Roche Diagnostics GmbH), 1 U of *Taq* polymerase, between 2-10 ng/µl of DNA and 17 µl of sterile SABAX water. The amplification conditions used were similar to those of Al-Subhi *et al.* (2006) and the conditions used to amplify the BotF15 locus were the same as those of Pavlic *et al.* (2009a). The PCR amplification products were separated by electrophoresis on 2 % agarose gels stained with ethidium bromide in a 1x TAE buffer and visualized under UV light.

2.5. DNA Sequencing

Amplified PCR fragments were cleaned using 6 % Sephadex G-50 columns with 50-150 µm bead size (Sigma, Steinheim, Germany) following the manufacturer's instructions. Thereafter, 25 amplification cycles were carried out for each sample on an Icycler thermal cycler to generate sequences in both the forward and reverse directions using 10 µl mixtures. Each mixture contained 1µl reaction buffer, 2 µl ready reaction buffer (Big dye), 1 µl primer (10 mM), 3 µl of the PCR product and 3 µl Sabax water. The following PCR conditions were followed: One step at 96 °C for denaturation of the double stranded DNA (10 s), followed by an annealing step at 50 °C (5 s) and primer extension at 60 °C (4 min). The BigDye Terminator v 3.1 Cycle sequencing Kit (PE Applied Biosystems) was used for sequencing reactions, following the manufacturer's protocols, on an ABI PRISM 3130xl genetic analyzer using Pop 7 polymer (Applied Biosystems, Foster City, California, USA).

2.6. DNA Sequence Analyses

Sequences of the Botryosphaeriaceae generated in this study were edited using MEGA version 4 (Tamura *et al.* 2007). For the phylogenetic analyses, DNA sequences from this study, together with those retrieved from published sequences in Genbank (http://www.ncbi.nlm.gov) were aligned online using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) version 6 (Katoh *et al.* 2005). The aligned sequences were transferred to PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2001) where a final manual alignment was made. All the ambiguously aligned regions within each data set were excluded from the analyses. Single gene phylogenetic analyses were run

for the datasets representing the different gene regions and three combinations of analyses were also done: ITS and *tef 1-α* for all the isolates; ITS, *tef 1-α*, *β-tub*, *rbp2* and BOTF15 for *Neofusicoccum* and ITS, *tef 1-α* and *β-tub* for *Lasiodiplodia* isolates. In the analyses, gaps were treated as fifth characters and all characters were unordered and of equal weight. The phylogenetic analyses for all the datasets were performed using the maximum parsimony (MP) option, with trees generated by heuristic searches with random stepwise addition in 1000 replicates, tree bisection and reconnection (TBR) as branch swapping algorithms, and random taxon addition sequences for the construction of maximum parsimonious trees. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. MAXTREES was set to auto-increase in all analyses. *Guignardia philoprina* (Berk. & M.A. Curtis) Van der Aa was used as outgroup in analyses of ITS and *tef 1-α* data sets whereas no outgroup was inserted in additional analyses for the *Neofusicoccum* and *Lasiodiplodia* groups of isolates as the trees generated were unrooted. The support for branches of the most parsimonious trees was assessed with a 1000 bootstrap replications (Felsenstein 1985). Other measures used to assess the trees were tree length (TL), consistency index (CI), rescaled consistency index (RC), and the retention index (RI) (Hillis and Huelsenbeck 1992). A partition homogeneity test (Farris *et al.* 1995) was conducted in PAUP to assess the possibility of combining the ITS and *tef 1-α* data sets in analyses of all the isolates whereas Incongruence Length Difference (Farris *et al.* 1995) was used in combined analyses for the groups of *Neofusicoccum* and *Lasiodiplodia* isolates.

Bayesian analyses using the Markov Chain Monte Carlo (MCMC) method were performed to ascertain the topology of trees obtained with PAUP. Before launching the Bayesian analyses, the best nucleotide substitution models for each dataset were separately determined with MrModelTest version 2.2 (Nylander 2004) and included in each partition in MrBayes v3.1.2. (Huilsenberg and Ronquist 2001). GTR+I+G and HKY+G were chosen as best-fitting models for the ITS and *tef 1-α* data sets respectively for the general analyses. In the following independent analyses, K80, HKY, GTR, and HKY + I, models were chosen for the ITS, *tef 1-α*, *rbp2*, *β-tub* and BotF15 data sets respectively to analyse sequences of species in the *Neofusicoccum* group. In the second analyses for species in the *Lasiodiplodia* group, the following models were chosen: K80, HKY + I and HKY for the ITS, *tef 1-α* and *β-tub* data sets respectively. The MCMC analyses, with four chains, started from random tree topology and lasted one million generations. Trees were saved every 100th generation. The burn-in

number was graphically estimated from the likelihood scores and trees outside this point were discarded in the analyses. The consensus trees were constructed in MEGA version 4 and posterior probabilities were assigned to branches after 50 % majority rule.

2.7. Pathogenicity

Two-year-old nursery grown *T. catappa* plants with stems ranging from 50-100 cm in height and 1- 1.5 cm in diameter, growing in peat moss soil in 20 L plastic bags were maintained in the greenhouse at 22 °C and watered once a day for pathogenicity experiments. For inoculations, 15 isolates of Botryosphaeriaceae representing all the species identified in the study (Table 1) were grown on 2 % MEA for 10 days prior to inoculation. To inoculate trees, wounds were made on the stems by removing the outer bark with a 7 mm diameter cork-borer. A 7 mm-diameter plug of the test isolates was placed into each wound, with the mycelium facing the cambium, and covered with a strip of Parafilm to prevent desiccation of the wound and inoculum. Five trees, arranged in a completely randomized design, were used for each isolate and the trial was repeated once. For the controls, sterile MEA plugs were used instead of a fungal culture. After six weeks, the lengths of the bark and cambium lesions were measured to obtain an indication of the pathogenicity of the isolates tested. Small pieces of necrotic tissue from the edges of lesions were incubated on MEA to show that the inoculated fungi were associated with the lesions. The trial was repeated once. As no significant differences were noticed between the two repeats of the pathogenicity test, the data for all isolates of a particular species were pooled in a single dataset for analyses. Variations in the lengths of the lesions were assessed through a one-way analysis of variance (ANOVA) using SAS (SAS systems, version 8.2; SAS Institute).

3. RESULTS

3.1. Isolates

In total, 79 isolates of Botryosphaeriaceae were obtained from 40 of the 83 *T. catappa* trees sampled in Cameroon, Madagascar and South Africa. Of these, 19 originated from branches on *T. catappa* in Kribi (Cameroon), 29 from Morondava, 8 from Mahajanga (Madagascar), and 25 from Richardsbay (South Africa). Only one isolate per tree was used for further

morphological and molecular studies. The isolates obtained were grouped according to their colony and conidial morphology, and representative isolates of each group were selected for DNA sequence comparisons.

3.2. Morphologic characterization

All the isolates from *T. catappa* could group into two categories based on conidial morphology (Table 2). The isolates in the first category (Group A) produced hyaline, elongate and thin-walled, fusoid conidia. In the second category (Group B), isolates were characterized by hyaline or dark, thick-walled, aseptate or one-septate, ovoid conidia sometimes exhibiting longitudinal striations. Only anamorph structures were produced by the isolates collected from *T. catappa* when incubated on pine needles.

Based on colony morphology, only one group could be distinguished for all isolates collected in this study. All isolates on MEA grew fast, filling the Petri dishes within five days. The aerial mycelium was originally white, turning dark greenish-grey or greyish after four to five days at 25 °C under near UV-light (Table 2). Based on a combination of colony morphology and morphology of conidia, it was possible to distinguish two groups of Botryosphaeriaceae from *T. catappa* with confidence and these were used in DNA sequence comparisons.

3.3. DNA extraction and PCR amplification

A total of 40 isolates, each originating from a separate *T. catappa* tree, were selected for ITS sequence comparisons to obtain a broad indication of their identities and to select isolates for the data sets used in the final analyses. These comprised 12 from Group A and 28 from Group B. Of these, 19 isolates were selected for *tef 1-α* sequence comparisons and were considered in the final analyses. Sequences from the *β-tub, rbp2* and BotF15 gene regions were used to clarify the relationships between isolates that could not be clearly resolved with ITS and *tef 1-α* sequences. DNA extraction and PCR was conducted successfully for all gene regions selected**.** PCR fragments for the ITS were ~ 580 bp in size, while those for *tef 1-α, β-tub, rbp2* and BOTF15 were 710 bp, 440 bp, 615 bp and ~350 bp, respectively.

3.4. DNA sequence analyses

ITS analyses. The ITS dataset comprised 82 sequences of which 40 originated from *T. catappa* and 42 sequences were retrieved from GenBank. Of the 543 characters present in the ITS sequence data set, 24 % were parsimony informative. The MP analyses generated 11 trees with identical topology (TL = 401, CI = 0.840, RI = 0.977, RC = 0.821). Isolates from *T. catappa* grouped into five well separated clades, representing *Neofusicoccum* [Bootstrap support (BS) = 74 % and Bayesian posterior probabilities (BPP) = 1] and *Lasiodiplodia* (BS = 51 %, BPP = 0.61), which also corresponded with the two groups defined based on isolate morphology (Figure 1).

Within the *Neofusicoccum* clade, isolates from *T. catappa* were divided into two groups. The first comprised only isolates from Cameroon, grouping in a single clade (with no Bootstrap value) close to the recently described *N. umdonicola* Pavlic, Slippers, & M.J. Wingf. The second clade accommodated isolates from *T. catappa* in South Africa, together with isolates of *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips., with no sequence variation among them. Bayesian analyses supported the separation of the isolates in the *Neofusicoccum* group as observed with MP analyses.

Isolates from *T. catappa* formed three clades within *Lasiodiplodia* based on ITS sequence data. Isolates in the first two clades grouped with *L. theobromae* (Pat.) Griff. & Maubl. and *L. pseudotheobromae* A.J.L. Phillips, A. Alves & Crous., and included isolates from all three countries. Three isolates (CMW27801, CMW27818, CMW27820) from *T. catappa* in Madagascar constituted a clade (BS = 63 % and BPP = 0.91), which did not group with any known *Lasiodiplodia* sp., suggesting a possibly undescribed species, most closely related to *L. parva* A.J.L. Phillips, A. Alves & Crous. The topology of the consensus tree generated with Bayesian analyses was similar in overall topology to the one obtained with MP analyses.

ITS and tef 1-α analyses. The partition homogeneity test for the ITS and *tef 1-α* data sets showed that they could be combined (P = 0.303) as no conflict was found between the gene genealogies. The combined dataset consisted of 59 isolates and contained 804 characters of which 38 % were parsimony informative. Gaps were treated as a fifth character. After heuristic searches, eight most parsimonious trees were obtained (TL = 884; CI = 0.782, RI =

0.960, RC = 0.750, TreeBase No: SN4517). The topology of the tree generated from the combined analyses with MP, as well as with the 50 % majority rule consensus tree (Figure 2), was congruent with the trees obtained with the individual analyses of ITS and *tef 1-α*, presenting the same clades. However, the Cameroonian isolates with hyaline, thin-walled conidia formed a single sub-group (BS = 56 % and BPP = 0.94) close to *N. umdonicola* within the larger clade containing species close to *N. ribis*, similar to that observed on the tree obtained for the ITS analyses. The clade (BS = 100 % and BPP = 1) accommodating the apparently undescribed *Lasiodiplodia* sp. from Madagascar (CMW27801, CMW27818, CMW27820) was basal to *L. plurivora* as observed on the tree obtained using *tef 1-α* analyses.

Analyses of both ITS and *tef 1-α* separately, as well as combined, identified the same groups amongst the isolates collected from *T. catappa*. These included *N. parvum*, *L. theobromae, L. pseudotheobromae* and two previously unidentified groups. Some uncertainty was, however, present regarding the Cameroonian isolates with hyaline, thin-walled conidia. Although these isolates consistently grouped in a unique clade within the *N. ribis/N. parvum* complex, only low statistical support was observed in all analyses. These results raised uncertainties regarding their relationship with other closely related species and prompted analyses using additional gene regions in an attempt to clarify their identity.

