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GENOTYPIC AND PHYLOGEOGRAPHIC INVESTIGATION OF INDIGENOUS AND ALIEN *TAMARIX* SPECIES IN SOUTHERN AFRICA

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A Dissertation submitted to the Faculty of Science, University of Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

School of Animal Plant and Environmental Sciences

Johannesburg, August 2013

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Masters of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or any examination at any other University.

_____

Samalesu Guelor MAYONDE

_____day of _____20_____

ABSTRACT

Tamarix (Tamaricaceae) is from the Old World, but has become naturalized and invaded other parts of the world including South Africa. *Tamarix usneoides* is the only species native to southern Africa, but the exotic species *T. aphylla*, *T. chinensis*, *T. parviflora* and *T. ramosissima* have been reported to be present in South Africa and these *Tamarix* species are hypothesized to be hybridizing among themselves and with the indigenous *T. usneoides*. Among the exotic species, *T. chinensis*, *T. ramosissima* and their putative hybrids have become invasive. *Tamarix usneoides* is used in southern African mines for phytoremediation as it has the ability to hyper-accumulate sulphate and heavy metals from Acid Mine Drainage from Tailing Storage Facilities and excretes gypsum ($CaSO_4$). *Tamarix* species are morphologically and ecologically similar, making them difficult to distinguish and hybridization adds to the taxonomic confusion. Identification of *Tamarix* species in South Africa is of great importance because of the invasive potential of *T. chinensis*, *T. ramosissima* and their putative hybrids, and also because of the potential usefulness of *T. usneoides*. This investigation aimed to identify populations of pure *T. usneoides* that can be cloned for cultivation for phytoremediation on the mines, and to reveal the geographic origin of the invasive species to facilitate a biological control programme. Nuclear (ITS) and plastid (*trn*S-*trn*G) DNA sequence data and the multilocus Amplified Fragment Length Polymorphisms (AFLPs) markers were used in this study to characterize southern African *Tamarix* species and their putative hybrids. Phylogenetic analyses and population genetic structure confirm the presence of three *Tamarix* species in South Africa (*T. chinensis*, *T. ramosissima* and *T. usneoides*) with admixed individuals (*Tamarix* hybrids). The indigenous *T. usneoides* is clearly genetically distant from the alien species *T. chinensis* and *T. ramosissima*. Although the exotic species remain largely unresolved in the phylogenies, they are distinctly separated through AFLP markers. The *Tamarix* infestation in South Africa is dominated by hybrids between *T. chinensis* and *T. ramosissima*, and the parent species match their counterparts from their places of origin in Asia. These places of origin in Asia can provide the source of potential biological control agents. Some remote populations, e.g. Witbank, Goodhouse and Henkries in the Northern Cape Province/South Africa at the border with Namibia, of pure breeding *T. usneoides* have been identified and these should be used as a source of genetic material that can be propagated for planting on the mines for phytoremediation programmes.

DEDICATION

I dedicate this work to my family, particularly my father Mukazu Robert MAYONDE and my mother Musenga Bernadette Musumba for always telling me that YES you can do it. To my sisters Samba Lillian Mayonde, Kainda Seraphine Mayonde, Sompo Huguette Mayonde who have urged me to persevere and never give up. To my brothers Makaya Daddy Mayonde and Tshibwidi Chris Musumba for encouraging me and challenging me to always do better. I LOVE you guys. You will always occupy a special place in my heart.

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I am grateful to my supervisors Prof. Glynis Goodman-Cron and Prof. Marcus J. Byrne for their valuable time, guidance, patience, for trusting and encouraging me to pursue independent investigations. To Prof. Marcus J Byrne, thank you for your invaluable advice, mentorship and making me believe that I could do "science". I owe a huge debt of gratitude to my collaborator and friend Dr J.F. Gaskin for allowing me to work in his laboratory at the USDA Agriculture Service, Sidney, Montana, USA and to his family for welcoming me into their home. I would like to thank Ms Kimberly Mann from the Molecular Ecology laboratory at the USDA in Sidney for her assistance in developing the AFLP markers and conducting the work in a predictable amount of time. Many thanks to Dr Kelsey Glennon for her valuable contribution and advice on the population structure analysis using AFLP data.

I would like to extend my special Thanks to Dr. Jenny Botha, Hayden Wilson, Miranda Muller and Lutendo Mugwedi for their field assistance. I would like to pay tribute to the all the AP&ES staff members for welcoming me and working with me throughout my Master's program and to all the staff of the C.E. Moss Herbarium, especially Dr Renée Reddy, for their assistance and support. Special thanks to my friends and colleagues, Pumeza Cingo, Else Uys, Solomon Newete, McDonald Wanenge, Kendall Hauptfleisch, Archibold Sasa, Seyram Agbemenya, Jubilee Bubala for their priceless words of encouragement, and to Naweji Katembo who is not only a colleague but a brother. Many thanks to Nobukhosi Ngwenya for being my companion and source of joy, your love and kindness will always be appreciated.

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CHAPTER ONE: INTRODUCTION

A. PROBLEM STATEMENT

Tamarix usneoides (E Mey. ex Bunge) is one among other woody plants that is useful for phytoremediation in the gold mines of South Africa, due to its ability to hyper-accumulate sulphate and metals from Acid Mine Drainage and in the process excrete gypsum (Weiersbye et al., 2006). In 2005 three *Tamarix* species, *T. usneoides, T. ramosissima, T. chinensis* and their putative hybrids were observed at most of the gold Tailing Storage Facilities and mine waste-water pans in Gauteng, North West and Free State in South Africa (Weiersbye et al., 2006). In 2010 Dr. Goodman-cro, Dr. Byrne and I confirmed the identity of three distinct *Tamarix* species and their putative hybrids based on a molecular marker (the internal transcribed spacer regions of the ribosomal nuclear DNA), and morphological features (Mayonde, 2010). However, the lack of adequate signal in the internal transcribed spacer (ITS) regions to distinguish closely related species such as *Tamarix ramosissima* and *T. chinensis*, and to separate hybrids from pure breeding species in a phylogeny indicated that multilocus DNA markers (AFLPs) were needed to improve the lack of resolution through population genetics analyses. Additional DNA sequence data from chloroplast regions could also be used to indicate reticulation events by comparing positions of specimens in resulting phylogenies based on the plastid and nuclear regions. A phylogeographic investigation was therefore carried out using the above mentioned molecular markers primarily to identify populations of pure native *T. usneoides* that could be cloned for use in phytoremediation in the mines of South Africa, and reveal the geographic extent of hybrids among the three parental species.

Identification of the various alien *Tamarix* populations and their distributions across South Africa would also be useful in that they would provide vital information for management of alien invasive *Tamarix* species and their putative hybrids (Gaskin and Schaal, 2003, Gaskin and Schaal, 2002). Molecular systematics and population genetics could elucidate information about the target invasive species that would be critical to enhance the success of biological control interventions. Accurate identification of the trees species, evidence of hybridization and introgression, presence of cryptic species, population structure and origin of invasions, and better development of test plant lists will facilitate the development of a host specificity test for a

biological control potential agent (Gaskin et al., 2011). When considering classical biological control as a management tool, additional biological and ecological information on the invasive *Tamarix* species and its putative hybrids will give insight into the historical processes associated with their colonization history. This information includes founder events, genetic bottlenecks and whether there have been multiple introductions (Zhang et al., 2010).

I. LITERATURE REVIEW

Tamarix is an Old World genus, but many of its species have become naturalized and invaded other parts of the world (Gaskin and Schaal, 2003). *Tamarix usneoides* is the only *Tamarix* species indigenous to southern Africa (Obermeyer, 1976). However, the exotic species *T. aphylla* (L.) Karst., *T. ramosissima* Ledeb., *T. chinensis* Lour., and *T. parviflora* DC., have been reported to occur in South Africa (Bredenkamp, 2003). The exotic *Tamarix* species are hypothesized to be hybridizing among themselves and with the indigenous species (Hoffman et al., 1995, Weiersbye et al., 2006), and among them *T. ramosissima* and *T. chinensis* have been declared invasive in South Africa (Henderson, 2001, Holmes et al., 2005). Recent molecular analysis of a single locus nuclear DNA sequence marker (ITS) has provided some evidence that *Tamarix* plants are hybridizing in South Africa (Mayonde, 2010). The genus *Tamarix* is characterised by considerable morphological and ecological similarity among its species, making it one of the more taxonomically challenging genera among the angiosperms (Baum, 1978) and it remains one of the few in the Tamaricaceae family that can grow in different climatic and edaphic conditions; hence the species exhibit considerable phenotypic variation (DiTomaso, 1998). Many taxa are almost indistinguishable in the vegetative state (Crins, 1989), making their morphological identification difficult. Hybridization between the various species also plays a role in the taxonomic confusion in *Tamarix* (Wilken, 1993).

1.1. TAXONOMY, ORIGIN AND DISTRIBUTION OF *TAMARIX*

Tamarix is one of four genera in the family Tamaricaceae and is represented by 55 species world-wide (Heywood et al., 2007). The family Tamaricaceae grows most successfully in temperate and sub-tropical regions. The Tamaricaceae family has previously been placed as a sister family to Frankeniaceae because they share characters such as secondary chemistry, gland structure, and scale-like leaves (Kubizki, 2003). These two families were classified until recently

in the order Violales (Thorne, 1992, Cronquist, 1988); and, they have been placed in their own order, Tamaricales (Takhtajan, 1997, Dahlgren, 1983). The Tamaricaceae currently contains three larger genera: *Tamarix* L., *Myricaria* Desv. (10 species of shrubs), and *Reaumuria* L. (12 small shrub species), and two smaller genera, *Myrtama* Ovcz. & Kinzikaeva and *Hololachna* Ehrenb. that are taxonomically problematic (Crins, 1989, Kubizki, 2003).

Among the genera of the Tamaricaceae, *Tamarix* is the largest with 55 species (Heywood et al., 2007). *Tamarix* are native to the Mediterranean countries, southern Europe, China, India, Mongolia, North Africa and south western Africa (Baum, 1967, Heywood et al., 2007). The genus is thought to have been named after the Tamaris River in Spain (DiTomaso, 1998). *Tamarix* plants are shrubs, semi-shrubs and tall trees that can grow up to 18 m in height. They are adaptable halophytic or xerophytic plants mostly with multiple stems and slender branches (Brotherson and Winkel, 1986). Young branches are reddish brown in colour, sometimes black with light-green coloured leaves. Leaves of *Tamarix* are taxonomically useful as their shape and attachment modes vary according to species, e.g. sessile vs. vaginate (Baum, 1978). They are scale-like, about 3 mm in length (Baum, 1978) and usually contain salt glands (Bredenkamp and Phepho, 2008). This explains *Tamarix*'s common name 'Saltcedar'. The fact that *Tamarix* exudes salts enables it to grow in and tolerate soils with high salt concentrations (ranging from 650 to 36000 ppm) (Brotherson and Winkel, 1986).

1.2. THE BIOLOGY AND ECOLOGY OF TAMARIX

1.2.1. Reproduction and seedling establishment

Tamarix produces flowers that are usually bisexual, rarely unisexual and *Tamarix* plants are either monoecious or dioecious. Usually the flowers have five or four sepals and a corresponding number of petals. Flowers have five or numerous stamens which are free or rarely fused and are inserted into a fleshy, glandular, hypogynous disc (Obermeyer, 1976). *Tamarix* trees can flower anytime during the entire growing season (Everitt, 1980), producing seeds that allow them to colonize areas soon after summer rains (DiTomaso, 1998). The plants can outcross or self-pollinate, and the small white to pink flowers are pollinated by many different species of insects and possibly also by wind (Brotherson and Field, 1987). *Tamarix* produces fruits that are three-to five-valved capsules (Brotherson and Field, 1987), containing about half a million light small viable seeds (0.1 mg each) (DiTomaso, 1998), with a tuft of hairs on the ends to aid in wind dispersal; they are also easily deposited along sand banks and riverbeds by water (Brotherson

and Field, 1987). The seeds of *Tamarix* have a high initial viability (Neill, 1985), but under normal circumstances, they remain viable for only five weeks. It is because of their short-lived viability that they must come into contact with water within a few weeks of dispersal (DiTomaso, 1998). Although the seeds can germinate rapidly, new seedlings usually require wet soils for several weeks (Horton, 1974). *Tamarix* seedlings can grow 3 to 4m in a single growing season given ideal conditions (Friederici, 1995). The roots of *Tamarix* seedlings appear to grow more slowly than most riparian plants which seems to affect the survival rate of the seedlings (Everitt, 1980). Various studies (Brotherson and Field, 1987, Brotherson and Winkel, 1986, Everitt, 1980) have shown that for most *Tamarix* seedlings to establish successfully they require a combination of saturated soil and open sunshine with little competition (e.g. river bank or sand bar) for the first two to four weeks of life.

1.2.2. Root growth and function

Tamarix is classified as a facultative phreatophyte because of its extensive deep root system that reaches underground water tables (Kerpez and Smith, 1987). Nonetheless, under certain circumstances *Tamarix* can grow where no ground water is accessible. The root system of T*amarix* is responsible for its competitiveness and survival under stress (DiTomaso, 1998). *Tamarix* trees are capable of surviving severe drought by dropping their leaves, thus reducing evapotranspiration rates (Horton and Campbell, 1974). *Tamarix* populations are therefore capable of withstanding lengthy periods of drought despite the reduction in growth rates (DiTomaso, 1998). Roots of *Tamarix* can vigorously resprout into new plants if the top growth is removed or damaged (Everitt, 1980).

1.2.3. Soil type, salinity and water use

Tamarix can grow on a wide variety of soils (silt loams and silt clay loams) with high organic matter, intermediate moisture and high water tables, mineral gradients and environmental stress conditions (Brotherson and Field, 1987), but very saline soils are best (DiTomaso, 1998). However, alkaline conditions (pH 7.5) are optimal, although *Tamarix* is also commonly found on more acidic soils (Brotherson and Winkel, 1986). The trees have high water consumption and one of the highest evapotranspiration rates of any phreatophyte (Brotherson et al., 1984, Dahm et al., 2002). Evapotranspiration of *Tamarix* (saltcedar) has been estimated to range from 0.7 to 3.4m/year, depending on the measurement technique and other parameters such as climatic conditions, and the duration of measurements (Shafroth et al., 2005). In severely infested areas *Tamarix* can dry up springs, drain pools, and even perennial streams due to their high water

consumption (Johnson, 1987). To the best of my knowledge, no intensive studies have been conducted in South Africa to evaluate the rate of *Tamarix* water consumption in comparison to the indigenous riparian plant species. Although there are no studies conducted, it is likely that there are significant negative impacts caused by alien invasive *Tamarix* to the riparian ecosystems and the biodiversity in South Africa.

1.3. *TAMARIX* IN SOUTH AFRICA

Tamarix usneoides occurs naturally in southern Africa and is found mostly in sandy river banks, riverbeds and desert paleaochannels (Kubizki, 2003, Obermeyer, 1976). Among the exotic species present, *T. ramosissima* has become invasive (Holmes et al., 2005), and *T. chinensis* is also thought to be part of *Tamarix* invasion in South Africa (Henderson, 2001). *Tamarix ramosissima* is native to temperate Asia distributed from Turkey to Korea, while *T. chinensis* is indigenous to China, Korea and Japan (Baum, 1978). Until this study there was no exact estimation of the extent of *Tamarix* invasions (Fig. 1.1) in South Africa and which were the most dominant invasive species. However, *Tamarix ramosissima* and *T. chinensis* occupy about 470 000–650 000 ha of the western USA since their introductions in the 1800s (Zavaleta, 2000). Within the USA invasion, 83–87% incidence of hybridization has been evidenced through multi locus DNA markers (amplified fragment length polymorphisms) (Gaskin and Kazmer, 2009). Their invasive potential, and the morphological confusion between *T. ramosissima* and *T. chinensis* and their ability to hybridize make them the focal alien *Tamarix* species in South Africa. They are difficult to separate morphologically (Crins, 1989), with some slight differences in sepal margin, petal shape, and filament insertion (Baum, 1978).



FIGURE 1. 1: An example of infestation of the pinkish flowering *Tamarix* hybrids (*T. chinensis* x *T. ramosissima*) in the Sondags River in the Eastern Cape, South Africa.

1.4.CLASSIFICATION AND DESCRIPTIONS OF THE *TAMARIX* SPECIES PRESENT IN SOUTH AFRICA

1.4.1. Tamarix usneoides

Tamarix usneoides forms part of Series 3 called Vaginates (Bge.) Bge., (Baum, 1978).This group has *T. aphylla* (L.) Karst. as the type species (Baum, 1978). It includes species such as *T. angolensis* Ndz., *T. bengalensis* Baum., and *T. dioica* Roxb., and *T. kermanensis* B.R. Baum. This group is characterised by leaves which are vaginate (sheathing around the stem) or pseudo-vaginate, and entirely glabrous with no papillae present. In this series *T. usneoides* is the only species native to southern Africa, where it occurs in Angola, Namibia and South Africa. *Tamarix aphylla* is native to North Africa and the Middle East (Baum, 1978). As noted previously *T. usneoides* is mostly found in sandy and salty dunes and flats, rocky deserts and river beds, and it is a dioecious tree that usually flowers from July to October, but sometimes between January or February and May. Often, *T. usneoides* is a shrub (Fig. 1.2), but it can grow to 18 m with brown, later grey bark that is entirely glabrous. The leaves are vaginate (1.25 cm long) with short to inconspicuous herbaceous vaginate bracts that are shorter than, or sometimes slightly exceed the pedicels. It bears a loose inflorescence with racemes that are 2–6 cm long and 5–6 cm broad. *Tamarix usneoides*' pedicels are half as long as their sepals which are entire and 1–1.25 cm in length. The corolla is pentamerous and persistent. The petals are 2.25 cm long, usually inequilateral, ovate to somewhat elliptic (Baum, 1978). Most importantly, *T. usneoides* has a haplostemonous (series of stamens that are equal in number to and aligned with the petals) androecium comprising five antesepalous stamens (stamens that are equal in number to and aligned with the sepals). The insertion of filaments is peridiscal (filaments attached at the periphery of the flower disc), while the disc shape is synlophic to para-synlophic in female flowers and hololophic to paralophic in male flowers (Baum, 1978).

Tamarix usneoides is mostly used for phytoremediation on the mines in southern Africa. The trees remove pollutants from the environment (Salt et al., 1998). It is known to hyper-accumulate sulphates and metals from Acid Mine Drainage (AMD) from Tailing Storage Facilities (TSFs) and in the process it excretes gypsum ($CaSO_4$) (Weiersbye, 2007, Weiersbye et al., 2006). Therefore, *Tamarix usneoides* reduces environmental pollutant caused by mining activities. Alien invasive *Tamarix* species and their putative hybrids are present on the majority of gold

Tailing Storage Facilities (TSFs) and mine waste pans in the Gauteng, North West and Free State (Weiersbye et al., 2006).

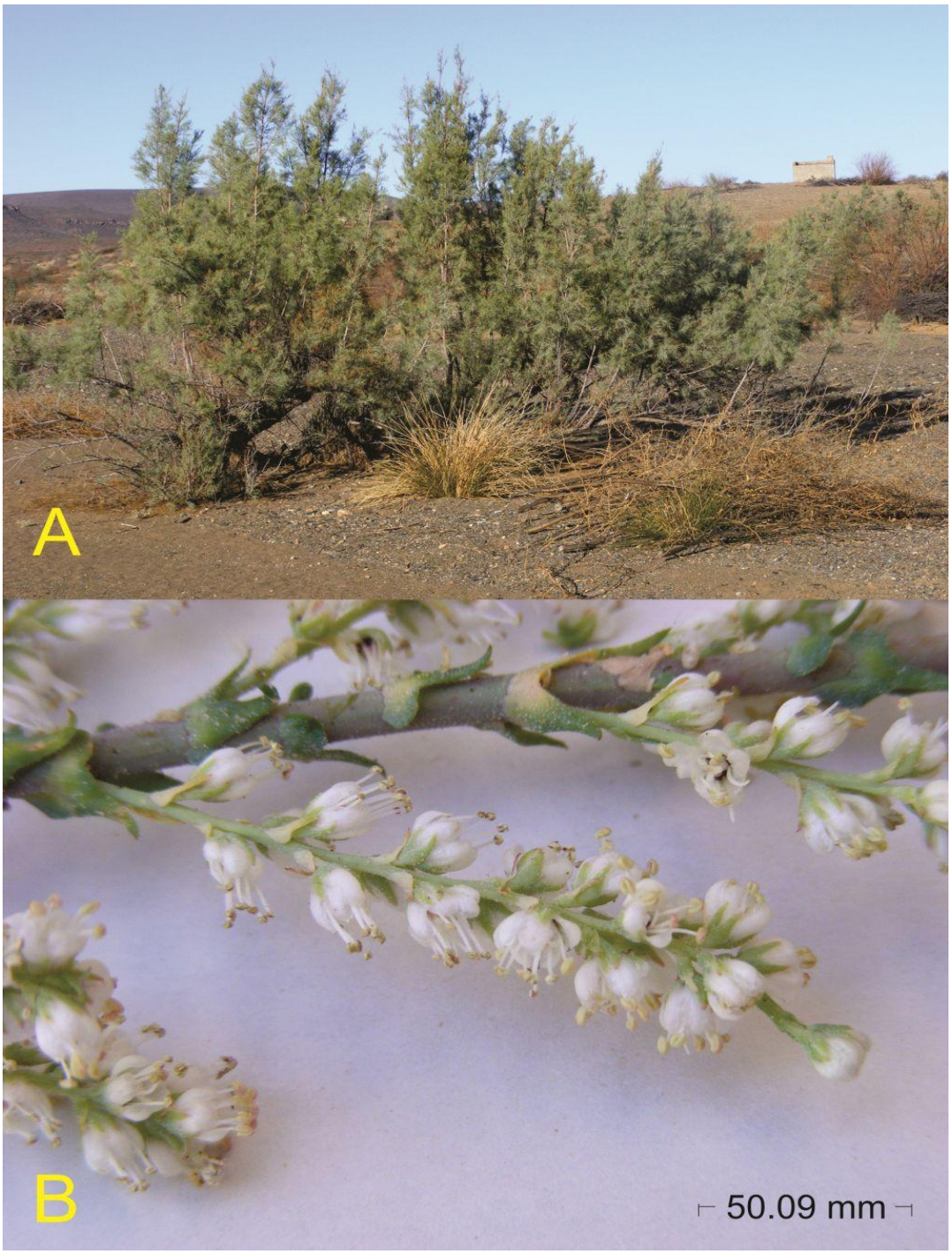

FIGURE 1. 2: Habitat and some morphological features of the indigenous *Tamarix usneoides*: A) growing alongside a road on a semi-desert plain in Northern Cape, South Africa; B) a *T. usneoides* raceme showing white flowers with white petals, five stamens, vaginate leaves and bracts.

1.4.2. Tamarix ramosissima

Tamarix ramosissima (Fig. 1.3) falls under Series I, Gallicae Baum, with *T. gallica* L. as the type species (Baum, 1978). This series is characterised by sessile leaves with a narrow base, and they are entirely glabrous with no papillae. *Tamarix ramosissima* is a shrubby tree of about 1 to 6 m tall. Morphologically, it has a reddish-brown bark which is entirely glabrous. In contrast to *T. usneoides*, its leaves are mostly sessile with a narrow base (1.5–3.5 mm long). Racemes are mostly 1.5–7 cm long and 3–4 cm broad, with bracts that are longer than the pedicels. However, the pedicels are shorter than the pentamerous calyx. Its petals are 1–1.75 cm long and are obovate to broadly elliptic-obovate and in-equilateral. Like *T. usneoides*, *T. ramosissima* has an androecium that is haplostemonous with five antesepalous stamens. Most importantly, the insertion of filaments in *T. ramosissima* is hypodiscal (filaments born under the disc); compared to peridiscal in *T. usneoides*; and it has a hololophic disc. The flowering period of *T. ramosissima* is usually between May and October (Baum, 1978). This particular plant species grows mainly on sandy shores of lakes, salty river banks and salty steppes (Baum, 1978, DiTomaso, 1998). *Tamarix ramosissima* is from Eurasia and is widely distributed from eastern Turkey to Korea (Baum, 1978; Heywood et al. 2007).

1.4.3. Tamarix chinensis

Tamarix chinensis is from series Laxae Baum and has *T. laxa* Willd as the type species (Baum 1978). The tree has brown to black bark which is entirely glabrous. Its leaves are sessile with a narrow base, similar to *T. ramosissima.* The vernal (spring flowering) inflorescences are pyramidal with many dense racemes, whereas the aestival (summer flowering) inflorescence is loose and has slender racemes. Racemes are mostly 2–6 cm long and 5–7 cm broad. Bracts are the same as pedicels but slightly longer, the lower bracts of the vernal racemes are oblong equalling the pedicels while the upper bracts and those of the aestival racemes are longer, narrowly triangular, acuminate, entire and herbaceous. *Tamarix chinensis* has sepals that are 0.75–1.25 cm long, subentire, trullate-ovate to narrowly trullate-ovate, and acute. The sepals are somewhat connate at the base in the aestival inflorescence. Its petals are elliptic to ovate, usually ovate-elliptic, rarely obovate and are 1.5–2.25 cm long. The insertion of filaments in *T. chinensis* is hypodiscal in the vernal flowers, hypo-peridiscal (with 1–3 hypo, 2–4 peridiscal) in the aestival flowers, and the disc is hololophic (Baum 1978). This species flowers between March and November and grows best on river banks, humid plains and mountain slopes. *Tamarix*

chinensis is mainly distributed in Mongolia, China and Japan (Baum 1978; Heywood et al. 2007).



FIGURE 1. 3**:** Shrubby *Tamarix ramosissima* with pink flowers growing along a road in Cape Town where it is mostly planted as ornamental.