Additional analyses using five loci: To resolve uncertainties in the relationships among the Cameroonian isolates (CMW28315, CMW28363, CMW28320, CMW28637) grouping close to *N. ribis*, additional independent multilocus analyses were used for taxa included in the genus. Twenty-one isolates taken from the *N. ribis* and *N. parvum* complex were included in the ITS, *tef 1-α*, BOTF15, *rbp2* and *β-tub* data sets (Table 5). For each data set, trees obtained from both MP and Bayesian analyses showed identical topologies. Isolates from *T. catappa* in Cameroon grouping in the *N. ribis/ N. parvum* complex formed a distinct clade in four of the five individual partitions (Figure 3a, 3b, 3d, 3e). These isolates were more closely related to *N. ribis* and *N. umdonicola* than to any other species of the complex. The clade accommodating the Cameroonian *Neofusicoccum* isolates was characterized by Bootstrap and Bayesian posterior probabilities values between 60 and 97 %. The ITS and BOTF15 gene regions contained one unique fixed polymorphism each, while nucleotide sequences of *tef 1-α* and *β-tub* showed a number of base variations (one deletion and two substitutions for *tef 1-α* and one substitution for *β-tub*) from those representing *N. ribis* but no fixed polymorphism

was observed (Table 3). No differences were observed in *rbp2* sequences between Cameroonian isolates and those representing *N. ribis* (Table 3) (Figure 3c). However, analysis of the data from the *rbp2* locus remained informative and could not cancel out the lineage sorting of the Cameroonian isolates. To provide a better resolution in the relationship of these isolates, we combined the data from each partition into one phylogenetic analysis.

The Incongruence Length Difference calculated for all the data sets related to all the isolates included in the *N. ribis* and *N. parvum* complex (I = 0) (Table 5) indicated that the gene phylogenies were congruent. This was illustrated by strong statistical (BS = 86 %; BPP = 1) support observed on the consensus tree obtained from both MP and Bayesian analyses of the combined data set (Figure 3f). Analyses of the combined data sets confirmed the grouping of the isolates from Cameroon into an undescribed and unique lineage (Figure 3f).

Additional analyses for the *Lasiodiplodia* clade, including nine isolates representing *L. theobromae, L. pseudotheobromae, L. parva* and isolates of the apparently undescribed species from Madagascar were conducted using part of the ITS, *tef 1-α* and *β-tub* gene regions (Table 6). From the MP tree topologies, which were identical to those obtained in Bayesian analyses, for each partition, as well as the combined analysis, it was clear that isolates from Madagascar consistently formed a clade distinct from *L. theobromae, L. pseudotheobromae* and *L. parva* (Figure 4a, 4b, 4c, 4d). There was considerable sequence variation across the three gene regions among isolates representing the undescribed species and those of *L. theobromae, L. pseudotheobromae* and *L. parva* (Table 4). The resolution in the relationship of these isolates was improved by combining individual data sets in one phylogenetic analysis. The Incongruence Length Difference calculated for all the data sets for this group of isolates (I = 2) (Table 6) indicated a congruence in the phylogenies of all three genes. Strong statistical (BS = 100 %; BPP = 1) support was observed for the consensus tree obtained for both MP and Bayesian analyses (Figure 3d) of the combined data set. Analyses of the combined data sets confirmed the monophyly of isolates (CMW27801, CMW27818, CMW27820) from Madagascar in the *Lasiodiplodia* clade (Figure 4d).

3.5. Taxonomy

DNA sequence data for the ITS, *tef 1-α*, BOTF15, *rbp2* and *β-tub* gene regions revealed the presence of two previously undescribed species of Botryosphaeriaceae amongst the isolates collected from *T. catappa* in this study. A study of the morphology of these isolates confirmed that they are distinct from previously described species and they are consequently described as new here:

Lasiodiplodia mahajangana Begoude, Jol. Roux, Slippers, sp. nov. MB514012
FIGURE 5.

Etymology: the name refers to the locality where this fungus was collected for the first time.

Conidia pycnidialia usque ad 300.0 µm lata, in foliis Pini in MEA in 14 diebus facta, solitaria mycelio tecta, superficialia conica. Conidiophorae ad cellulas conidiogenas reductae. Cellulae conidiogenae holoblasticae discretae hyalinae cylindricae. Conidia primo non septata, hyalinae ellipsoideae vel ovoideae parietibus crassis < 2.5 µm, contentis granularibus, demum semel septataet liberata colorata, matura verticaliter striata 17.5 x 11.5 µm.

Conidiomata: pycnidial (up to 300 µm wide), produced on pine needles on MEA within 14 days, solitary and covered by mycelium, superficial, conical, unilocular, with long necks (up to 200 µm) and single ostioles at the tips, locule walls thick, consisting of two layers: an outer dark brown *textura angularis*, lined with inner thin-walled, hyaline cells. *Paraphyses*: rare, cylindrical, hyaline, aseptate 1-celled (27.5) 33.5-52.5 (66.0) x (2) 2.5-3.5 (5.0) µm, (average 50 paraphyses 43.0 x 3.0 µm), rounded at the tips, unbranched. *Conidiophores*: reduced to conidiogenous cells. *Conidiogenous cells*: holoblastic, discrete, hyaline, cylindrical, proliferating percurrently to form a periclinal thickening (10.0) 10.5-18.0 (26.0) x (3.0) 3.5-5.5 (6.0) µm (average 50 conidiogenous cells 14.5 x 4.5 µm, l/w 3.2). *Conidia*: initially aseptate, hyaline, ellipsoid to ovoid, thick-walled (< 2.5 µm), granular content, becoming one-septate and pigmented after release, vertical striations observed at maturity, (13.5) 15.5-19.0 (21.5) x (10.0) 11.5-13.0 (14.0) µm (average 50 conidia 17.5 x 11.5 µm, l/w 1.4). *Cultural characteristics*: white fluffy and abundant aerial mycelium, becoming pale olivaceous grey (23’’’’’f) after 4 days, with the reverse sides of the colonies olivaceous grey (23’’’’’b).

Optimum temperature for growth 25-30 °C, covering a 90 mm diameter Petri dish after 3 days on MEA in the dark, no growth observed at 10 °C.

Teleomorph: not observed

Host: *Terminalia catappa*.

Distribution: Madagascar, Mahajanga.

Specimen examined: Madagascar, Mahajanga, 15° 43'.084 N, 46° 19'.073 E, 0 m asl: isolated from healthy branches of *Terminalia catappa*, Oct 2007, J. Roux, holotype (PREM 60288), a dry culture on pine needles CMW27801 = CBS124925; ex-type culture CMW27820 = CBS124927.

Additional specimens: Madagascar, Mahajanga, 15° 43'.084 N, 46° 19'.073 E, 0 m asl: isolated from healthy branches of *Terminalia catappa*, Oct 2007, J. Roux, ex-paratype (PREM 60289) CMW27818 = CBS124926.

Neofusicoccum batangarum Begoude, Jol. Roux, Slippers, sp. nov. MB514013 FIGURE 6.

Etymology: Name refers to the Batanga people who live in the area where the type specimen was collected.

Conidia pycnidialia in foliis Pini in 14 diebus facta, solitaria mycelio tecta, primo immersa, matura ¾ per foliis emergentia, obpyriformia vel ampulliformia. Conidiophorae ad cellulas conidiogenas reductae. Cellulae conidiogenae holoblasticae hyalinae cylindricae. Conidia non septata, hyalinae fusoideae vel ovoideae parietibus tenuis, 15.5 x 5.5 µm.

Conidiomata: pycnidial produced on pine needles within 14 days, solitary and covered by mycelium, initially embedded, ¾ erumpant through the pine needles at maturity, obpyriform to ampulliform with a central and circular ostiole at the neck, unilocular, locule wall thick consisting of two layers: an outer layer of dark brown *textura angularis*, lined with an inner layer of thin-walled, hyaline cells. *Conidiophores*: reduced to conidiogenous cells. *Conidiogenous cells*: holoblastic, hyaline, cylindrical, proliferating percurrently, sometimes forming a periclinal thickening, smooth producing a single conidium, (11.0) 12.5-19.0 (27.0) x (2.0) 2.5-3.0 (3.5) µm (average of 50 conidiogenous cells 15.5 x 2.5 µm, l/w 6). *Conidia*: aseptate, hyaline, smooth, fusoid to ovoid, thin-walled, (12.0) 14.0 -17.5 (20.0) x (4.0) 4.5-6.0

(6.5) µm (average 50 conidia 15.5 x 5.5 µm, l/w 2.9). *Cultural characteristics*: colonies forming concentric rings on MEA, mycelium white and immersed at the leading edge, becoming smokey grey (21''''d) to grey olivaceous (21''''b) from the old ring after 5 days on MEA. *Optimum temperature for growth* 25 °C, covering the 90 mm diameter Petri plate after 4 days on MEA in the dark, little growth observed at 10 and 35 °C.

Teleomorph: not observed

Host: *Terminalia catappa*.

Distribution: Cameroon, Kribi.

Specimen examined: Cameroon, Kribi, Beach , 2° 58'.064 N, 9° 54'.904 E, 7 m asl, isolated from healthy branches of *Terminalia catappa*, Dec 2007, D. Begoude and J. Roux, ex-paratype (PREM 60285), a dry culture on pine needles CMW28315 = CBS124922; ex-type culture (PREM 60286) CMW28363 = CBS124924.

Additional specimens: Cameroon, Kribi, Beach, 2° 58'.064 N, 9° 54'.904 E, 7 m asl, isolated from healthy branches of *Terminalia catappa*, Dec 2007, D. Begoude and J. Roux ex-paratype (PREM 60284) CMW28320 = CBS124923; (PREM 60287) CMW28637.

3.6. Distribution of the Botryosphaeriaceae

In total, five species of Botryosphaeriaceae were isolated from *T. catappa* in South Africa, Madagascar and Cameroon. Two cosmopolitan species, *L. pseudotheobromae* the most commonly isolated species, which represented 42 % of the isolates collected and *L. theobromae*, were collected from trees in all three countries. The other three species, *N. parvum*, *N. batangarum* and *L. mahajangana,* were each isolated only in South Africa, Cameroon and Madagascar respectively (Figure 8).

3.7. Pathogenicity

All inoculations with isolates of Botryosphaeriaceae collected in this study resulted in visible lesions on the bark and cambium of *T. catappa* trees after six weeks. Analysis of variance showed that there were significant differences in the pathogenicity among species ($P < 0.0001$). Overall *L. pseudotheobromae*, *L. theobromae, N. parvum* and *N. batangarum* produced the longest lesions on both bark and cambium, whereas *L. mahajangana* produced the smallest lesions (Figure 9,10). Considerable variation in levels of pathogencity was also

observed among isolates of the same species. There was a positive correlation (R^2 = 75 %) between lesions produced on the bark and those on the cambium. Re-isolations from lesions on the inoculated trees resulted in the recovery of the inoculated fungi.

4. DISCUSSION

This study presents the first consideration of the possible fungal pathogens of *T. catappa*. It is also the first study of the Botryosphaeriaceae on these popular ornamental trees. In total**,** five species of the Botryosphaeriaceae were identified and two of these were new taxa that were described and provided with the names *N. batangarum* and *L. mahajangana.*

Slippers *et al.* (2004a), in comparing the assemblage of Botryosphaeriaceae on native and introduced *Eucalyptus* trees in Australia and South Africa, emphasized the importance of individually identifying species affecting a specific host in every country or environment where it occurs. This was because they found more pathogenic fungal species on *Eucalyptus* spp. outside their native environment (South Africa), than in the area (Australia) where these trees were native. Although the assemblage of the Botryosphaeriaceae found in the current study varied from one country to another, colonization patterns on *T. catappa* in the three areas showed similar trends. In each country, three species of Botryosphaeriaceae were found, one of which was specific to that country and two species occurring in all three countries. These patterns might be explained by climatic differences as has been shown for the distribution of Botryosphaeriaceae in California (Urbez-Torrez *et al.* 2006).