The above morphological descriptions of the three principal *Tamarix* species occurring in southern Africa are based on the study by Baum (1978). As noted previously, *T. ramosissima* and *T. chinensis* are difficult to separate morphologically (Crins, 1989). The morphological confusion between *T. ramosissima* and *T. chinensis* (Crins 1989) exacerbated by hybridization (Gaskin and Schaal, 2002, Mayonde, 2010) and their invasive potential (Henderson, 2001) make them the focal alien *Tamarix* species in South Africa. The natural ranges of the two alien *Tamarix* species in South Africa (*T. ramosissima* and *T. chinensis*) overlap for approximately 4 200 km across China to Korea (Gaskin and Schaal, 2002).

1.5.CONTROL OF INVASIVE *TAMARIX*

Various control measures have been implemented to control the alien invasive *Tamarix* species in South Africa, and this include mechanical clearance through, cutting trees at ground level or uprooting them, or even burning them (Holmes et al., 2005). Herbicides are used to control the trees in riparian zones. None of these methods is known to be efficient as the alien invasive *Tamarix* resprout and grow rapidly after being treated (Holmes et al., 2005). However, the introduction of classical biological control agents (such as *Diorhabda elongata* Brullé), is known to be an effective way of controlling *Tamarix* in the USA (Milbrath and DeLoach, 2006). The United States Department of Agriculture-Agricultural Research Service (USDA-ARS) initiated a biological control in the late 1960s to help combate the *Tamarix* invasion (Hudgeons et al., 2007b). *Diorhabda elongata* Brullé is a group of *Tamarix*-feeding leaf beetles from the genus *Diorhabda* Weise and comprised of five sister species *D. elongata*, *D. carinata*, *D. sublineata*, *D. carinulata* and *D. meridionalis* (Tracy and Robbins, 2009). Four species of the *Diorhabda* have been approved for release as biological control of invasive *Tamarix* in the US (Knutson et al., 2012). The establishment of the *Diorhabda* beetles seems to be dependant to the climate. Tracy et al., (2009) developed a climate-matching model to predict areas that will be suitable for each one of the four introduced beetle species in the U.S. *Diorhabda elongata* and *D. carinata* have been observed to establish better and defoliate *Tamarix* populations which are between 32.5 and 34.0°N/southern parts of the US (Hudgeons et al., 2007a, Hudgeons et al., 2007b). On the other hand *Diorhabda sublineata* and *D. carinulata* are known to establish better in the northern part of the country (Hudgeons et al., 2007b). Thus far no biological control agent has been introduced into South Africa to control invasive *Tamarix* populations and not much research has been done on the entofauna on invasive *Tamarix* species in South Africa (Buckham, 2011).

1.6. IMPACTS OF *TAMARIX*

In riparian zones *Tamarix* may have positive effects on the ecosystem: stabilization of stream banks by its extensive root system and prevention of flooding by taking up water are some of the major useful effects (DiTomaso 1998). However, heavy investation can result in flooding and inundation incidences which can be caused by the narrowing of the water channel (Blackburn et al., 1982). *Tamarix* is used also to desalinate the lower soil profiles through the process of excreting salts (Brotherson and Field, 1987). Its crowding behaviour provides favourable shade for birds and animals and its stems are an important source of firewood (Friederici, 1995). For

example, a southwestern sub-species of willow flycatcher (*Empidonax traillii extimus*) has been documented to use *Tamarix* foliage as nesting habitat in several locations in the lower Colorado River system and new Mexico, USA (Dudley and Bean, 2012). *Tamarix pentendra* and *T. aphylla* are grown as hedges or windbreaks in Mediterranean coastal regions (Kubitzki 2003). Beekeepers utilise Salt cedar trees for their pollen and nectar for honey production (Everitt, 1980). *Tamarix articulate* and *T. gallica* have some insects that make galls on the trees which can be a source of tannin, dyes and medical extracts (Kubitzki 2003). *Tamarix* species (i.e. *T. gallica, T. africana, T. ramosissima*) are frequently used as ornamental plants for their feathery appearance and their catkin-like inflorescences (DiTomaso 1998; Kubitzki 2003). Their aesthetic value is so appealing that these plants have been brought into many countries and planted in the gardens; as a result their introduction and subsequent spread has rendered some of the alien species (especially the pink flowering species) invasive in certain places (DiTomaso, 1998). These features were considered desirable until the twentieth century (DiTomaso 1998).

Alien invasive *Tamarix* species are thought to have tremendous negative side effects on both the ecosystem and the environment because of their high water consumption, deposition of salts on underlying soils, and modification of hydrologic regimes (Bailey et al., 2001, Zavaleta, 2000). Large infestations have caused significant changes in flooding and erosion patterns, fire frequency, and both plant and animal diversity (DiTomaso 1998). Therefore, the negative attributes of the alien species of *Tamarix* far out-weigh its desirable features noted above.

1.6.1. Flooding and erosion

Several authors (e.g. Friederici 1995), have noted that narrowing of a water channel increases the rate of water flow and the potential and severity of subsequent floods; and this happens when stream channels are stabilized as they become more immobile and inflexible, which progressively restricts channel width by increasing sediment deposition. Heavy infestations of *Tamarix* also significantly alter the hydrology of an area because of their extensive root system (DiTomaso, 1998). *Tamarix* plants usually establish themselves farther out into the river channel, and this process will continue until stream flow is severely reduced. Although the extensive root system of *Tamarix* species is stable and resistant to erosion, a *Tamarix* infested river, e.g. the Gila River in Arizona, USA, resulted in a 30% increase in water flow velocity and a 13% increase in water depth than a normal non-infested transect on the same river according to the bureau of reclamation study (DiTomaso 1998). Further *Tamarix* infestations established in the

river beds until the stream was severely reduced which finally increased the incidence of flooding and water inundations (Blackburn et al., 1982).

1.6.2. Animal and plant diversity

Tamarix infestations can form dense stands which may comprise 80% of the vegetation cover in riparian zones (DiTomaso, 1998). However, such infestation can cause significant reductions in native woody and herbaceous diversity and abundance (Hughes, 1993). The displacement of native plant species and some habitat changes caused by invasive *Tamarix* species negatively affect most wildlife species (Brotherson and Winkel, 1986). Although some wildlife successfully survives in *Tamarix* infestations, most species are negatively impacted by the displacement of native riparian plant species. For example, *Tamarix* can provide nesting sites for doves, but their populations are usually higher in nonsaltcedar communities (Shrader, 1977), and *Tamarix* trees provide less food for these birds than riparian trees such as mesquite (Kerpez and Smith, 1987). However, aside from the doves, bird species such as Gambel's quail prefer honey mesquite and cottonwood habitats but can utilise *Tamarix* communities for shelter, although not for nesting (Shrader 1977). Some riparian bird species are known to continue breeding in *Tamarix* infested habitats, but the breeding densities for most bird species are reported to have dramatically declined in the western U.S.A (DiTomaso 1998).

Insect species in South Africa have the capacity to discern between the *Tamarix usneoides* and the exotic *Tamarix* species, where the exotic species were observed to have less insect herbivore pressure compared to the indigenous one and the insect diversity on them was low on *T. ramosissima* and its hybrids (Buckham, 2011). Therefore, these impacts signify that alien *Tamarix* infestations are not conducive for optimal bird or insect biodiversity althopugh further investigation needs to be conducted on the entomofauna of *Tamarix* in South Africa.

1.7. BIOLOGICAL INVASION AND ALIEN INVASIVE *TAMARIX*

Biological invasion has increasingly been a major subject of research in ecology. Over recent years, the increase in international travel and trade has facilitated the movement of species around the globe, and has increased their level and frequency in the introduced areas (Alpert, 2006, Mack, 2003). Invasive species are defined as organisms that expand beyond their natural range and population density, and they normally cause ecological or economic harm (Richardson et al. 2000). Ecological impacts of biological invasions are mostly on indigenous species,

community dynamics and the overall structure and function of ecosystems (Catford et al. 2009) through various mechanisms (e.g., competition, predation, hybridization). Such impacts by invasive species have rendered biological invasion a subject of much interest. The theory of biological invasions was first described in the 19th century by Darwin in *The Origin of Species*. In his treatise, Darwin knew about and discussed biological invasions. Contemporary ecologists and evolutionary biologists of the 20th century have had similar thoughts and ideas as Darwin on the theory of biological invasions (Ludsin and Wolfe, 2001). Research studies aimed at describing, understanding, and predicting species invasions have dramatically increased in the 20th century because of the increase in biological invasions and their impacts (Williamson, 1996). Clearly, biological invasions cause serious environmental, economic, and health damage such that the invasive weeds require control or eradication. Early efforts to control alien invasive plant species were based on physical removal (mechanical) and the use of non-selective inorganic compounds (Briese, 2004). However, these two methods are expensive, have tremendous environmental side effects (i.e. soil erosion, damage of non-target organisms and spread of toxic substances such as arsenic, copper and sodium salt over the landscape), and they only provide temporary control of the weeds. An alternative control method has been sought and the discipline of biological control has evolved (Briese, 2004).

1.7.1. Classical biological control of alien invasive plant species

Biological control is defined as the use of an biological organism to reduce the population density of another organism (Bale et al., 2008). It implies the deliberate release of specialist natural enemies from the alien organism's native range to reduce its abundance in the introduced range below an ecological and/or economic threshold (Muller-sharer and Schaffner, 2008). Therefore, this includes the control of weeds, animals and diseases (Bale et al., 2008). Molecular based techniques have much to offer to assist in reaching this goal (Briese, 2004, Goolsby et al., 2006) by resolving taxonomic issues, elucidating geographic sources of invaders, detecting hybridisation and introgression events, tracking dispersal and spread of alien invasive plants and assessing the importance of genetic diversity in invasion success (Le Roux and Wieczorek, 2008).

1.7.2. Hybridization as a driving force of invasion biology

When new species are introduced into a new region, usually they come into contact with closely related species or genotypes which were previously isolated from that species for a long time. This interaction creates potential for hybridization events, often producing individuals with high genotypic fitness in the newly-invaded habitat (Gaskin and Kazmer, 2009). High genetic variation in plants may result in the creation of novel hybrids or genotypes (Abbott, 1992) through base substitutions (single nucleotide substitutions), insertion or deletion events (indels), inversion of DNA segments and/or their rearrangement (Le Roux and Wieczorek 2008). Hybridization is regarded as an extremely rapid mechanism for increasing genetic variation by producing novel gene combinations that can potentially enhance the evolution of invasiveness (Lee, 2002, Schierenbeck and Ellstrand, 2009). For example, a case of three hybrid species of *Helianthus* in the USA, which have unique combinations of genes acquired from each of their parent species, survive in extreme habitats that are not suitable for either of the parent taxa (Riesberg et al., 2003). A number of recent studies using molecular tools have revealed the hybrid status of plant invasions, showing them to be a significant proportion of an invasion (Gaskin and Kazmer, 2009, Moody and Les, 2007, Gaskin, 2003, Gaskin and Schaal, 2003). Interspecific hybridization occurs either between an indigenous and an invading plant species or between two invading species. Hybridization and introgression (natural back-crossing between hybrids and parental lineages) between native and invasive taxa create difficulties in determining the natural status of indigenous species (Petit, 2004). This situation can be critical to invasive species management, especially when considering a biological control program.

1.8. IDENTIFICATION OF *TAMARIX*

Identification of *Tamarix* species is of great importance in South Africa because of the prospective invasiveness of some species and the potential for their biological control, and the probable usefulness of the indigenous species. *Tamarix* species can potentially be identified either morphologically or by molecular data. Morphological identification of *Tamarix* has received extensive attention in the recent past (Obermeyer 1978; Baum 1978, Bredenkamp and Phepo 2008; Mayonde 2010). However, the molecular method of plant species identification offers a more reliable and consistent method of identifying *Tamarix* species, which is important for critical disciplines such as conservation biology and biodiversity research (DeSalle et al., 2005).

1.8.1. Morphological identification of Tamarix

Willdenow (1816) was the first person to monograph *Tamarix*; he described 16 species (Gaskin and Schaal, 2003). Bunge (1852) later monographed the genus *Tamarix*, identifying 51 species. His taxonomic diagnosis was based on whether the racemes were produced on the old branches (vernal) or on the newly produced branches (aestival). In contrast, Baum (1967) considered this character diagnostically unreliable. The last revision of the genus *Tamarix* was conducted by Baum (1978) and later supplemented by Qaiser (1983) who worked on the Pakistani species. Various scientists (e.g. Bredenkamp and Phepo 2008) have morphologically described and confirmed the identity of the four *Tamarix* species presently known in South Africa. Mayonde (2010) identified eight reliable morphological characters (Table 1) from Obermeyer (1976), Baum (1978), and Bredenkamp and Phepo (2008) to distinguish between three of these *Tamarix* species (pure breeding) and also recognise some *Tamarix* hybrids.

TABLE 1. 1: Important stable characters for morphological identification of Tamarix species and their literature sources (from Mayonde 2010).

Character	*Tamarix usneoides*	*Tamarix ramosissima*	Literature source
1. Salt glands	Present and abundant	Absent	Bredenkamp and Phepho 2008
2. Petal shape	Ovate-elliptic	Obovate-elliptic	Henderson 2001; Baum 1978
3. Insertion of filaments	Peridiscal	Hypodiscal	Baum 1978
4. Petal colour	White	Pink-purple	Henderson 2001
5. Leaf shape and attachment	Vaginate	Sessile	Baum 1978
6. Leaf shape	Over-lapping	Not overlapping	Obermeyer 1976; Henderson 2001
7. Bracts shape and attachment	Vaginate	Sessile	Baum 1978
8. Disc shape	Male: Hololophic to paralophic	Bisexual: Hololophic	Baum 1978
	Female: Synlophic to para-synlophic		

1.8.2. Molecular approaches and population genetics of alien invasive plants

Molecular tools in systematics provide the means to investigate the identity of different plant species at the DNA level, showing genetic variation within and among populations, and can also detect hybridization and introgression patterns between closely related species (Le Roux and Wieczorek 2008). These attributes make molecular systematics one of the most exciting tools in the field of plant systematics. Population genetics deals with the variations of allele frequencies between and within populations (Evanno et al., 2005). A fundamental prerequisite of any inference on the genetic structure of populations is the definition of populations themselves, which are determined mostly upon geographical origin or phenotypes. However, the population genetic structure is not always reflected in the geographical proximity of individuals (Evanno et

al. 2005). Even individuals that are not discretely distributed based on geographical origin or phenotypes can be genetically structured due to restricted barriers to gene flow. Molecular systematics and population genetics are both useful in elucidating problems faced in biological invasions and have already shed light on management, prevention and introduction of efficient biological control of some invasive plants (Sakai et al., 2001). Molecular systematics and population genetics can be applied to the management of alien invasive species by resolving taxonomic issues, revealing their geographic source(s) and revealing the role of genetic diversity in the invasion success.

1.8.2.1. Taxonomic identification

Taxonomic identification of a target weed is necessary at several levels. From the intra-specific detail of the structure of invasive and indigenous populations for survey purposes, to the higher classification and phylogeny of the weed and its close relatives in order to design appropriate host specificity test plant lists (Gaskin et al., 2011). Integrated management of invasive species can be useful across multiple taxa. However, management practice such as biological control may be effective only on certain species or variants within species (Goolsby et al., 2006). Morphological identification is sometimes unable to distinguish such inter- and intraspecific variations, whereas molecular systematics can reliably, rapidly and accurately reveal variants and cryptic species (Le Roux and Wieczorek, 2008). In some case molecular systematics can be used to evaluate the risk of an exotic plant species pose by comparing it genetically to the already established invasive counterpart populations. For example, Jousson et al. (2000) used phylogenetic analysis based on the ITS regions to show the relatedness of some invasive seaweed species. Taxonomic confusion among plants occurs because of high hybridization rates between and within species, introgression among closely related species and regular instances of phenotypic plasticity (Le roux and Wieczorek 2008). Thus, the importance of molecular techniques when dealing with alien invasive species as taxonomic identification is the first step towards making effective management decisions.

1.8.2.2.Population structure and native source of invasive species

The exact geographical origin of invasive species is never easy to determine because of their large extent area in their native ranges (Moody and Les, 2007). However, discovering the genetic structure of invasive weed populations, within both the introduced and native ranges can provide

useful information for a biological control program (Gaskin et al. 2011). Knowing the geographic source of an invasive plant species, and whether the invasion was established by single or multiple introductions are highly relevant aspects for management purposes (Ascunce, 2011). The presence of genetic variation not associated with adaptive value (i.e. neutral genetic variation) within and between sites can determine if there were multiple introductions, leading to multiple genetic variants in the introduced range (Gaskin et al., 2011). Multiple introductions can be inferred if alleles that do not occur in native populations are found in introduced samples like in the case of tiger salamander (*Ambystoma tigrinum*) (Johnson, 2011). Although, the absence of certain alleles in the native population can be due to insufficient samples collected. The quantification of population genetics in the native range may reveal the specific geographic provenance of a weed and prove useful in finding candidate biological control agents adapted for these biotypes. This information may reveal the area from where invasive plants were introduced, and these areas should be *a priori* targets when collecting candidate biological control agents. While it may be true in some cases that natural enemies from the area of origin might be locally adapted to the particular genotype of a plant that has invaded a new range (Goolsby et al. 2006), this should be regarded as a hypothesis that can be tested to some extent (Hufbauer and Roderick, 2005). Just as the main focus of classical biological control has been to ensure a successful control programme, determining the geographic sources of invaders is also important to understand the basic mechanisms in invasion ecology (Le Roux and Wieczorek, 2008).

1.8.2.3.Hybridization, introgression and invasiveness

Hybridization results in genetic recombination, increases genetic diversity, and creates novel genotypes by masking deleterious alleles and transferring ones favoured by natural selection (Lewonton and Birch, 1966), and is thought to promote invasiveness in some cases (Abbott, 1992). This phenomenon therefore has ecological consequences and might be a strong determinant of high fitness in invasive species (Abbott et al., 2009). Introgressed plant species complicate taxonomic identification by producing a continuum of phenotypes and genotypes (Abbott, 1992), which may lead to the creation of cryptic hybrid individuals that can most conclusively and reliably be identified using molecular techniques (Gaskin et al. 2011). Multi locus molecular DNA marker may reveal the genetic distances in plants providing visual evidence of hybrids in dendograms or scatterplots (Saitou and Nei, 1987); or by assigning hybrids into classes such as parental, F_1, F_2, and back-crosses (Nason and Ellstrand, 1993).

Hybrids are mostly identified depending on the presence or absence of species specific markers, but this might not always be the case (Vaha and Primmer, 2006), especially with the recent development of statistical methods such as Bayesian-based models implemented in software such as STRUCTURE (Pritchard et al., 2000) and NewHybrids (Anderson and Thompson, 2002) which do not necessarily require that any alleles be unique to either parental type. Whether novel hybrids are created after the introduction (crosses between or among closely related alien taxa or even native taxa) (Gaskin and Schaal 2002, 2003; Gaskin et al. 2009), or if they represent the majority of the plant infestation (Moody and Les, 2007), they should be included in host use and efficacy testing of biological control agents (Gaskin et al. 2011). This is of great importance in a biological control programme of alien invasive plants considering the fact that hybrids may create a genetic 'bridge' allowing host specific insect to expand their host range from one parental species to another (Floate and Whitham, 1993). More especially, the inclusion of hybrids in the assessment of non-target risk should be compulsory when hybrids are formed from natives and aliens.

1.8.3. DNA-based markers for molecular systematic and population genetics studies of Tamarix species

There are many molecular techniques and markers that can be used to study the genetic variation in populations and/or species. This study focuses on the following DNA based markers: nuclear DNA sequence markers (ITS1 and ITS2 regions), the plastid marker (*trn*S–*trn*G), and the multilocus nuclear DNA marker, amplified fragment length polymorphisms (AFLPs).

1.8.3.1.Internal transcribed spacer (ITS) regions

The internal transcribed spacers are located between the small subunit (16*S*–18*S*) and the 5.8*S* rRNA coding regions (ITS1), and between the 5.8*S* and large subunit (23*S*–28*S*) rRNA coding regions (ITS2) of the nuclear ribosomal DNA (nrDNA) (Baldwin et al., 1995). The total length of the ITS1, ITS2 and the 5.8*S* regions of the nrDNA (rDNA) is about 900 base pairs (bp) including the flanking subunits. The spacer regions are evolving rapidly and show intraspecific variation, whereas the 5.8*S* regions is usually conserved (Baldwin et al., 1995). ITS sequences have been widely used to construct phylogenies of angiosperms at lower taxonomic levels (Baldwin et al., 1995) because they evolve rapidly and can easily be amplified using universal primers in the flanking genes (Baldwin, 1992). ITS1 and ITS2 can be amplified by the Polymerase Chain Reaction (PCR) process and sequenced using universal or species specific primers. Sequencing the ITS regions of the nrDNA unveils polymorphisms (double base

readings) within plant individuals (Campbell et al., 1997). Polymorphisms in some individuals can occur because concerted evolution is not fast enough to homogenize repeats of mutations among the multiple copies in the genome, and/or because of recent hybridization events. However, the presence of many polymorphisms originating from gene flow between divergent lineages is more likely to be due to high incidence of hybridization (Campbell et al., 1997).

1.8.3.2.Chloroplast DNA (*cpDNA*) *sequences*

Chloroplast (cp) DNA sequences have served as a primary source of data for plant molecular systematic studies (Baldwin, 1992, Gaskin, 2003, Taberlet et al., 1991). Plastid genomes have a relatively high degree of conservation in size, structure, gene content, and linear order of the genes in angiosperms. Nevertheless, there are numerous intraspecific variations at the genome level despite its conservative mode of evolution. cpDNA sequencing has been recognized, for some years, as a valuable molecular tool for studying phylogenetic relationships at the generic level (e.g. since Taberlet et al. 1991). Analysing the non-coding regions of cpDNA can reveal the utility of the genome at lower taxonomic levels, as the non-coding regions tend to evolve more rapidly than the coding sequences. Hence they may be useful below the family level (Taberlet et al., 1991).

The plastid and nuclear DNA markers are used to compare the evolutionary dynamics of two independent genomes (i.e. the nuclear genome being biparental whereas the plastid genome is uniparental reflecting the maternal lineage) (Taberlet et al. 1991). Comparisons of the phylogenies based on these two data sources can provide information on hybridization events (Gaskin and Schaal, 2003, Soltis and Kuzoff, 1995).

1.8.3.3.Amplified fragment length polymorphisms (AFLPs)

Amplified fragment length polymorphisms (AFLPs) are a dominant marker which can provide a rapid and affordable approach to collect polymorphic data on a genomic scale (Campbell et al., 2003) for surveying variation at many loci in different organisms, including plants. The AFLP technique is based on selective PCR amplification of restriction endonuclease fragments from a total digest of genomic DNA involved the following steps: restriction and ligation of the DNA; pre-selective amplification; selective amplification and electrophoretic separation of the amplified fragments.

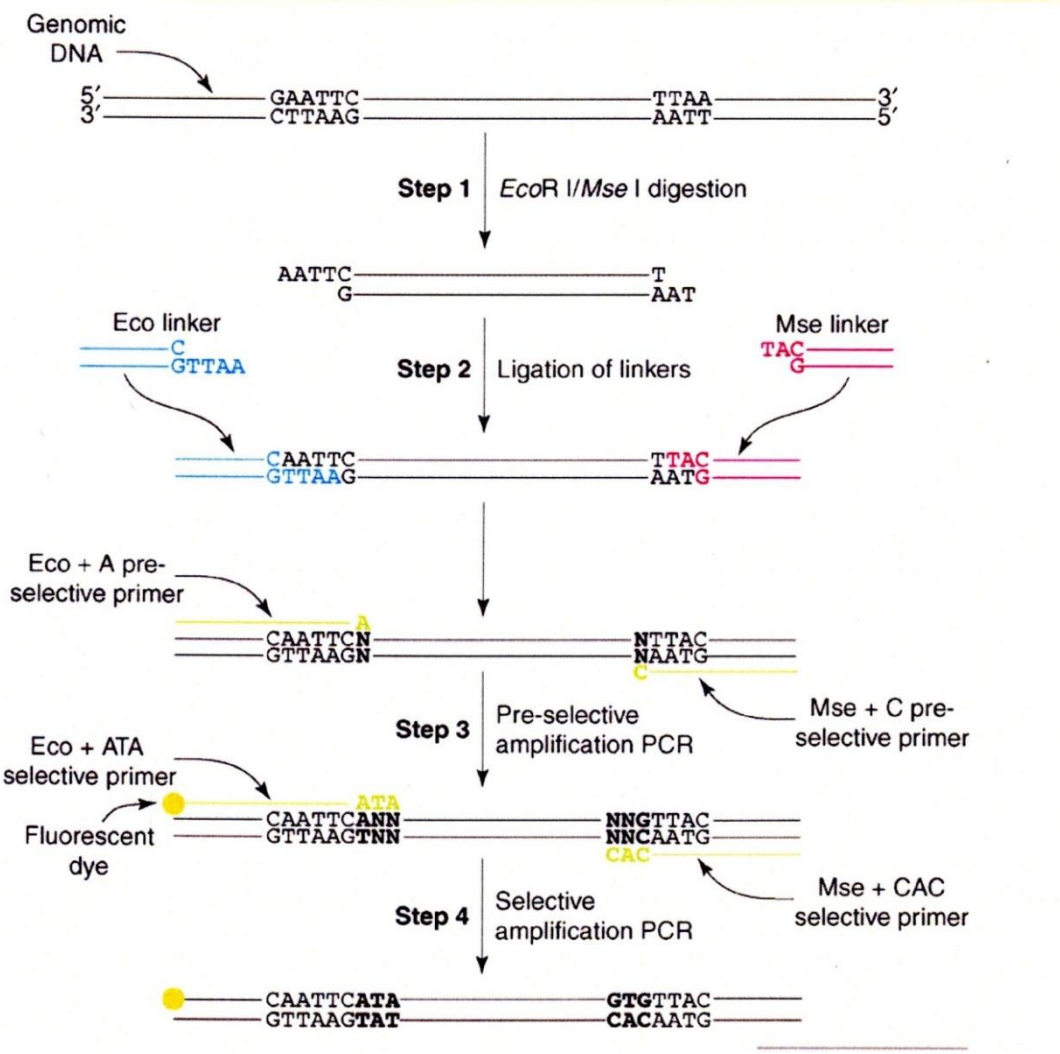

FIGURE 1. 4: Summary of different steps of DNA Amplified Fragment Length Polymorphisms (AFLPs) markers using Eco and Me adaptors with selective primers (Meudt and Clark, 2007).