Phylogenetic relationships for the Botryosphaeriaceae from *T. catappa* and other known members of this fungal family were determined using combined sequence data sets of the ITS and *tef 1-α* gene regions. However, the resulting phylogenies did not clearly separate all the species. This was especially true for isolates from Cameroon grouping in the *N. ribis / N. parvum* complex. Within the Botryosphaeriaceae, species in the *N. ribis / N. parvum* complex have been difficult to distinguish based on phylogenies of single gene regions (Pavlic *et al.* 2007; Slippers *et al.* 2004b). A recent study by Pavlic *et al.* (2009a) thus made use of the Genealogical Concordance Phylogenetic Species recognition (GCPSR) approach (Taylor *et al.* 2000) to resolve species boundaries in the complex. These authors

were able to identify three cryptic species in the *N. ribis / N. parvum* complex. The same approach was used in the present study, to confirm the unique nature of *N. batangarum*. Isolates of *N. batangarum* were distinct from *N. ribis* based only on four fixed unique single nucleotide polymorphisms (SNPs) out of 86 informative characters across four gene regions. The gene genealogies across the five different loci were not different, as illustrated by the similarity in the sums of the length of the gene trees for the observed and resampled data. Under these conditions, a recent clonal mutation, most likely due to geographical and host isolation (Geiser *et al.* 1998), provides the best explanation for these results.

Even though DNA sequence data provided the most important basis used to discriminate *N. batangarum* from other species in the *N. ribis / N. parvum* complex, some morphologically informative characters were also found. The most obvious of these were the fact that colonies of *N. batangarum* formed concentric rings on MEA (Figure 7), a characteristic that was not observed in any other species of the complex.

Neofusicoccum batangarum was found as an endophyte on healthy twigs of *T. catappa* in Cameroon. Although no more information regarding its ecology is available, *N. batangarum* was able to produce lesions on young *T. catappa* in pathogenicity trials. This suggests that *N. batangarum* lives in a latent phase in plant organs and is able to convert to being a virulent pathogen when environmental conditions become unfavorable for the tree host.

The second previously undescribed species, *L. mahajangana*, was found in samples from Madagascar, a country where very few studies of microfungi have been conducted. *L. mahajangana* is phylogenetically most closely related to *L. theobromae* and *L. parva*. However, six and 14 SNPs amongst 60 informative characters across ITS, *tef 1-α* and *β-tub* gene regions distinguish *L. mahajangana* from *L. theobromae* and *L. parva* respectively. Moreover, *L. mahajangana* can also be distinguished from these fungi based on conidial size, its paraphyses and growth characteristics. Conidia of *L. mahajangana* are smaller than those of its closest relatives, *L. theobromae* and *L. pseudotheobromae*, but larger than those of *L. parva*. The paraphyses in this species are aseptate while those of *L. theobromae* and *L. parva* are septate. Moreover, *L. mahajangana* exhibited growth at temperatures as high as 35 °C.

Isolates of *L. mahajangana* were obtained from healthy plant material where they occurred as endophytes. Besides this particular feature, there are no data relating to its ecology, distribution and host range. Our consideration of its pathogenicity on *T. catappa* trees showed that *L. mahajangana* was less pathogenic than the other Botryosphaeriaceae found on this host. Lesions produced by *L. mahajangana,* although smaller than those produced by the other species collected from *T. catappa* in this study, were also significantly different from the control inoculations. The relatively small lesions produced by *L. mahajagana,* together with the fact that is was isolated only from healthy material, provides an indication that it is not a primary pathogen of these trees.

Lasiodiplodia theobromae is considered to be a pantropical pathogen that occurs on numerous hosts worldwide (Punithalingam 1980). Thus, it was not surprising to isolate it from the tropical *T. catappa*. The relatively common occurrence of *L. theobromae* in Cameroon, compared to the other regions sampled in this study could also reflect a climatic influence. *Lasiodiplodia theobromae* appears to occur most commonly in consistently warm areas (Taylor *et al.* 2005; Urbez-Torrez *et al.* 2008) and the climatic conditions in the localities where samples were collected in this study apparently support the findings.

Neofusicoccum parvum was the most common species collected form *T. catappa* in South Africa and produced lesions on young trees of *T. catappa* in pathogenicity trials. *N. parvum* is a well known pathogen of forest and fruit trees (Davidson and Tay 1983; Mohali *et al.* 2007; Slippers *et al.* 2004a; van Niekerk *et al.* 2004). In the current study, isolates of this species were obtained from branches of *T. catappa* showing symptoms of die-back. This might indicate that it is the pathogen responsible for branch die-back and death of *T. catappa* in South Africa. In previous studies conducted in South Africa, *N. parvum* was common on non-native *Eucalyptus* trees and on native *Syzygium cordatum*, where it has been shown to be pathogenic to these hosts (Pavlic *et al.* 2007; Slippers *et al.* 2004a). The common occurrence and wide host range of *N. parvum* in South Africa suggests that this fungus might be native to this area.

Lasiodiplodia pseudotheobromae emerged from a recent separation of cryptic species originally identified as *L. theobromae* (Alves *et al.* 2008). It is known from Africa, Europe and Latin America, where it occurs on forest and fruit trees. However, no

information concerning its pathogenicity to these trees is available. *L. pseudotheobromae* was the most abundant species isolated from *T. catappa* and it occurred in all the sampled areas. The known host range of *L. pseudotheobromae* is very limited, with single isolates obtained from *Rosa* sp. in the Netherlands, *Gmelina arborea* and *Acacia mangium*. in Costa Rica, *Coffea* sp. in Democratic Republic of Congo and *Citrus aurantium* in Suriname (Alves *et al.* 2008). Results of this study have substantially increased the geographic areas from which the fungus is known and they suggest that *L. pseudotheobromae,* like *L. theobromae,* has a worldwide distribution and a very wide host range.

The inoculation trials conducted in this study have shown that, *L. pseudotheobromae* was the most pathogenic of all the species tested. *Lasiodiplodia theobromae* and *N. parvum* have previously been shown to be pathogens of several hosts (Davidson and Tay 1983; Mohali *et al.* 2007; Pavlic *et al.* 2007; Slippers *et al.* 2004a). It was, therefore, not surprising that they caused lesions on *T. catappa* in this study. However, this study has provided the first data for the pathogenicity of *L. pseudotheobromae,* which suggests that its importance has been overlooked in the past, most likely because it was considered collectively with *L. theobromae*. It will now be important to determine its host range and distribution in order to understand the threat that it might pose as a pathogen, as well as to guide possible quarantine and other control measures.

The origins of the species of Botryosphaeriaceae collected from *T. catappa* in this study are unknown. However, its common occurrence on both introduced and native plants has led to suggestions that *N. parvum* might be part of the indigenous fungal flora of South Africa (Pavlic *et al.* 2007; 2008; 2009a). In contrast, *L. theobromae,* which has a wide host range and has been reported on native and introduced hosts on many continents, may have been introduced to Africa. Population genetic studies on this fungus will likely provide answers to the questions related to its origin and movements. As limited information is available regarding the recently described *L. pseudotheobromae*, the origin of this species cannot be considered here. In this study, the close relationship between *N. batangarum* and *N. ribis* suggests that *N. batangarum,* which was commonly isolated form *T. catappa* in Cameroon, could be derived from a clonal mutation possibly arising from geographical and host isolation of *N. ribis,* a fungus that has been reported with certainty only from the United States of America on *Ribes* sp. (Slippers *et al.* 2004b).

More sampling, both in other areas and hosts is clearly needed to address the question of its origin.

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Table 1.Botryosphaeriaceae used for phylogenetic analyses in this study.

Species	Culture number[a]	Origin[b]	Host	Collectors	Genbank Accession No.				
					ITS	*tef 1-α*	*rbp2*	*β-tub*	BotF15
Botryosphaeria dothidea	CMW7999	Switzerland	*Ostrya* sp.	B. Slippers	AY236948	AY236897			
	CMW8000	Switzerland	*Prunus* sp.	B. Slippers	AY236949	AY236898			
Dichomera eucalyptii	CMW15952	Australia	*Eucalyptus diversicolor*	T. Burgess/K.LGoei	DQ093194	DQ093215			
	CMW15953	Australia	*E. diversicolor*	T. Burgess/K.LGoei	DQ093194	DQ093216			
Diplodia mutila	CBS112553	Portugal	*Vitis vinifera*	A.J.L. Phillips	AY259093	AY573219			
	CBS230.30	USA	*P. dactylifera*	L.L. Huilllier	DQ458886	DQ458869			
D. seriata	CMW7774	USA	*Ribes* sp.	B.Slippers/G.Hudler	EF445343	EF445382			
	CMW7775	USA	*Ribes* sp	B.Slippers/G.Hudler	EF445344	EF445383			
Guignardia philoprina	CMW7063	Netherlands	*T. baccata*	H.A. van der Aa	AY236956	AY236905			
Lasiodiplodia crassispora	WAC12533	Venezuela	*E. urophylla*	S. Mohali	DQ103552	DQ103556			
	WAC12534	Australia	*Santalum album*	T.I. Burgess/B. Dell	DQ103550	DQ103557			
	WAC12535	Australia	*S. album*	T.I. Burgess/B. Dell	DQ103551	DQ103558			
L. gonubiensis	CBS115812	South Africa	*Syzigium cordatum*	D. Pavlic	DQ458892	DQ458877			

	CBS116355	South Africa	*S. cordatum*	D. Pavlic	AY639594	DQ103567	
L. mahajangana	**CMW27801**	Madagascar	*Terminalia catappa*	J. Roux	FJ900595	FJ900641	FJ900630
	CMW27818	Madagascar	*T. catappa*	J. Roux	FJ900596	FJ900642	FJ900631
	CMW27820	Madagascar	*T. catappa*	J. Roux	FJ900597	FJ900643	FJ900632
L. margaritacea	CMW26162	Australia	*Adansonia gibbosa*	D. Pavlic	EU144050	EU144065	
	CMW26163	Australia	*A. gibbosa*	D. Pavlic	EU144051	EU144066	
L. parva	CBS356.59	Sri Lanka	*Theobroma cacao*	A. Riggenbach	EF622082	EF622062	EU673113
	CBS494.78	Colombia	Cassava-field soil	O. Rangel	EF622084	EF622064	EU673114
L. plurivora	STEU-5803	South Africa	*Prunus salicina*	U.Damm	EF445362	EF445395	
	STEU-4583	South Africa	*V. vinifera*	F.Halleen	AY343482	EF445396	
L. pseudotheobromae	**CMW26721**	South Africa	*T. catappa*	D. Begoude/J. Roux	FJ900598	FJ900644	
	CMW26716	South Africa	*T. catappa*	D. Begoude/J. Roux	FJ900599	FJ900645	
	CMW27802	Madagascar	*T. catappa*	J. Roux	FJ900600	FJ900646	
	CMW27817	Madagascar	*T. catappa*	J. Roux	FJ900601	FJ900647	
	CBS116459	Costa Rica	*Gmelinea arborea*	J.Carranza/Velásquez	EF622077	EF622057	EU673111
	CBS447.62	Suriname	*Citrus aurantium*	C. Smulders	EF622081	EF622060	EU673112