Use of AFLPs has come a long way since its initial publication by (Vos et al., 1995). AFLP analysis is a firmly established multilocus PCR-based technique with a wide application in population genetics, reconstruction of shallow phylogenies, linkage mapping, parentage analyses, and single-locus PCR marker development (Meudt and Clark, 2007). The AFLP approach is a fingerprinting method that takes into account information distributed over the whole genome of an organism (Schmidt et al., 2004). It is a technique that can be used in the following situations: where there is no sequence data in a molecular study; for intra-specific studies; when genomic heterogeneity is high; when genetic variability is low; when hybridization is occurring; and for rapid generation of data (Mueller and Wolfenbarger, 1999). Among the fingerprinting techniques (e.g. RAPD, RFLP and AFLPs) AFLPs are valued for their robustness,

reproducibility, and high discriminatory power (Vos et al. 1995). Moreover, the modern development of data evaluation software allowing typing, clustering, and identification of organisms with high throughput (Myburg et al., 2001) has rendered the AFLP technique more exciting.

AFLPs are generated through a complete restriction endonuclease digestion of total genomic DNA using a pair of restriction endonuclease assays (usually *Eco*R I and *Mse* I). This is then followed by the ligation of known synthetic DNA adaptors (Eco and Mse) sequences onto the complementary ends of the resulting DNA fragments (Fig. 4). During pre-selective amplification, the subset of all the fragments is amplified, using primers that are complementary to the linker sequences with the addition of one nucleotide (A,G,C or T) at the 3′ end of the primer (usually Eco+A and Mse+C). These pre-amp primers will only prime DNA synthesis of fragments with bases flanking the restriction sites that are complementary to the selective nucleotide of the primers, thus reducing the number of fragments to 1/16 of the initial amount. The selective amplification process will see the additional base pairs (Eco+ATA and Mse+CAC) from the primers linked to the DNA fragment by extending past the fragment adaptor ligated sites through into the DNA fragment (Meudt and Clark, 2007). AFLP markers are usually widely distributed throughout the genome, allowing the assessment of genome wide variation, even though they are often concentrated in the centrometric regions (Mueller and Wolfenbarger, 1999).

AFLP data can be applied in population genetics studies in various ways to infer: parentage analysis (Gerber et al., 2000); measuring of genetic diversity (Mariette et al., 2002, Nybom, 2004), identifying hybrids (Gaskin et al., 2011, Goldman, 2004); population genetics (Barluenga, 2006, Belaj, 2003, Woodhead, 2006), reconstruction of shallow (unresolved) phylogenies (Després, 2003, Kardolus, 1998, Perrie, 2003); and population assignments (Campbell et al., 2003). Identification of hybrids, population genetics and population assignments among *Tamarix* species in southern Africa are the main application of AFLPs markers in this study.

1.9.RESEARCH AIM, OBJECTIVES AND QUESTIONS

1.9.1. Research aim

The aim of this study is to characterize *Tamarix* species and biotypes in southern Africa and investigate levels of hybridization and introgression among them using molecular markers, and to trace the phylogeographic distribution of native and alien invasive species.

1.9.2. Research objectives

- To characterize *Tamarix* species and their putative hybrids in southern Africa using molecular markers such as amplified fragment length polymorphisms (AFLPs), and sequences from a nuclear DNA region viz. the internal transcribed spacer regions (ITS), and a plastid marker (*trn*S-*trn*G)
- To investigate the phylogenetic relationships and the level of hybridization and/or introgression in *Tamarix* species and their putative hybrids in South Africa using AFLPs and DNA sequence markers.
- To determine whether the native *T. usneoides* hybridizes with a non-native *Tamarix* species and to understand if hybrid *Tamarix* populations can be found in places where *T. usneoides* co-occur with any one of the exotic species.
- To determine whether alien invasive *Tamarix* species found in South Africa are predominantly pure parental genotypes, F_1 hybrids, or introgressed individuals
- To reveal the geographic origin of the invasive *Tamarix* species (e.g. *T. ramosissima*) in South Africa.
- To assess the usefulness of morphological features that will best separate various *Tamarix* species present in southern Africa and facilitate field identification of pure *Tamarix* species and their putative hybrids in South Africa.

1.9.3. Research question

- What is the genetic identity (genotype) of the pure indigenous South African *Tamarix usneoides*?

- Can the different *Tamarix* species and their putative hybrids in southern Africa be distinguished using amplified fragment length polymorphism (AFLP) markers together with selected DNA sequence markers?

- What is the phylogeographic pattern of *Tamarix* species and their putative hybrids in southern Africa?

- Can the additional information of nrDNA and cpDNA regions provide greater phylogenetic resolution of the various *Tamarix* species and their putative hybrids? And can *Tamarix* species and/or population relationships be indicated by analysis of AFLP markers using e.g. Neighbour joining approach.

- What are the native ranges of exotic *Tamarix* species in South Africa that could provide sources of potential biological control agents for the alien invasive *Tamarix* species?

CHAPTER II: MATERIALS AND METHODS

2.1. FIELD SAMPLING AND SAMPLE PREPARATION

2.1.1. Sampling methods, sample preparation and identification

Tamarix usneoides, T. ramosissima, T. chinensis and possible hybrids were collected across South Africa and from a few localities in Namibia. In South Africa, samples were collected from the Eastern Cape, Free State, Gauteng, North West, Northern Cape and Western Cape (Fig. 2.1). Samples were collected from the indigenous and exotic species in both cultivated (in gardens and on mines) and wild populations. It should be noted that plant sample collection and transport permits from the Northern Cape, Eastern Cape and Western Cape were obtained prior to sample collection. Five *Tamarix* plants were sampled in each population, with a minimum of three samples where the population was small. The minimum distance between samples in a population ranged from 10 to 15 m. The minimum distance between populations was 50 km or more.

Young stems with leaves (i.e. branch tips) were collected for DNA material, placed into coffee filter paper, then put into silica gel and sealed in a zip lock plastic bag to facilitate quick drying and preservation of the DNA, and then stored at −20°C prior to molecular diagnosis. Additional branches (three per sample) with flowers/and or fruits were collected for voucher specimens which were pressed, oven dried, frozen for sanitation purposes and then deposited at the C.E. Moss Herbarium (J) at the University of Witwatersrand, Johannesburg. Duplicates were sent to PRE (Pretoria National Herbarium) and the appropriate provincial herbarium. The voucher specimens were examined using a Stereo microscope (Wild Heerbrugg M-series). The identities of all *Tamarix* individuals were determined using the morphological characters described in (Table 1). The identification of putative hybrids was based on the presence and the number of intermediate characters.

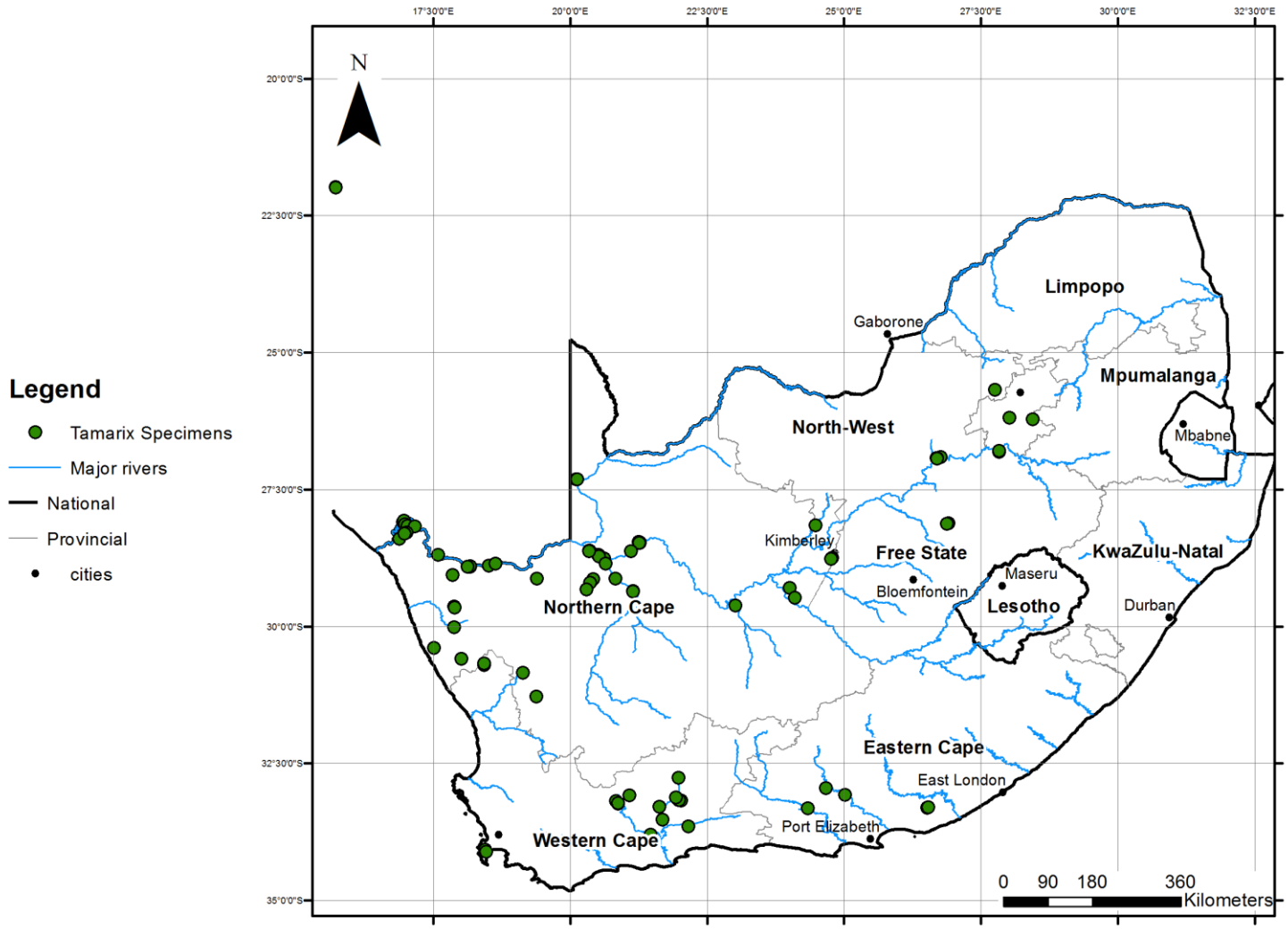

FIGURE 2. 1: South African collection sites of *Tamarix usneoides, T. ramosissima* and *T. chinensis* and putative hybrids for this study.

2.2. MOLECULAR METHODS FOR IDENTIFICATION OF *TAMARIX* SPECIES

2.2.1. *DNA extraction*

Genomic DNA was obtained from silica-dried leaf samples using the Qiagen DNeasy Plant Mini Kit (Qiagen®). The DNA was obtained from 20 mg of lyophilized young stem and leaf tissue, ground to fine powder using a mortar and pestle with liquid nitrogen.

The ground material was put into 400 µl of AP1 Buffer, then vortexed to eliminate tissue clumps, after which 4 µl of RNase A was added and the product was incubated in a heating block for 10 minutes at 65°C, inverting the tubes 3 to 4 times every three minutes. Then 130µl of AP2 buffer containing acetic acid was added to the lysate which was vortexed, then incubated for 5 min on ice. To precipitate detergents, proteins, and polysaccharides the lysate was centrifuged at 20,000 x g (14,000 rpm) for 5 min, then pipetted into a QIAshredder Mini spin column in a 2 ml collection tube, and centrifuged for 2 min at 14,000 rpm. The flow-through fraction (450 µl of the lysate) was transferred into a new tube without disturbing the pellet, then 675 µl of AP3/E Buffer containing guanidine hydrochloride was added and mixed by pipetting. After this 650 µl of the mixture was transferred into a DNeasy Mini spin column in a 2 ml tube and centrifuged for 1 minute at 8,000 rpm, then the flow-through was discarded and this step was repeated with the remaining mixed sample. The spin column was placed into a new 2 ml collection tube and 500 µl of Wash Buffer AW was added and centrifuged for 1 min at 8,000 rpm, then the flow-though was discarded. Another 500 µl of the Wash Buffer was added, and centrifuged for 2 min at 14,000 rpm (maximum available speed). Then the spin column was carefully removed from the collecting tube and transferred into a new 2 ml microcentrifuge tube where 100 µl nuclease free water was added for elution. This was incubated for five minutes at room temperature to increase the yield followed by centrifuging for 1 min at 8,000 rpm. This step was repeated with 50 µl nuclease free water to increase the DNA yield. Thus the DNA was eluted with a total of 150 µl nuclease free water. The extracted DNA product was ready for amplification and was stored at -20^0C. The DNA extraction procedure followed the manufacturer's protocol (Qiagen®).

2.2.2. *Amplification of DNA regions using Polymerase Chain Reaction (PCR)*

2.2.2.1. Nuclear Internal Transcribed Spacer (ITS) regions

The internal transcribed spacer (ITS) regions and intervening 5.8S subunit of the nuclear ribosomal DNA between the 18S–26S genes were amplified using primer pairs AB101 (5′-ACGAATTCATGGTCCGGTGAAGTG TTCG-3′) and AB102 (5′-TAAATTCCCCGGTTCGCTCGCCGTTAC-3′) (Sun et al. 1994). After optimization of the PCR protocol, the master mix (cocktail) contained 5 µl PCR Buffer (10X), 1 µl dNTPs (10 mM), 0.25 µl (10 mM) of both the forward and reverse primers (AB101 and AB102), 4.5 µl of the $MgCl_2$ (25 mM), 0.4 µl (5 U/µl) of Truestart *Taq* polymerase (Fermentas®) and 0.5µl or sometimes 1µl of the DNA template (depending on the concentration of the DNA) in a total volume of 50 µl. The following cycling parameters were used for PCR amplification: premelting at 95°C for 2 min; followed by 30 cycles of denaturation at 95°C for 50 s; annealing at 54°C for 45 s; extension by a thermostable DNA polymerase at 72°C for 1.30 min. Final extension was at 72°C for 7 min. The successfully amplified product was then ready for purification.

*2.2.2.2. Chloroplast intergenic region between trn*S *and trn*G

The chloroplast intergenic region between the *trn*S (GCU) and *trn*G (UCC) genes with intraspecific variation was amplified using the primer pairs trnS (GCU) (GCCGCTTTAGTCCACTCAGC) and trnG (UCC) (GAACGAATCACACTTTTACCAC) (Hamilton 1999). The *trn*S-*trn*G regions were amplified in one of the following three ways: (i) using Truestart taq: a 25 µl reaction containing 2.5 µl PCR buffer (10X), 0.5 µl dNTPs (10mM), 0.125µl of both the forward and the reverse primers (10mM), 2µl $MgCl_2$, 0.2µl polymerase Taq (Truestart), 0.5 µl DNA template, and the corresponding water to make the volume up to 25 µl. The following cycling parameters were used: premelting at 95°C for 2 min, followed by 33 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1.30 min, with a final extension at 72°C for 7 min; (ii) using Biolase, an 11µl reaction contained, 1 µl of 10X PCR buffer (Biolase), 0.50 µl $MgCl_2$ at 50 mM, 0.08 µl of dNTP mix (Biolase) at 25 mM, 1µl of each *trn*S-*trn*G primer pairs at 2 mM, 0.05 µl of biolase *Taq* at 5 U/µl, and 1 µl of DNA template, plus the relevant amount of water (6.40 µl). The following cycling parameters were applied: premelting at 95°C for 2 min, then 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, and then final extension at 75°C for 5 min; (iii) using Phusion® High-Fidelity DNA Polymerase (F-530): a 20 µl reaction

contained 2 µl of the 5x Phusion HF buffer which has 7.5 mM $MgCl_2$; 0.4 µl dNTPs (10 mM); 1 µl of both the forward and the reverse primers (10 µM); 0.2 µl (2 U/µl) of Phusion High-Fidelity DNA Polymerase (Thermo Scientific®); and 1 µl of the DNA template with the following cycling parameters: premelting at 98°C for 1 min; then 33 cycles of denaturation at 98°C for 5 s; annealing at 56°C for 35 s; extension at 72°C for 25 s followed by final extension at 72°C for 7 min.

2.2.3. DNA Purification

The PCR material was purified using a Zymo Clean and Concentrate Kit (Zymoclean™) from Zymo Research. The 20 µl of purified PCR product was stored at -20°C until sequencing. See Appendix I for more details on the purification of DNA. Most amplification of the plastid regions (*trn*S-*trn*G) yielded multiple bands. Therefore, the Gel DNA recovery method was used to extract the desired band using the ZymoClean Gel DNA recovery Kit. The detail on this procedure is provided in the appendix I below.

2.2.4. DNA sequencing

The purified PCR product was sequenced following the standard DNA sequencing protocol for the BigDye® Terminator v3.0 cycle sequencing kit (Life Technologies) at the University of Stellenbosch, Central Analytical Facilities (CAF) DNA sequencing Unit. Sequences were cleaned using Princeton separations Centri-sep clean-up plates and samples were run on a 3730xl Genetic Analyser following the standard protocols (ABI Applied Biosystems, 2002®).

2.2.5. Amplified Fragment Length Polymorphisms (AFLPs) analysis

The AFLP method followed Vos et al., (1995) with a few modifications such as restriction and ligation performed as a single step in an 11 µl reaction. The restriction-ligation reaction mix was then incubated at room temperature for at least 12 hours. The restriction-ligation processes were modified (combined) to reduce the error and time for completion due to the sensitivity of the AFLP analyses.

The restriction and ligation processes were performed during a single step in an 11µl reaction containing 2 µl of 500ng genomic DNA, 0.1 µl of 2 U *Mse*I enzyme, 0.25 µl of 1 U *Eco*RI enzyme, 0.15 µl of 1 x T4 DNA ligase buffer, 1.10 µl of 0.45 U T4 DNA ligase, 1.10 µl of 0.05 M NaCl, 0.55 µl of 0.5 x BSA, 1 µl of 4.5 µM *Mse*I adaptor, 1 µl of 0.45 µM *Eco*RI adaptor, and

water. The mixture was incubated at room temperature overnight, and then 5.5 µl of the product was diluted to 100 µl in TE (15 mM Tris and 0.1 mM EDTA).

2.2.5.1. Pre-selective polymerase chain reaction (PCR)

The pre-selective amplification was performed in a 20 µl reaction containing 4 µl of the diluted restricted-ligated product, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM of each pre-selective amplification primer (*Mse*I + C and *Eco*RI + A), 0.5 U *Taq* polymerase and water. The pre-selective PCR consisted of 20 cycles of: 94°C for 30 s, then 56°C for 60 s, and then 72°C for 60 s. Ten microlitres of the pre-selective amplification product was diluted to 200 µl in TE.

2.2.5.2. The selective polymerase chain reaction (PCR)

The selective amplification was performed in a 20 µl reaction containing 3 µl of the diluted pre-selective amplified product, 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.1 µM *Mse*I selective primer, 0.05 µM *Eco*RI selective primer dye-tagged with D4 (blue), 0.5 U of *Taq* polymerase and water. The selective PCR protocol was at 94°C for 120 s; 10 cycles of: 94°C for 20 s, then at 66°C for 30 s (decreasing by 1°C each cycle), and then at 72°C for 1.30 min; lastely 25 cycles of: 94°C for 20 s, 56°C for 30 s, and at 72°C for 1.30 min. One microlitre of each selective PCR product was combined with 0.3 µl of 600 bp size standard and 28.7 µl of de-ionized formamide and loaded into a Beckman Coulter CEQ 2000 fragment analyser.

2.3. ANALYSES OF DATA

2.3.1. *Phylogenetic analyses*

The forward and reverse sequences were aligned and edited using Sequencher™ version 4.1 (Gene Codes Corporation®) and electropherograms were viewed and compared to confirm the base readings. The consensus sequences were aligned and compared within species, hybrids and then among all samples. The alignment was refined manually, and mutations were confirmed by checking them against the electropherograms. Gaps caused by insertion and/or deletion (indel) events were treated as missing data, and multiple states (heterozygotes) in the nuclear region were scored as polymorphisms which were also treated as missing data as they do not contribute towards phylogenetic tree reconstruction, but are a good indicator of hybridization (Bailey et al.,

2003). Indels were coded as separate matrix at the end of each data set, as per Simmons and Ochoterema (2000) and analyses were run including and excluding coded indels.

Parsimony analysis of the nuclear (ITS) and chloroplast (*trn*S-*trn*G) DNA data sets was performed using PAUP* version 4.0b10 (Swofford, 2002). The biological replicates were not included in the phylogenetic analysis but were considered for AFLP analysis. The phylogenetic trees were rooted using the Genbank sequence of *Myricaria alopecuroides* Schrenk this has been shown to be the sister genus to *Tamarix* (Zhang et al., 2006). Heuristic searches comprising 10,000 random repetitions holding 20 trees at each step were performed with the maximum number of trees set at 10,000. Swopping on best trees only was used with Tree Bisection Reconnection (TBR) branch swopping, saving multiple trees. Bootstrap analyses (excluding coded indels) were conducted to assess clade support (Felsenstein 1985), using the same settings as above with 100 replicates.

2.3.2. Partition Homogeneity Test

The partition homogeneity test (in PAUP* v4.0b10) was used to test for congruency between the chloroplast and nuclear data sets comprising the same 30 samples (Farris, 1995). Phylogenetic trees resulting from the analyses of these plastid and nuclear DNA data sets were compared to trace the evolutionary dynamics of two independent genomes in *Tamarix* species. The two data sets (plastid and nuclear) were analysed separately.

2.3.3. *Principal Co-ordinates Analysis (PCoA)*

Principal coordinates analysis (PCoA) is a computational alternative to Principal Component Analysis (PCA) (Rohlf, 1998). Both are methods of ordination analysis, in which points are projected onto orthogonal coordinate axes with maximum variance in as few dimensions as possible (Rohlf 1998). These ordination analyses have as their objective to position points (specimens) along coordinate axes. Principal coordinates analysis is recommended for data sets that include qualitative data (Legendre and Legendre, 2003). Therefore PCoA is used in this study to investigate genetic differences between the *Tamarix* parent species (alien and indigenous), and where the South African hybrids are placed along the continuum between them. The PCoA was performed using the AFLP data matrix (data not shown here) and NTSYS-pc Ver. 2.2 software (Rohlf 1992). The Dice similarity coefficient was used in the analysis as suggested by Bonin et al. (2007); it is the same as the (Nei and Li, 1979) coefficient: $2a/(2a + b$

+ *c*) where *a* = number of bands present in both samples, *b* and *c* = number of bands present in only one or the other sample. The DICE similarity coefficient was preferred here because it does not assume that samples that are missing the same band are equal for that band (Rohlf 1998). The DCENTER and EIGEN options of NTSYS were then used to complete the ordination analysis.

The DCENTER option "double-centres" the similarity matrix (i.e. among the *Tamarix* specimens), followed by the EIGEN option which computes eigenvectors and eigenvalues of the symmetric similarity matrix. A two dimensional PCoA plot was then computed using the eigen vector values from the ordination.

2.3.4. *Bayesian model-based clustering analysis*

The Bayesian/Markov Chain Monte Carlo model-based clustering algorithm has become a prominent computational tool for inferring population structure in population genetics and in molecular ecology (Beaumont. and Rannal., 2004). The Bayesian clustering methods assign individuals/samples to a number of clusters based on their multilocus genotypes, and use genetic information to ascertain this without assuming predefined populations (Falush, 2007, Pritchard et al., 2000). This assignment of each individual genotype to its population of origin is done probabilistically (Pritchard et al. 2000), and can generally be achieved by using the Markov Chain Monte Carlo (MCMC) approaches.

Tamarix clustering and assignment tests were performed using the software STRUCTURE V. 2.3.4 (Pritchard et al. 2000; Falush et al. 2007). The AFLP results (southern African specimens combined with Asian samples from Gaskin et al. 2011) were diploidized (Falush et al. 2007) to determine the number of clusters (K), or species, represented both in the native Asian plants and southern African plants. No population information was included to determine if the genetic identification of individuals supported the morphological and field identification. The admixture model was assumed with consideration given to correlate allele frequencies and recessive allele model for the dataset. This model allows individuals to belong to any number of clusters, in varying proportions. Therefore, individuals with a proportion of their genotype assigned to both clusters are presumed to represent potential hybrids. Allele frequencies were considered to be independent in each population, and a 100,000 run burn-in (α stabilized at approximately 1 000 runs) and a 1,000,000 run length were used. The number of clusters was tested as (K=1-5) with 10 replicates per K. Selection of K from this data was done by using the software Structure

Harvester (Evanno et al., 2005), a web-based program that implements the algorithm outlined by Evanno et al. (2005) to calculate the most optimal value of K (ΔK), as suggested by Pritchard et al. 2000, which examines changes in the slope of plotted values of log P(X/K). Average estimate admixture coefficient (Q, or assignment value) of each specimen to *Tamarix usneoides*, *T. ramosissima* and *T. chinensis* was determined. The admixture option was assumed to be possible, allele frequency was considered to be independent in each population, and 10,000 burn-in and 10,000 run lengths were used. The software also calculated 95% probability intervals around the average assignment value of each sample using the 100,000 runs performed after burn-in. Average assignment values for each sample were calculated using Microsoft Excel 2010.