L. rubropupurea	WAC12535	Australia	*E. grandis*	T.I. Burgess/G.Pegg	DQ103553	DQ103571			
	WAC12536	Australia	*E. grandis*	T.I. Burgess/G.Pegg	DQ103554	DQ103572			
L. theobromae	**CMW28317**	Cameroon	*T. catappa*	D. Begoude/ J.Roux	FJ900602	FJ900648			
	CMW28319	Cameroon	*T. catappa*	D. Begoude/J. Roux	FJ900603	FJ900649			
	CMW26715	South Africa	*T. catappa*	D. Begoude/J. Roux	FJ900604	FJ900650			
	CMW27810	Madagascar	*T. catappa*	D. Begoude/J. Roux	FJ900605	FJ900651			
	CMW9074	Mexico	*Pinus* sp.	T. Burgess	EF622074	EF622054		AY236930	
	CBS164.96	New Guinea	Fruit along coral reef coast	Unknown	AY640255	AY640258		EU673110	
L. venezuelensis	WAC12539	Venezuela	*Acacia mangium*	S. Mohali	DQ103547	DQ103568			
	WAC12540	Venezuela	*A. mangium*	S. Mohali	DQ103548	DQ103569			
Neofusicoccum batangarum	**CMW28315**	Cameroon	*T. catappa*	D. Begoude/ J.Roux	FJ900606	FJ900652	FJ900614	FJ900633	FJ900622
	CMW28363	Cameroon	*T. catappa*	D. Begoude/ J. Roux	FJ900607	FJ900653	FJ900615	FJ900634	FJ900623
	CMW28320	Cameroon	*T. catappa*	D. Begoude/ J.Roux	FJ900608	FJ900654	FJ900616	FJ900635	FJ900624
	CMW28637	Cameroon	*T. catappa*	D. Begoude/ J. Roux	FJ900609	FJ900655	FJ900617	FJ900636	FJ900625
N. cordaticola	CMW13992	South Africa	*S. cordatum*	D. Pavlic	EU821898	EU821868	EU821928	EU821838	EU821802
	CMW14056	South Africa	*S. cordatum*	D. Pavlic	EU821903	EU821873	EU821933	EU821843	EU821807
	CMW14054	South Africa	*S. cordatum*	D. Pavlic	EU821906	EU821876	EU821936	EU821846	EU821810
N. kwambonambiense	CMW14023	South Africa	*S. cordatum*	D. Pavlic	EU821900	EU821870	EU821930	EU821840	EU821804
	CMW14025	South Africa	*S. cordatum*	D. Pavlic	EU821901	EU821871	EU821931	EU821841	EU821805
	CMW14123	South Africa	*S. cordatum*	D. Pavlic	EU821924	EU821894	EU821954	EU821864	EU821828

N. parvum	CMW9081	New Zealand	*P. nigra*	G.J. Samuels	AY236943	AY236888	EU821963	AY236917	EU821837
	CMW9079	New Zealand	*A. deliciosa*	S.R. Pennicook	AY236940	AY236885	EU821961	AY236915	EU821835
	CMW26714	South Africa	*T. catappa*	D. Begoude/ J. Roux	FJ900610	FJ900656	FJ900618	FJ900637	FJ900626
	CMW26717	South Africa	*T. catappa*	D. Begoude/ J. Roux	FJ900611	FJ900657	FJ900619	FJ900638	FJ900627
	CMW26718	South Africa	*T. catappa*	D. Begoude/ J. Roux	FJ900612	FJ900658	FJ900620	FJ900639	FJ900628
	CMW26720	South Africa	*T. catappa*	D. Begoude/ J. Roux	FJ900713	FJ900659	FJ900621	FJ900640	FJ900629
N. ribis	CMW7772	USA	*Ribes* sp.	B. Slippers/G.Hudler	AY236935	AY236877	EU821958	AY236906	EU821832
	CMW7773	USA	*Ribes* sp.	B. Slippers/G Hudler	AY236936	AY236878	EU821959	AY236907	EU821833
N. umdonicola	CMW14106	South Africa	*S. cordatum*	D. Pavlic	EU821899	EU821869	EU821929	EU821839	EU821803
	CMW14058	South Africa	*S. cordatum*	D. Pavlic	EU821904	EU821874	EU821934	EU821844	EU821808
	CMW14060	South Africa	*S. cordatum*	D. Pavlic	EU821905	EU821875	EU821935	EU821845	EU821809

[a] **CMW**, Research collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa;

[b] Country of collection.

Table 2. Conidial dimensions of *Neofusicoccum* spp. and *Lasiodiplodia* spp. from *Terminalia catappa* and comparison with those reported in previous studies.

Species	Conidial size (µm)		Source of data
	This study	Previous studies	
N. parvum	(10.5-)14.0-19.0(-20.5) x (4-)5.5-6.5(-7.5)	(12-)15-19(-24) x 4-6	Slippers *et al.* 2004b
N. batangarum	(12.0-)14.0-17.5(-20.0) x (4.0-) 4.5-6.0(-6.5)		This study
L. pseudotheobromae	(21.5-)24.5-29.5(-31.0) x (13.5-)14.0-16.5(-18.0)	(22.5-)23.5-32(-33) x (13.3-)14-18(-20)	Alves *et al.* 2008.
L. theobromae	(20.5-)22.5-26.0(-30.5) x (11.5-)12.5-15.0(-17.0)	(19-)21-31(-32.5) x (12-)13-15.5(-18.5)	Alves *et al.* 2008.
L. mahajangana	(13.5-)15.5-19.0(-21.5) x (10.0-)11.5-13.0(-14.0)		This study

Table 3. Polymorphic nucleotides from sequence data of the ITS, *tef 1-α*, *rbp2*, *β-tub* and BOTF15gene regions for isolates in the *Neofusicoccum ribis, N. kwabonambiense*, *N. umdonicola, N. cordicola* and *N. parvum* clade introduced from outgroup comparisons.

Identity	Culture number	ITS									*tef1-α*										*rbp2*																			*β-tub*											BOTF15										
		51	115	141	163	168	173	372	389	416	43	44	66	67	77	81	153	211	227	257	10	22	49	97	100	112	205	265	280	343	382	397	409	421	475	526	42	50	93	106	125	167	185	245	261	326	389	407	97	98	102	105	166	215	252	301	311
N. ribis	CMW 7772	A	G	T	C	T	A	A	A	T	G	I	T	G	T	C	C	A	-	G	T	T	C	T	G	C	T	G	C	C	G	T	T	G	C	T	C	C	C	G	T	C	G	G	C	T	T	G	A	0	C	0	C	G	C	G
	CMW 7773	.	.	.	.	.	.	.	.	.	.	I	.	.	.	.	.	A	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
N . batangarum	**CMW 28315**	.	.	.	.	.	.	.	G	.	.	0	.	.	.	.	.	G	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.
	CMW 28320	.	.	.	.	.	.	.	G	.	.	0	.	.	.	.	.	G	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.
	CMW 28363	.	.	.	.	.	.	.	G	.	.	0	.	.	.	.	.	G	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.
	CMW 28637	.	.	.	.	.	.	.	G	.	.	0	.	.	.	.	.	G	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.
N. kwabonambiense	CMW 14023	.	-	.	T	.	G	.	.	.	-	0	.	.	.	.	.	G	-	.	.	C	G	C	.	.	C	.	.	.	A	C	.	A	.	C	.	.	.	.	A	C	T	A	A	.	C	C	.	T	I	T	I	.	.	T	.
	CMW 14025	.	-	.	T	.	G	.	.	.	-	0	.	.	.	.	.	G	-	.	.	C	G	C	.	.	C	.	.	.	A	C	.	A	.	C	.	.	.	.	A	C	T	A	A	.	C	C	.	T	I	T	I	.	.	T	.
	CMW 14123	.	-	.	T	.	G	.	.	.	-	0	.	.	.	.	.	G	-	.	.	C	G	C	.	.	C	.	.	.	A	C	.	A	.	C	.	.	.	.	A	C	T	A	A	.	C	C	.	T	I	T	I	.	.	T	.
N. umdonicola	CMW 14106	.	.	.	.	C	.	.	.	.	-	0	.	.	.	T	.	G	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	CMW 14079	.	.	.	.	C	.	.	.	.	.	0	.	.	.	T	.	G	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	CMW 14096	.	.	.	.	C	.	.	.	.	.	0	.	.	.	T	.	G	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
N. cordaticola	CMW 13992	.	-	.	.	.	.	G	.	C	.	0	.	.	C	.	.	G	-	.	.	C	.	C	A	T	C	A	.	.	.	C	C	.	.	.	T	.	.	T	.	C	.	.	.	G	C	C	T	C	.	T	.	.	.	T	.
	CMW 14056	.	-	.	.	.	.	G	.	C	.	0	.	.	C	.	.	G	-	.	.	C	.	C	A	T	C	A	.	.	.	C	C	.	.	.	T	.	.	T	.	C	.	.	.	G	C	C	T	C	.	T	.	.	.	T	.
	CMW 14054	.	-	.	.	.	.	G	.	C	.	0	.	.	C	.	.	G	-	.	.	C	.	C	A	T	C	A	.	.	.	C	C	.	.	.	T	.	.	T	.	C	.	.	.	G	C	C	T	C	.	T	.	.	.	T	.
N. parvum	CMW 9081	T	-	.	.	.	.	.	.	.	.	0	C	A	.	.	T	G	-	A	C	C	.	C	.	.	C	.	.	T	.	C	.	.	T	.	.	.	T	.	A	C	.	.	.	.	.	.	.	.	.	T	.	.	T	.	T	A
	CMW 9079	T	-	.	.	.	.	.	.	.	.	0	C	A	.	.	T	G	-	A	C	C	.	C	.	.	C	.	.	T	.	C	.	.	T	.	.	.	T	.	A	C	.	.	.	.	.	.	.	.	.	T	.	.	T	.	T	A

Table 4. Polymorphic nucleotides from sequence data of the ITS, *tef 1-α* and *β-tub* gene regions for isolates in the *Lasiodiplodia theobromae, L. parva, L. pseudotheobromae* and *L. mahajangana* clade introduced from outgroup comparisons.

Identity	Culture number	ITS	*tef 1-α*	*β-tub*
		42 46 98 116 439	20 22 23 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 50 53 55 58 59 61 62 68 82 99 232 267 282	85 124 143 157 177 189 230 244
L. theobromae	CBS 164.96	A C C T C	G C C C A C G C A T G T C G T T T T T T A T C A T C - - 1 C C A A A	C C G T T A C C
	CMW 9074	. . . . .	. . . . . - - 1	. . . . .
L. parva	CBS 356.59	. T . . .	- G A A . G A T T . T C - - C C - - - C . G T G C T C A 0 . T . . G	T . C C C G A .
	CBS 494.78	. T . . .	- G A A . G A T T . T C - - C C - - - C . G T G C T C A 0 . T . . G	T . C C C G A .
L. pseudotheobromae	CBS 447.62	G T . C T	- T . A T T T . C C C C - - C C . C C C . G T G C T T A 0 . . G G G	T . . C C . . .
	CBS 116459	G T . C T	- T . A T T T . C C C C - - C C . C C C . G T G C T T A 0 . . G G G	T . . C C . . .
L. mahajangana	CMW 27801	. T T . .	. . . . . G . . . . . T . . . . . - - 0 T T . . .	T T C C C G . A
	CMW 27818	. T T . .	. . . . . G . . . . . T . . . . . - - 0 T T . . .	T T C C C G . A
	CMW 27820	. T T . .	. . . . . G . . . . . T . . . . . - - 0 T T . . .	T T C C C G . A