CHAPTER III RESULTS

3.1. PHYLOGENETIC ANALYSES OF NUCLEAR RIBOSOMAL (ITS1 AND ITS2) SEQUENCE DATA

The data matrix of 86 specimens (total sample size after removing replicates) comprised 44 *Tamarix usneoides* samples, ten *T. ramosissima*, six *T. chinensis*, and 26 potential hybrid individuals (19 *T. chinensis* hybrids, nine *T. ramosissima* hybrid and one possible *T. usneoides* hybrid). The aligned nuclear ribosomal DNA sequence data matrix contained 828 aligned bases. The full ITS data matrix had 675 (82%) constant characters which included those in the 5.8S gene region and the flanking portions of the rRNA large and small subunit genes where mutation does not usually take place at the species level (Baldwin et al., 1995). The sequences of the 5.8S gene region were thus invariant across southern African *Tamarix* species despite the differences in species composition. 153 (18.5%) characters were variable across the 86 specimens, of which, 50 (6%) characters were parsimony informative (i.e. synapomorphic or derived shared mutations) across 86 samples and the remaining 103 characters were parsimony uninformative.

The parsimony analysis of the ITS nuclear sequence data of the 87 specimens resulted in 10 000 trees (maximum limit set) of 176 steps with a Retention Index (RI) of = 0.99. The Consistency Index (CI) was 0.93 excluding uninformative characters and the Rescaled Consistency Index (RC) was 0.92. The strict consensus of the 10,000 MP trees generated excluding indels is shown in Fig 3.1.

The strict consensus tree including 86 *Tamarix* samples has two main clades (A and B, Fig. 3.1) separating *T. usneoides* specimens (including a potential hybrids) from *T. ramosissima* and *T. chinensis* samples (as well as some hybrids) with little resolution within each clade (Fig. 3.1). The two main clades are separated by 21 point mutations of which 12 synapomorphies group *T. ramosissima* and *T. chinensis* specimens together; and nine synapomorphies (shared derived characters) unite the *T. usneoides* samples together with one specimen identified as potential hybrids (Fig 3.1). Both clades A and B are strongly supported with 100% bootstrap support (BS) values.

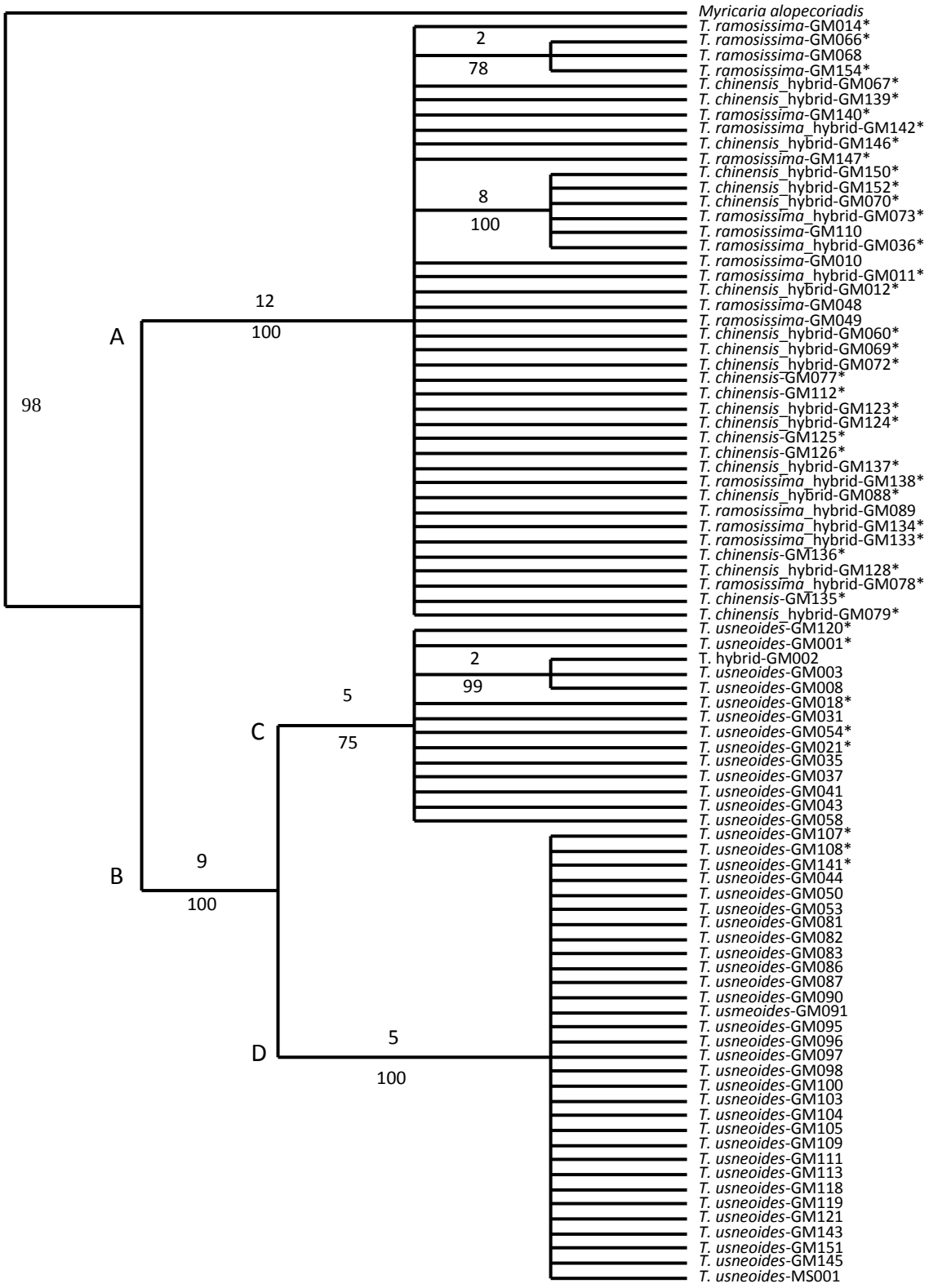

FIGURE 3. 1: Strict consensus of 10 000 equally most parsimonious (EMP) trees of 86 *Tamarix* specimens and the outgroup *Myricaria alopecuroides* based on ITS sequences with gaps coded as missing data. 176 steps in length with RI = 98, and CI = 0.93. Bootstrap values, based on 100 replicates with 20 trees per replicate are below the branches. Numbers above lines are minimum branch lengths. *Identification changed according to AFLP Structure analysis (Appendix IV).

All the *Tamarix ramosissima* specimens are grouped together with those of *T. chinensis* in clade A. The majority of specimens identified as hybrids have also been grouped in this same clade. Just as their morphological similarity suggests that these two species are closely related, their almost identical nuclear DNA ITS sequence data confirms this relationship. Within Clade A, there is remarkably little resolution, but six specimens have grouped together (with 100% BS) as they share eight characters distinct from the other individuals. They include three specimens identified as *T. chinensis* hybrids, two as *T. ramosissima* hybrids and a single pure breeding *T. ramosissima* GM110. Within clade A, there is a branch comprising three specimens of *T. ramosissima* united by two synapomorphies (shared characters) with a 100% bootstrap support.

Clade B comprises all of the *T. usneoides* specimens and one putative hybrid (Fig 3.1). Unlike clade A, clade B has some resolution (branching) with the specimens grouping into two sub-clades (C and D), with fair (75%) to very strong (100%) bootstrap support respectively. However, there appear to be two levels (categories) of pure *T. usneoides* in clade B, (Fig 3.1). There are five synapomorphies (shared point mutations) supporting sub-clade C, in which one specimen (GM002) which 'morphologically' appears to be a *T. usneoides* hybrid is grouping together with some specimens that were identified as pure *T. usneoides*. Other individuals in clade C that were identified as putative hybrids of *T. usneoides* based on morphology and the presence of multiple polymorphisms in their ITS sequences (Appendix IV) were not assigned as hybrids in the Structure analysis based on the AFLP data. (Note: These individuals are denoted by an asterisk in Fig. 3.1.). The relatively weak bootstrap support (75%) here reflects homoplasy in the sequence data which may be due to the presence of these hybrids which share some based with the exotic species in clade A. Sub-clade D is composed mainly of *Tamarix usneoides*, but there are three *Tamarix* specimens which were previously identified as *T.usneodies* hybrids present as well. The exact final identities of these hybrid samples were however indicated by the AFLP markers to be pure breeding *T. usneoides*.

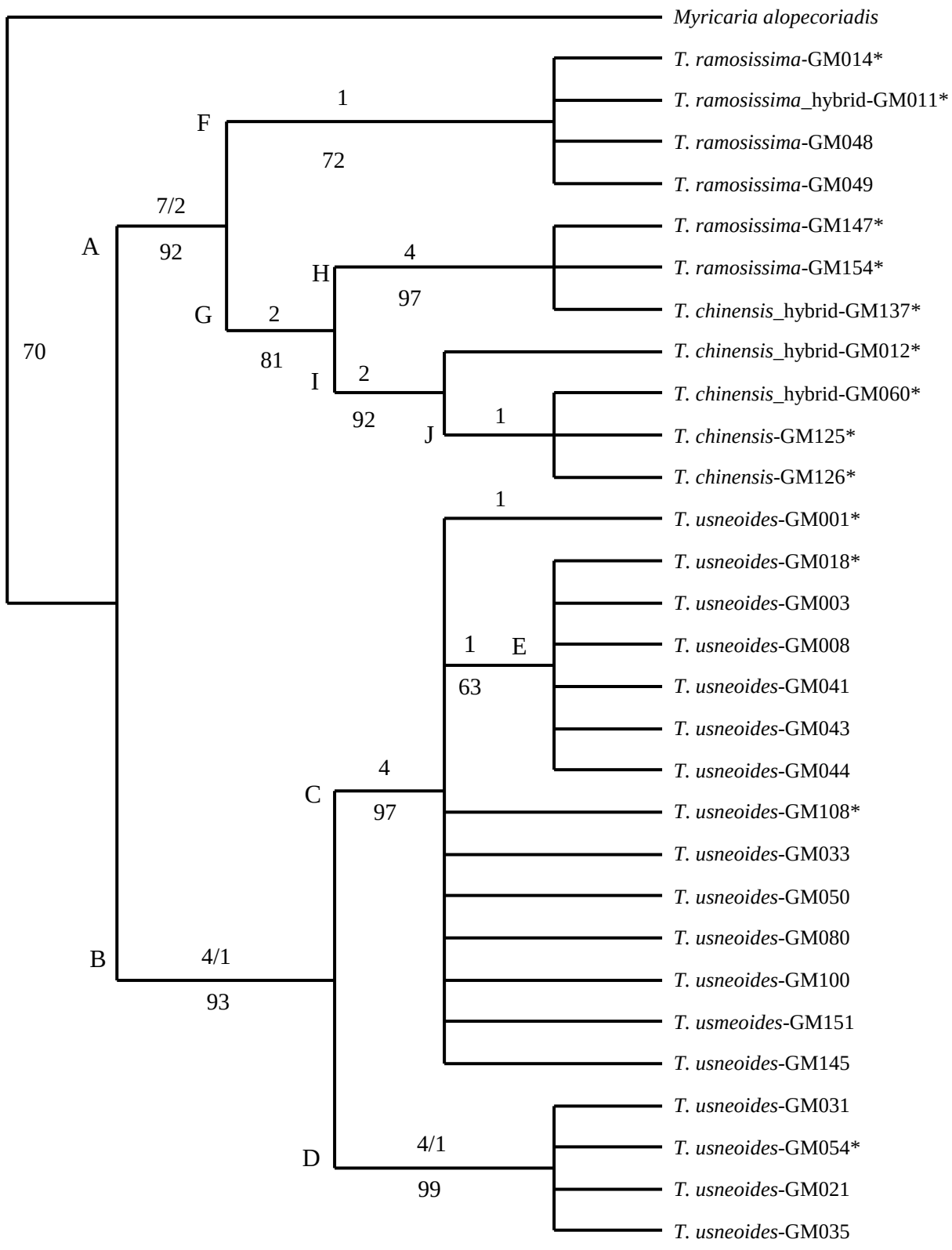

FIGURE 3. 2: Strict consensus of 10 000 EMP trees of 29 *Tamarix* specimens and the outgroup *Myricaria alopecuroides* based on *trn*S-*trn*G plastid sequence data including coded indels. Bootstrap values (100 replicates with 20 trees per replicate) are below the branches. Numbers above lines are minimum branch lengths (point mutations/indels). (CI = 0.9348; RI = 0.9879). *Identification changed according to AFLP structure analysis (Appendix IV).

3.2. PHYLOGENETIC ANALYSIS OF THE PLASTID SEQUENCE DATA FROM *trn*S-*trn*G DNA REGIONS

The plastid DNA data set of *Tamarix* species in South Africa consisted of 940 aligned data bases of which 847 characters were constant across the region and 98 (10.37%) characters were variable. Of the variable characters, only 36 (36.73%) characters were parsimony informative. There were 17 indels across the plastid data set of which five indels (Table 3.1) were synapomorphic within *Tamarix*. The data matrix of 29 ingroup taxa comprised 18 *T. usneoides*, six *T. ramosissima,* and five *T. chinensis* specimens (this sample size was dependent on the success of PCR amplification).