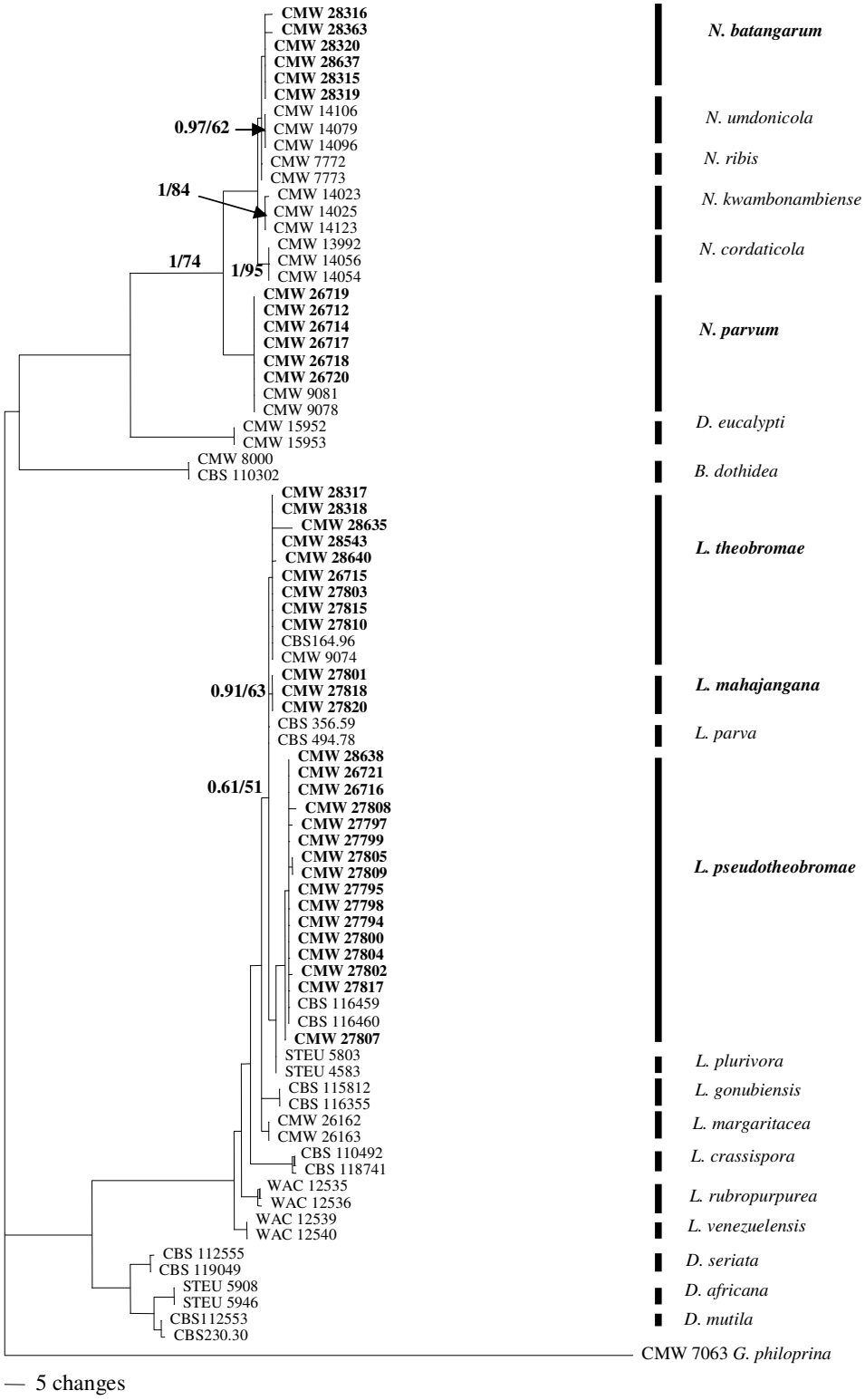
Table 5. Sequence dataset characteristics and phylogenetic information for ITS, *tef 1-α*, *rbp2, β-tub* and BotF15 and combined data sets of *Neofusicoccum* spp.

Data set	Sequence range (bp)	No. variable sites	No. informative sites	No. most parsimonious trees	Tree length	Consistency index	Retention index	Monophyletic taxa
ITS	502	15	10	1	15	1	1	*N. kwambonambiense, N. cordaticola, N. batangarum, N. umdonicola, N. ribis, N. parvum*
tef 1-α	263	24	23	6	25	0.960	0.977	*N. kwambonambiense, N. cordaticola, N. batangarum, N. umdonicola, N. ribis, N. parvum*
rpb2	566	19	16	1	19	1	1	*N. kwambonambiense, N. cordaticola, N. umdonicola, N. parvum*
β-tub	420	12	12	2	13	0.923	0.974	*N. kwambonambiense, N. cordaticola, N. batangarum, N. umdonicola, N. ribis, N. parvum*
BotF15	364	26	25	1	26	1	1	*N. kwambonambiense, N. cordaticola, N. batangarum, N. parvum*
Combined data	2115	96	86	1	98	0.980	0.992	*N. kwambonambiense, N. cordaticola, N. batangarum, N. umdonicola, N. ribis, N. parvum*

Table 6. Sequence dataset characteristics and phylogenetic information for ITS, *tef 1-α* and *β-tub* and combined data sets for *Lasiodiplodia* spp.

Data set	Sequence range	No. of variable sites	No. of informative sites	No. of most parsimonious trees	Tree length	Consistency index	Retention index	Monophyletic taxa
ITS	461	5	5	1	5	1	1	*L. theobromae, L. pseudotheobromae, L. parva, L. mahajangana*
tef 1-α	276	51	47	1	55	0.982	0.990	*L. theobromae, L. pseudotheobromae, L. parva, L. mahajangana*
β-tub	422	9	8	1	9	1	1	*L. theobromae, L. pseudotheobromae, L. parva, L. mahajangana*
Combined data	1159	65	60	1	71	0.958	0.976	*L. theobromae, L. pseudotheobromae, L. parva, L. mahajangana*

Figure 1. One of the most parsimonious trees obtained from Maximum Parsimony analyses of the ITS sequence data of the representative taxa of the Botryosphaeriaceae. Posterior probabilities followed by Bootstrap support (%) from 1000 replications are given on the branch (PP/BS). Isolates marked in bold represent those obtained from *T. catappa*.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CMW 7773
1/84
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CMW 14025
CMW 14123
CMW 13992
1/74
CMW 14056
1/95
CMW 14054
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CMW 9078
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CMW 15953
CMW 8000
CBS 110302
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CMW 28318
CMW 28635
CMW 28543
CMW 28640
CMW 26715
CMW 27803
CMW 27815
CMW 27810
CBS164.96
CMW 9074
CMW 27801
0.91/63
CMW 27818
CMW 27820
CBS 356.59
CBS 494.78
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CMW 27797
CMW 27799
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CBS 115812
CBS 116355
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CBS 110492
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WAC 12536
WAC 12539
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CBS 112555
CBS 119049
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CBS112553
CBS230.30
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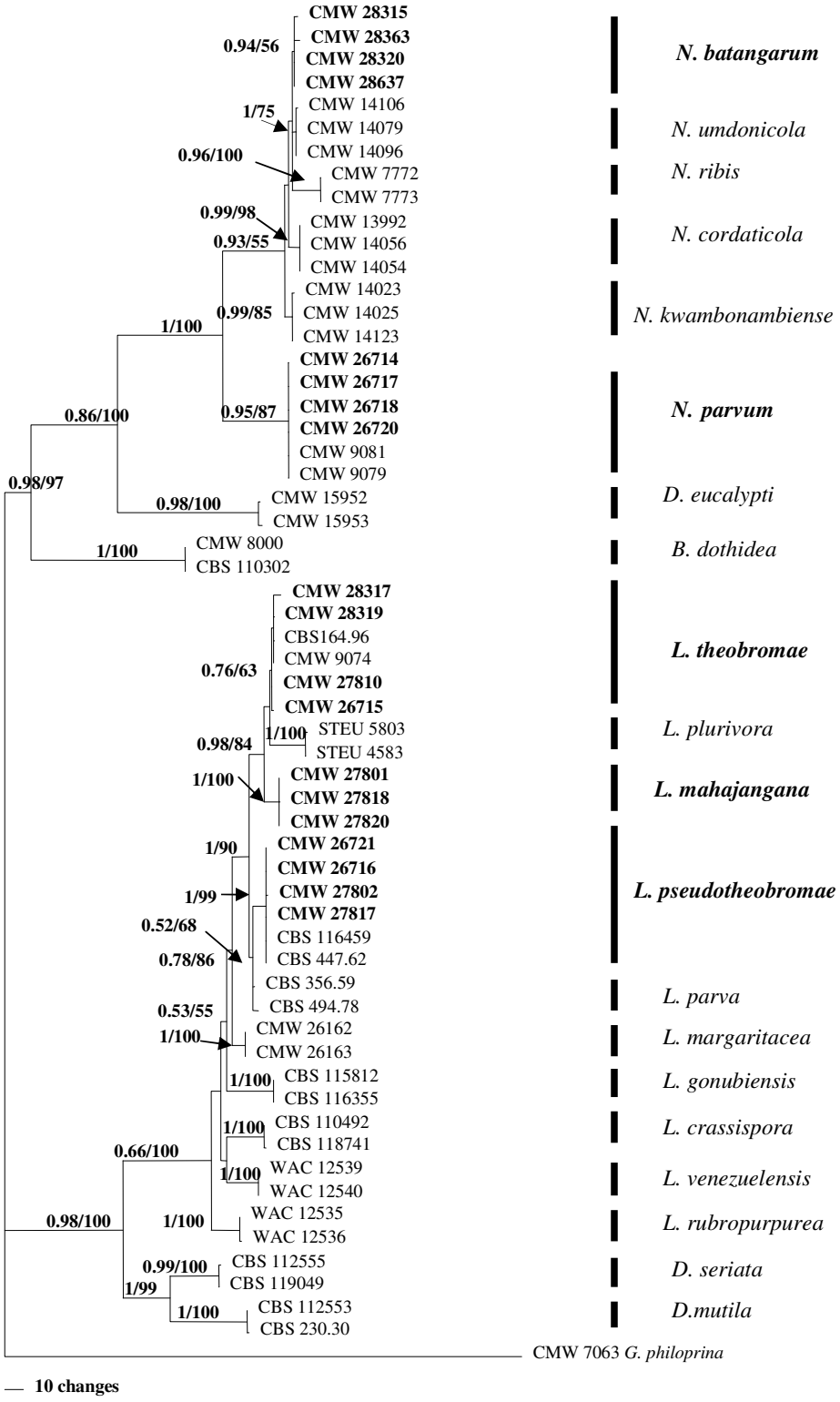
N. batangarum
N. umdonicola
N. ribis
N. kwambonambiense
N. cordaticola
N. parvum
D. eucalypti
B. dothidea
L. theobromae
L. mahajangana
L. parva
L. pseudotheobromae
L. plurivora
L. gonubiensis
L. margaritacea
L. crassispora
L. rubropurpurea
L. venezuelensis
D. seriata
D. africana
D. mutila
5 changes

Figure 2. One of the most parsimonious trees obtained from Maximum Parsimony analyses of the combined ITS and *tef 1-α* sequence data of the representative taxa of the Botryosphaeriaceae. Posterior probabilities followed by Bootstrap support (%) from 1000 replications are given on the branch (PP/BS). Isolates marked in bold represent those obtained from *T. catappa*.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CMW 14123
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CMW 26717
CMW 26718
CMW 26720
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CMW 15953
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WAC 12540
WAC 12535
WAC 12536
CBS 112555
CBS 119049
CBS 112553
CBS 230.30
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0.99/98
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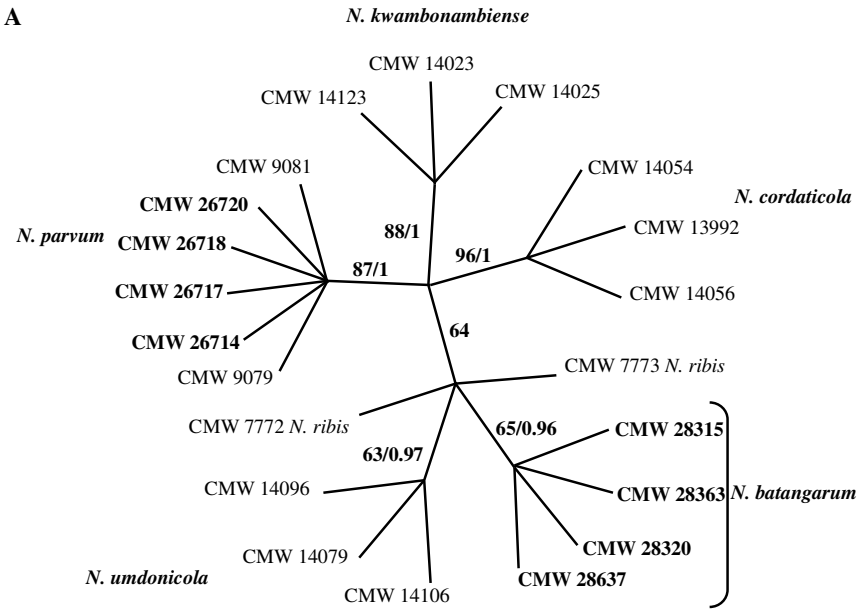
0.99/85
1/100
0.95/87
0.86/100
0.98/97
0.98/100
1/100
0.76/63
1/100
0.98/84
1/100
1/90
1/99
0.52/68
0.78/86
0.53/55
1/100
1/100
1/100
0.66/100
1/100
0.98/100
1/100
0.99/100
1/99
1/100
N. batangarum
N. umdonicola
N. ribis
N. cordaticola
N. kwambonambiense
N. parvum
D. eucalypti
B. dothidea
L. theobromae
L. plurivora
L. mahajangana
L. pseudotheobromae
L. parva
L. margaritacea
L. gonubiensis
L. crassispora
L. venezuelensis
L. rubropurpurea
D. seriata
D.mutila
CMW 7063 G. philoprina
10 changes

Figure 3. One of the most parsimonious unrooted trees inferred from independent analyses of each data set (A = ITS; B = *tef* 1-α; C = *rpb2*; D = *β-tub*; E = BOTF15; F = combination of sequences of the five loci) in the *Neofusicoccum* spp. group of the Botryosphaeriaceae from *T. catappa*. Bootstrap support (%) from 1000 replications followed by Posterior probabilities are given on the branches (BS/PP). Isolates marked in bold represent those obtained from *T. catappa*.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CMW 14054
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CMW 26718
88/1
CMW 13992
87/1
96/1
CMW 26717
CMW 14056
64
CMW 26714
CMW 9079
CMW 7773 N. ribis
CMW 7772 N. ribis
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CMW 28315
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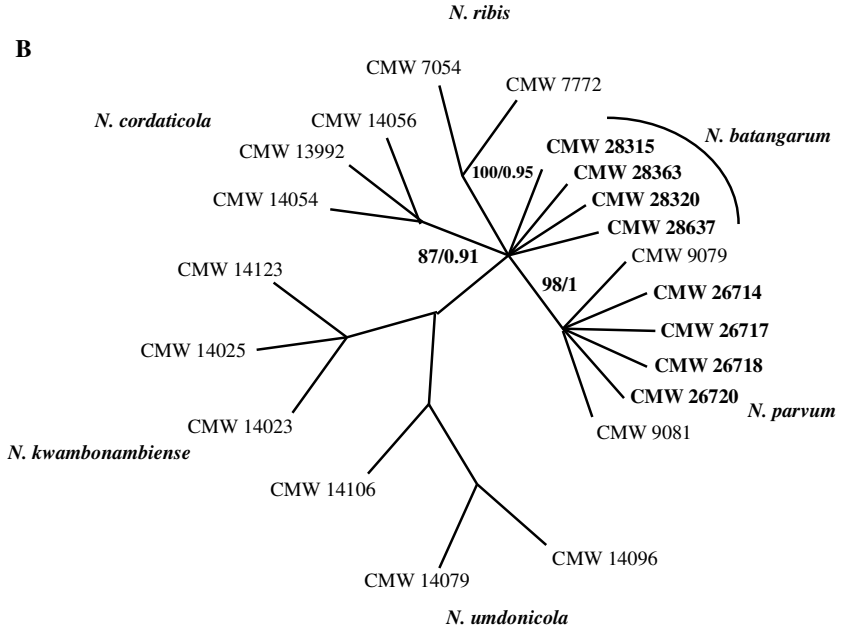
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98/1
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CMW 26720
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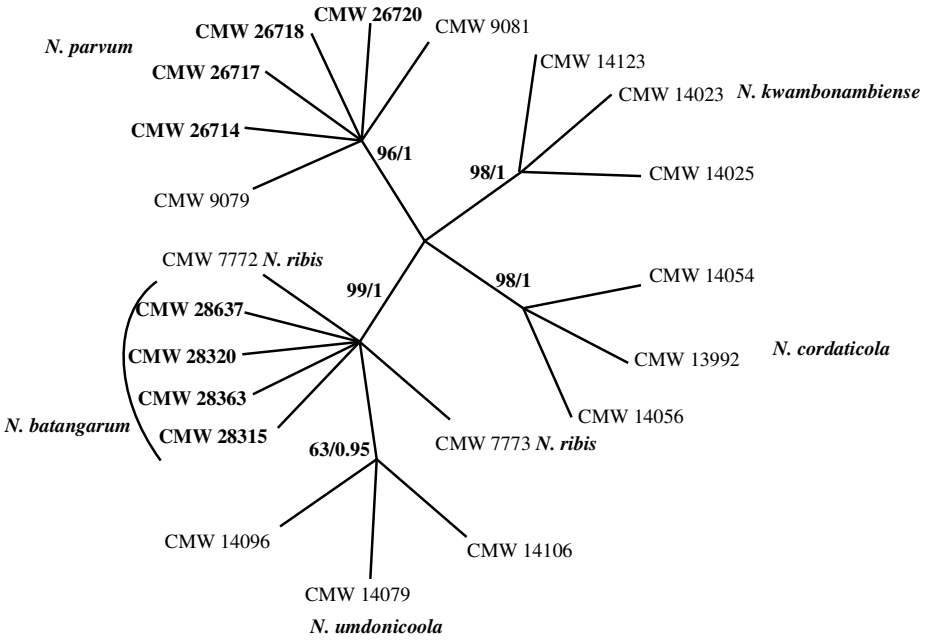
N. umdonicola

C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99/1
98/1
CMW 14054
CMW 28637
CMW 28320
CMW 13992
N. cordaticola
CMW 28363
N. batangarum
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63/0.95
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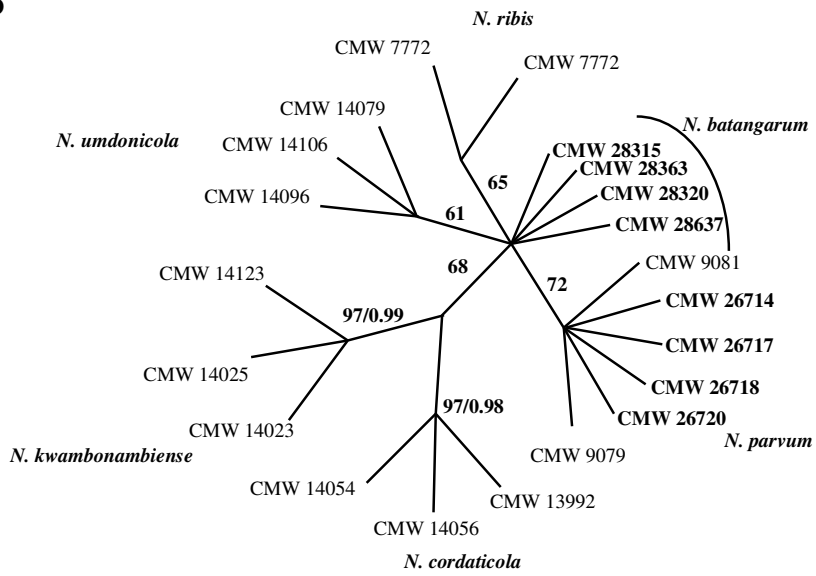
N. umdonicoola

D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CMW 14025
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97/0.98
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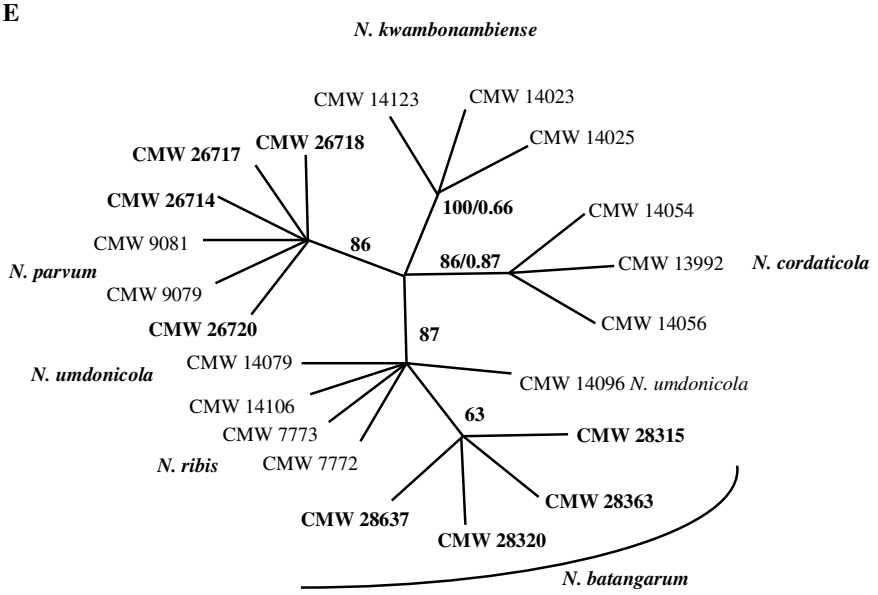
N. cordaticola

E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CMW 13992
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N. parvum
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CMW 14079
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N. ribis
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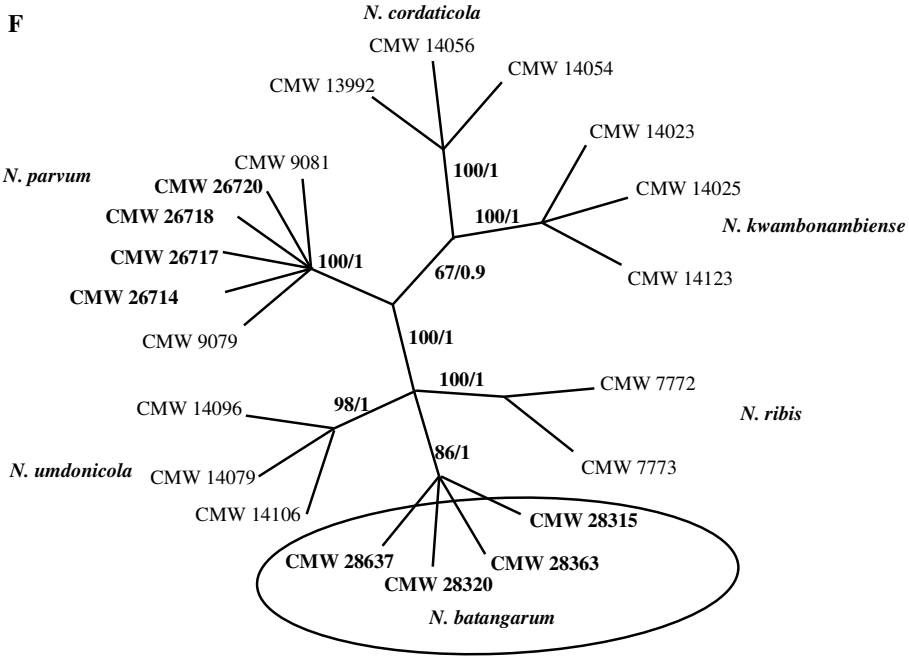
N. batangarum

F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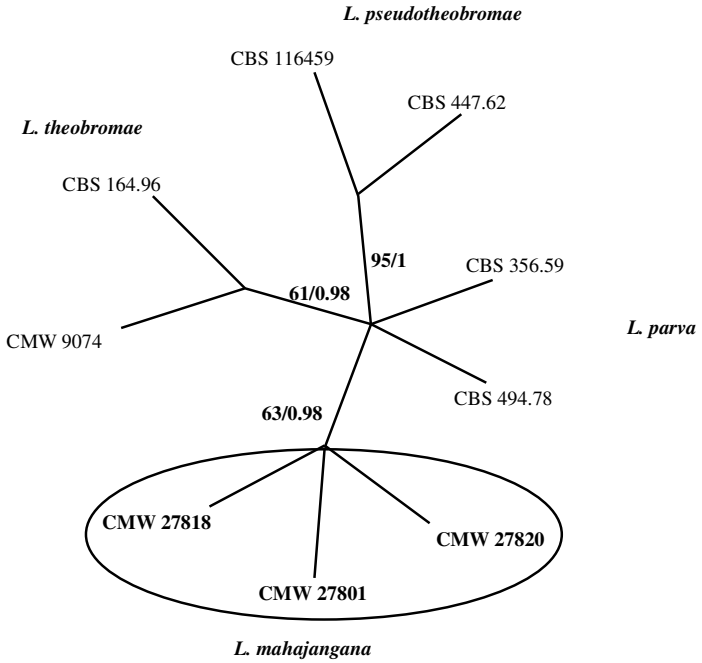
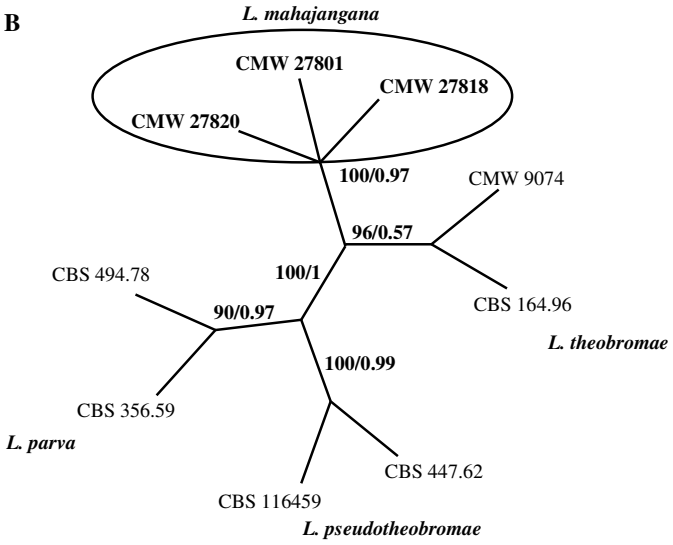
100/1
N. kwambonambiense
CMW 26717
100/1
67/0.9
CMW 14123
CMW 26714
CMW 9079
100/1
100/1
CMW 7772
CMW 14096
98/1
N. ribis
86/1
CMW 7773
N. umdonicola
CMW 14079
CMW 14106
CMW 28315
CMW 28637
CMW 28363
CMW 28320
N. batangarum

Figure 4. Most-parsimonious unrooted trees inferred from independent analyses of each data set (A = ITS; B = *tef* 1-α; C = *β-tub*; D = combination of sequences of the three loci) of the *Lasiodiplodia* spp. from *T. catappa* and related species. Bootstrap support (%) from 1000 replications followed by Posterior probabilities are given on the branch (BS/PP). Isolates marked in bold represent those obtained from *T. catappa*.

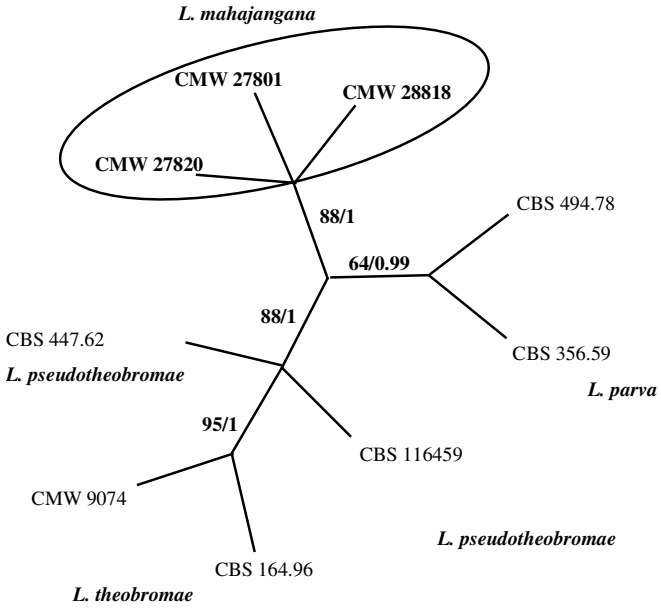
A


L. pseudotheobromae
CBS 116459
CBS 447.62
L. theobromae
CBS 164.96
95/1
CBS 356.59
61/0.98
L. parva
CMW 9074
CBS 494.78
63/0.98
CMW 27818
CMW 27820
CMW 27801
L. mahajangana

B


L. mahajangana
CMW 27801
CMW 27818
CMW 27820
100/0.97
CMW 9074
96/0.57
CBS 494.78
100/1
CBS 164.96
90/0.97
L. theobromae
100/0.99
CBS 356.59
L. parva
CBS 447.62
CBS 116459
L. pseudotheobromae

C


L. mahajangana
CMW 27801
CMW 28818
CMW 27820
88/1
CBS 494.78
64/0.99
88/1
CBS 447.62
L. pseudotheobromae
CBS 356.59
L. parva
95/1
CBS 116459
CMW 9074
L. pseudotheobromae
CBS 164.96
L. theobromae

D

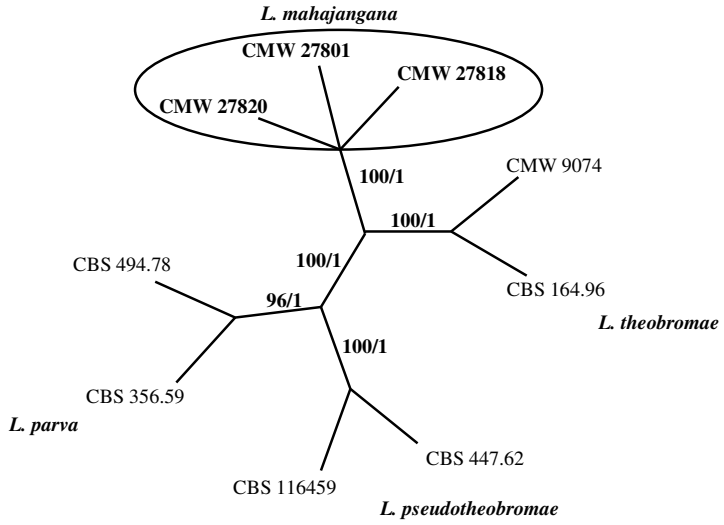

L. mahajangana
CMW 27801
CMW 27818
CMW 27820
100/1
CMW 9074
100/1
CBS 494.78
100/1
CBS 164.96
L. theobromae
96/1
100/1
CBS 356.59
L. parva
CBS 447.62
CBS 116459
L. pseudotheobromae

Figure 5. *Lasiodiplodia mahajangana.* (a) Pycnidium formed on pine needle in culture. (b) Paraphyses. (d) Conidiogenous cells with developing conidia. (d) conidia. (e) mature conidium showing septum. Bars: a = 500 µm; b, c, d, e = 10 µm.

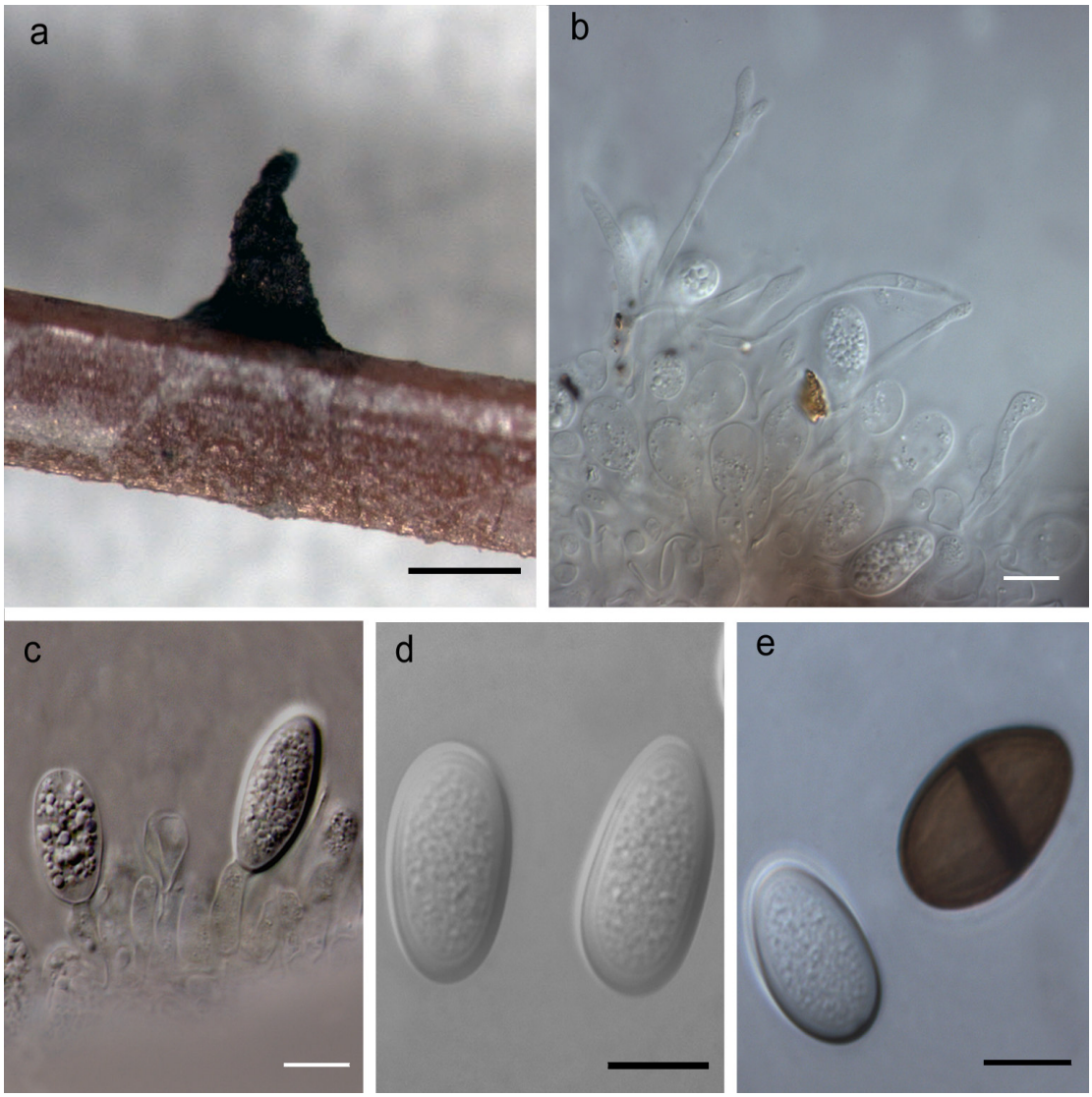

a
b
c
d
e

Figure 6. *Neofusicoccum batangarum.* (a) Pycnidium formed on pine needle in culture. (b, d) conidia. (c,e,f) Conidiogenous cells with developing conidia. Bars: a = 500 µm; b, c, d, e, f = 10 µm.

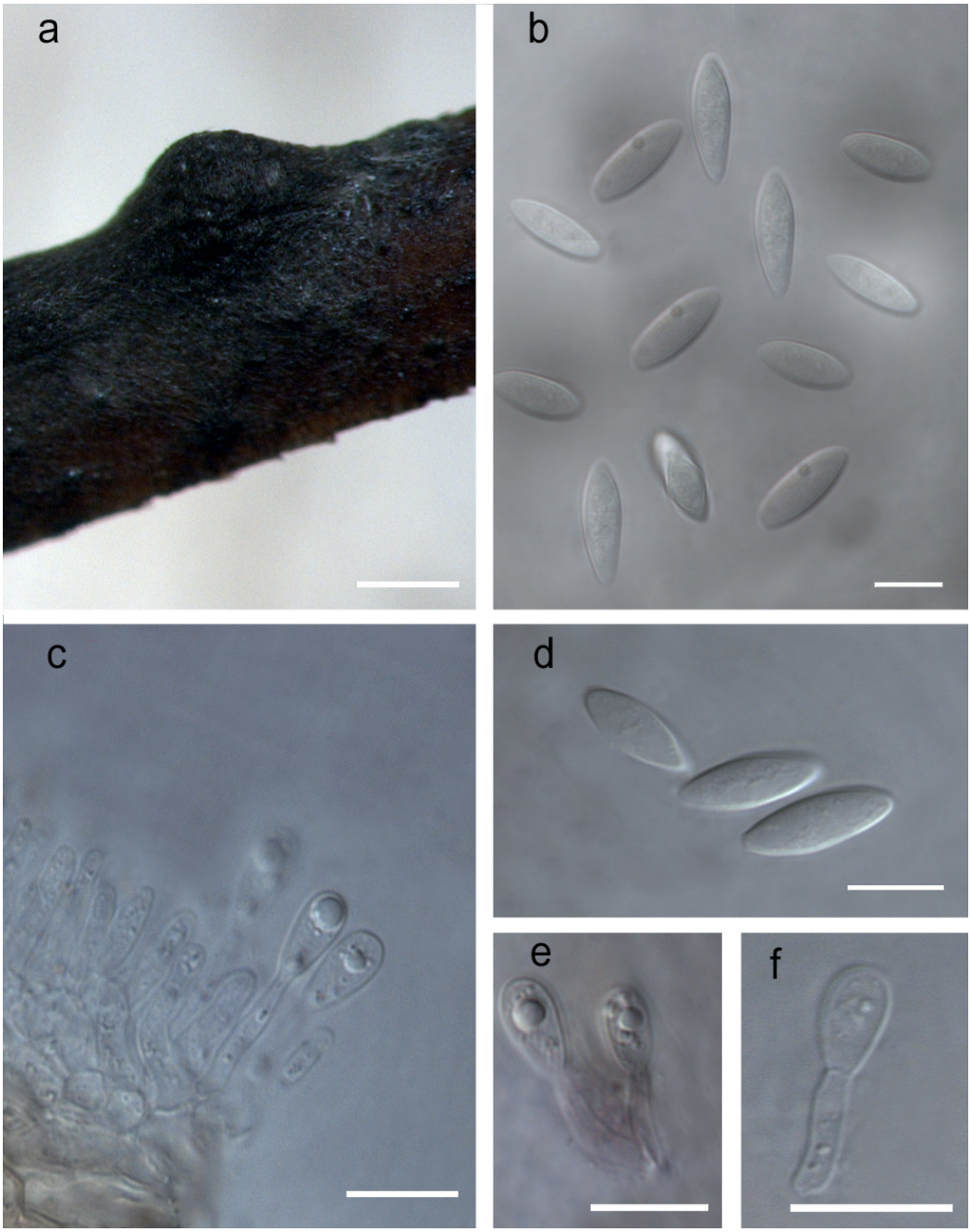

a
b
c
d
e
f

Figure 7. *Neofusicoccum batangarum* culture on MEA. (a) Front plate. (b) Reverse plate.

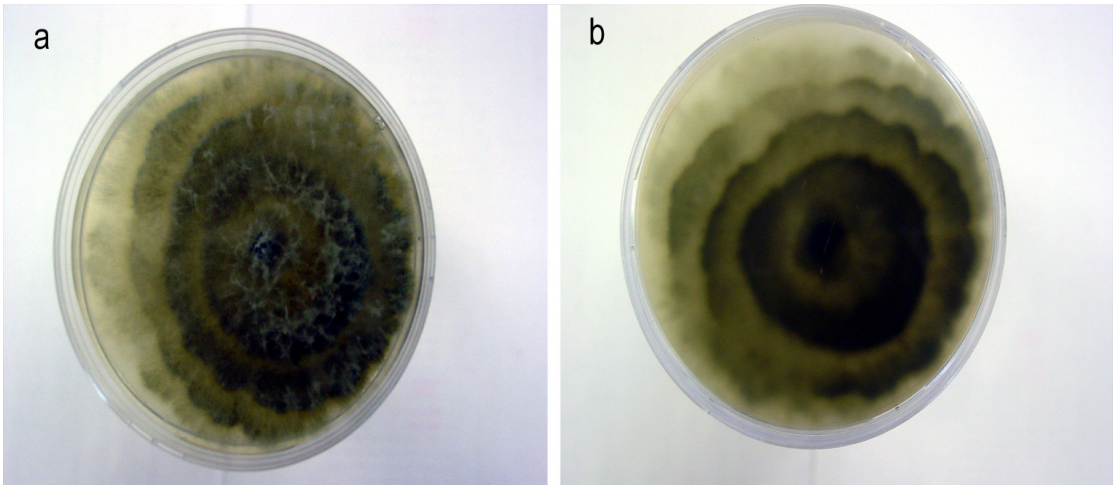

a
b

Figure 8. Distribution of Botryosphaeriaceae collected from *T. catappa* per locality.

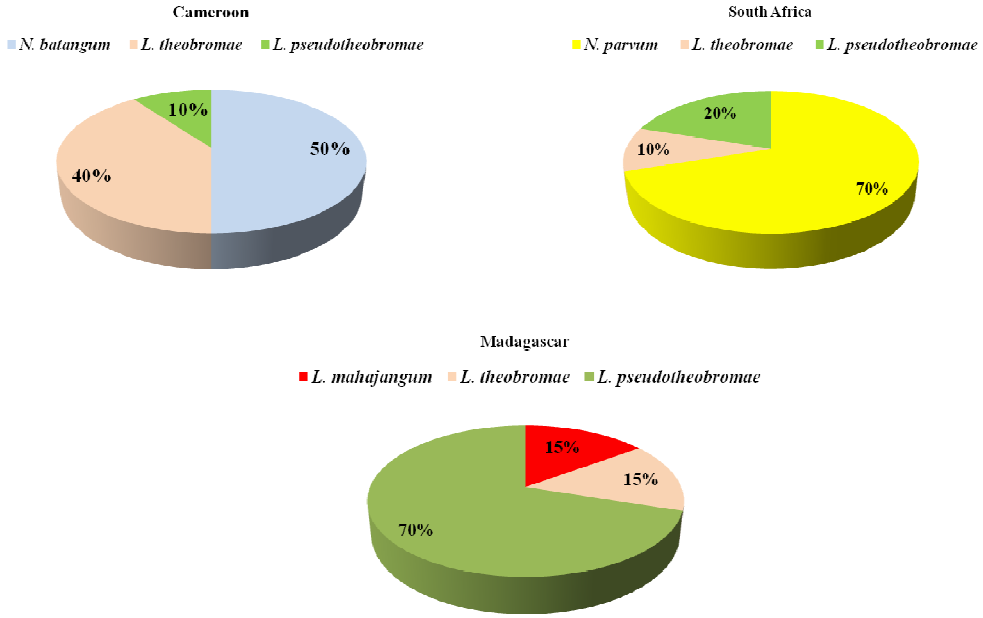

Cameroon
N. batangum
L. theobromae
L. pseudotheobromae
10%
50%
40%
South Africa
N. parvum
L. theobromae
L. pseudotheobromae
20%
10%
70%
Madagascar
L. mahajangum
L. theobromae
L. pseudotheobromae
15%
15%
70%

Figure 9. Mean lesion lengths (mm) of bark lesions for each Botryosphaeriaceae isolate six weeks after inoculation on *T. catappa* ($P < 0.0001$). *L. pseudotheobromae* (LPs), *N. parvum* (NP), *L. theobromae* (LT), *L. mahajangana* (LM), *N. batangarum* (NB), Control.

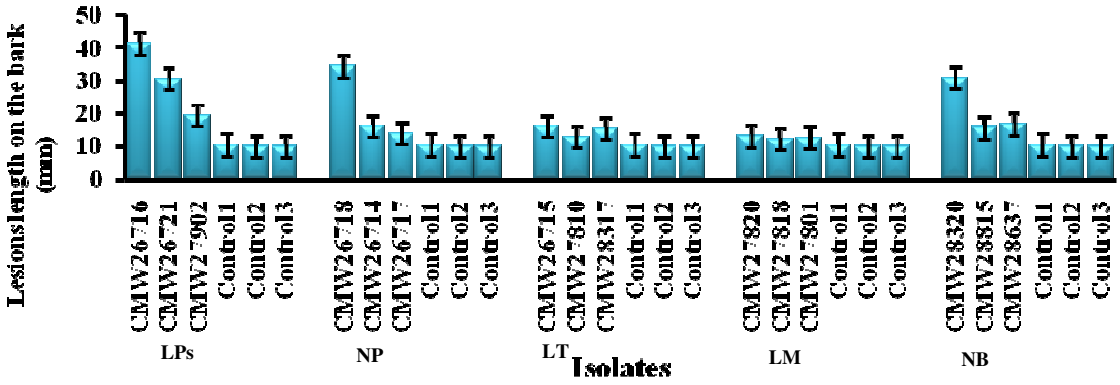
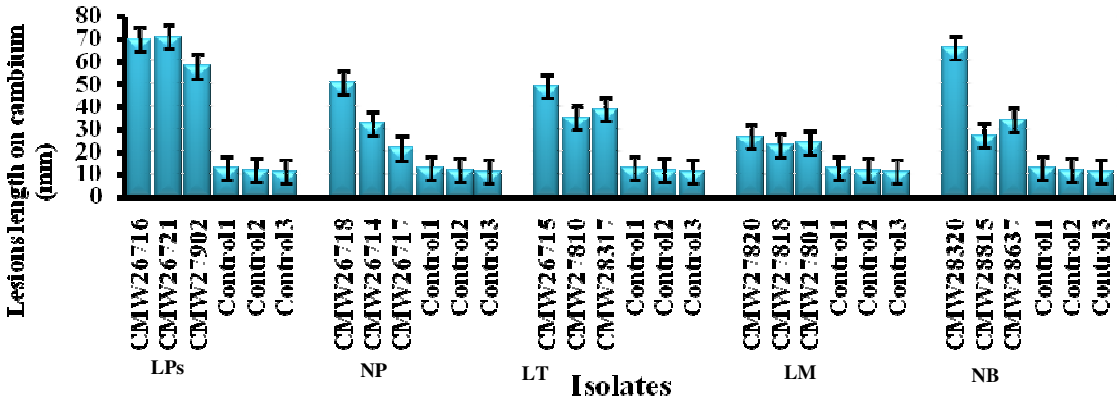

Lesionslength on the bark (mm)
50
40
30
20
10
0
CMW26716
CMW26721
CMW27902
Control1
Control2
Control3
CMW26718
CMW26714
CMW26717
Control1
Control2
Control3
CMW26715
CMW27810
CMW28317
Control1
Control2
Control3
CMW27820
CMW27818
CMW27801
Control1
Control2
Control3
CMW28320
CMW28815
CMW28637
Control1
Control2
Control3
LPs
NP
LT
LM
NB
Isolates

Figure 10. Mean lesion lengths (mm) on cambial lesions for each Botryosphaeriaceae isolate six weeks after inoculation on *T. catappa* ($P < 0.0001$). *L. pseudotheobromae* (LPs), *N. parvum* (NP), *L. theobromae* (LT), *L. mahajangana* (LM), *N. batangarum* (NB), Control.


Lesions length on cambium (mm)
80
70
60
50
40
30
20
10
0
CMW26716
CMW26721
CMW27902
Control1
Control2
Control3
CMW26718
CMW26714
CMW26717
Control1
Control2
Control3
CMW26715
CMW27810
CMW28317
Control1
Control2
Control3
CMW27820
CMW27818
CMW27801
Control1
Control2
Control3
CMW28320
CMW28815
CMW28637
Control1
Control2
Control3
LPs
NP
LT
LM
NB
Isolates