The parsimony analysis of the plastid region (*trn*S-*trn*G) including 30 specimens (29 *Tamarix* samples and *Myricaria alopecuroides* as outgroup) resulted in 10,000 trees (maximum limit) of 109 steps with a Retention Index (RI) of = 0.99. Excluding uninformative characters, a Consistency Index (CI) of = 0.94, and a Rescaled Consistency Index (RC) of = 0.96 were obtained.

The strict consensus tree is shown in Fig 3.2. Two strongly supported clades separate *T. usneoides* specimens and *T. ramosissima*, *T. chinensis* (including some hybrids) samples with some further resolution within each clade. There are 14 mutations separating the two main clades (A and B) of which nine shared mutations (synapomorphis) are distributed among *T. ramosissima*, *T. chinensis* samples and five characters are shared among *T. usneoides* samples and the hybrids.

Clade A (Fig. 3.2) groups *Tamarix ramosissima* together with *T. chinensis* with strong bootstrap support (92%) and comprises two clades (F and G) of which one (clade F) comprises only specimens of *T. ramosissima* with one of its hybrids. All of these are cultivated individuals, including specimens from a garden in Johannesburg (Gauteng), a nursery in Upington (Northern Cape) and a single *T. ramosissima* hybrid specimen from a mine at Vaal River (North West) united by a single point mutation. The other clade (G) branches further and sub-clade H includes a mixture of *T. ramosissima* samples and a *T. chinensis* × *T. ramosissima* hybrid, while sub-clade I consists of one *T. chinensis* specimen and putative hybrids (*T. chinensis* × *T. ramosissima*). This sub-clade (H) consists of garden collected plants from Prince Albert (Western Cape), Steytlerville and Grahamstown (Eastern Cape), with four shared point mutations uniting these

three specimens with strong Bootstrap support (97%, Fig. 3.2). Sub-clade I consists of cultivated *T. chinensis* and hybrids (two garden specimens and two from the mines) which have been united by two shared point mutations with fairly strong bootstrap support (92%), and three of the specimens group together in sub-clade J due to one synapomorphic indel (Table 3.1).

Clade B comprises all *Tamarix usneoides* specimens united by four synapomorphic point mutations and an indel (Table 3.1) with 93% BS (Fig. 3.2). Clade B is further subdivided into two sub-clades. Sub-clade C is united by four synapomorphies with 97% BS, and includes most of the cultivated *T. usneoides* specimens from the mines, three of which were identified as putative hybrids based on morphology and presence of polymorphisms reflecting dual parentage. There are eight specimens (GM041, GM043, GM044, GM080, GM100, GM108, GM145 and GM151) which represent wild populations from the Northern Cape and Western Cape. These wild populations were observed to be growing in proximity to the exotic pink flowering *Tamarix* species (*T. ramosissima* and/or *T. chinensis*). Furthermore, there are six specimens that group together (sub-clade E) with relatively weak bootstrap support (63%). Sub-clade D within clade B, groups four specimens (two cultivated specimens from the mines and two wild specimens) which have four shared point mutations and one indel resulting in very strong bootstrap support (99%, Fig. 3.2).

TABLE 3. 1: Coded indels that are parsimony informative in the plastid region

Base positions	**nucleotides**	**Presence in taxa**
154—158	ATTAT	Deletion in all *T. chinensis* and *T. ramosissima* and their hybrids (in clade A)
163—168	TAAAAA	Insertion in *T. usneoides* hybrids (GM031 and GM054) and *T. usneoides* (GM021 and GM035)
189—190	TA	Deletion in *T. chinensis* (GM125, GM126) and *T. chinensis* (GM060)
191—196	TATATA	Deletion from all *T. chinensis, T. ramosissima* and hybrids
548—554	TTTTTCA	Insertion in all *T usneoides*

3.3. PHYLOGENETIC ANALYSIS OF THE 29 *TAMARIX* RESTRICTED ITS SEQUENCE DATA

A restricted nuclear ribosomal ITS (biparental) data matrix was set up to match the specimens in the plastid data (maternal inheritance) set to enable comparison of placement of the specimens in the phylogenies generated from the two data sources and thereby assess the level of congruence in the data sets. The aligned DNA matrix of 30 specimens, 29 ingroup taxa (18 *Tamarix usneoides*, five *T. ramosissima*, two *T. chinensis*, four hybrids of *T. chinensis* and *T. ramosissima*) and one out-group taxon (*Myricaria alopecuroides*), comprised 806 aligned bases, with 43 (45.3%) parsimony informative variable sites. There are 95 (11, 78%) variable characters that are parsimony uninformative, i.e. are autapomorphic point mutations occurring among individuals within the populations.

Phylogenetic analysis of this ITS (restricted) data set for these 30 samples using parsimony resulted in 10,000 trees of 142 steps with RI = 0.97, CI = 0.87 and RC = 0.94. The strict consensus of the 10,000 EMP trees is shown below in Fig 3.3.

As in the more complete analysis of the ITS data set (Fig. 3.1), the consensus tree resulting from ITS restricted data set (Fig 3.3) has two clear clades separating the indigenous *Tamarix* (*T. usneoides*) specimens from the exotic (*T. ramosissima* and *T. chinensis*) specimens. Unlike the phylogenetic tree of the plastid regions (Fig 3.2), the restricted ITS phylogenetic (Fig 3.3) analysis has less resolution in the *T. ramosissima*/*T. chinensis* clade. However, it has a slightly more resolved *T. usneoides* clade when compared to the consensus tree from phylogenetic analysis of the ITS full data set (Fig 3.1). There are a minimum of 25 point mutations separating the two main clades (Fig 3.3), where 15 shared characters are distributed among *T. ramosissima*/*T. chinensis* clade (clade A) and the *T. usneoides* clade (clade B, Fig 3.3) is united by 10 shared mutations.

Clade A (Fig 3.3) is well supported with a bootstrap value of 80% and includes both mine and garden planted *Tamarix chinensis* and *T. ramosissima* specimens and their hybrids (e.g. GM011 and GM014) together with the wild specimens and hybrids (e.g. GM137). However, as there is no resolution within this clade, it is not possible to compare the placement of these specimens with that in the consensus tree resulting from parsimony analysis of the plastid data set (Fig. 3.2).

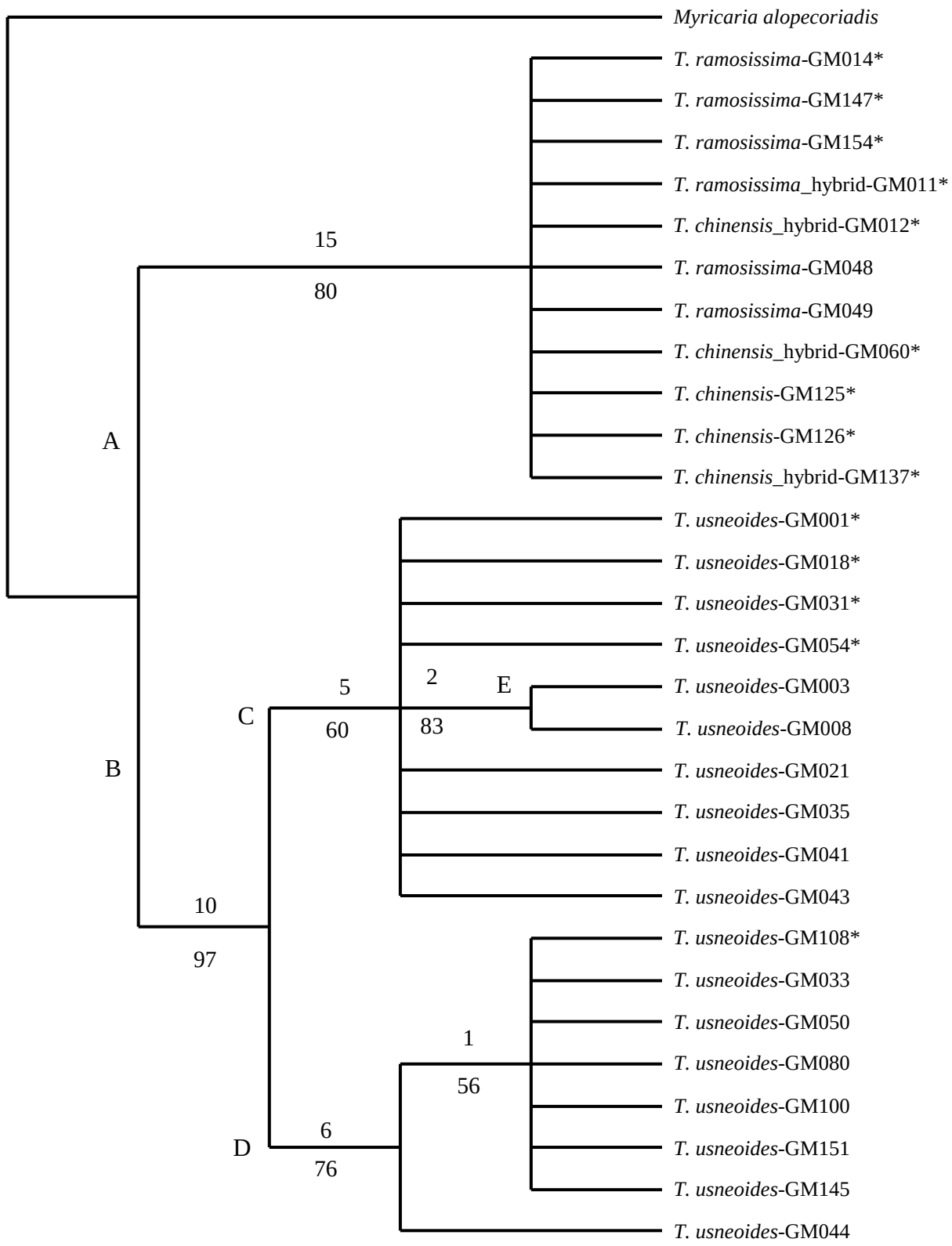

FIGURE 3. 3: Strict consensus of 10 000 EMP trees of a restricted ITS data set comprising 29 *Tamarix* specimens and the outgroup *Myricaria alopecuriodes*. Bootstrap values are below the branches. Numbers above lines are minimum branch lengths. CI = 0.874; RI = 0.965. *Identification changed according to AFLP structure analysis (Appendix IV).

As in the consensus tree of the analysis of the larger ITS data set (Fig 3.1), clade B (Fig 3.3) comprises all *T. usneoides* specimens united by 10 synapomorphic mutations with strong bootstrap support (97% BS). There is some branching within clade B: the weakly supported (60% BS) sub-clade C comprises all of the specimens from the mines as well as some from the wild. Within sub-clade C two specimens from the mines (GM003 and GM008, sub-clade E) have two synapomorphic characters that unite them with good bootstrap support (83%). Sub-clade D comprises only pure breeding *T. usneoides* from the wild populations. The sub-structuring of clade B is not the same in the plastid and nuclear consensus trees (Figs. 3.2 and 3.3 respectively). The four specimens in sub-clade D in the plastid consensus tree (Fig. 3.2) do not form a separate clade in the nuclear consensus tree (Fig. 3.3) and only two of the six specimens in sub-clade E in the plastid phylogeny (Fig. 3.2) form a distinct clade (sub-clade E, Fig. 3.3) in the nuclear phylogeny. The specimens in sub-clade D of the nuclear consensus tree (Fig. 3.3) are placed together in a single sub-clade (C) in the plastid consensus tree (Fig. 3.2) with those from sub-clade C of the nuclear consensus tree (Fig. 3.3). There is therefore a lack of congruence evident in the consensus trees resulting from these two data sets, which is possibly due to the presence of hybrids.

3.4. PARTITION HOMOGENEITY TEST OF THE SEQUENCE DATA OF THE PLASTID REGION (*TRN*S-*TRN*G) AND NUCLEAR (ITS) REGIONS

The partition homogeneity test of the ITS and *trn*S-*trn*G data sets resulted in a P value = 1− (99/100) = 0.01, which is on the cusp of rejecting the null hypothesis of congruence. Analysis of a combined data matrix (1751 bases, 236 variable) resulted in an unresolved phylogenetic tree (not shown here) which confirmed the lack of congruence between these plastid and nuclear data sets. Comparison of the *T. usneoides* clades in both restricted ITS and *trn*S-*trn*G phylogenies (Figs 3.4 and 3.4 respectively) shows that there are changes in placement of some of the specimens in the two main clades in each consensus tree. However, there is no consistent pattern that differentiates *T. usneoides* specimens from their closely similar individuals. For example: pure breeding *T. usneoides* specimens such as GM050, GM080 and GM044 in clade E, Fig 3.3 are monophyletic and clearly separate from specimens in clade C; whereas the same individuals in Fig 3.2 are grouped in sub-clade C with specimen GM044 further grouping with the individuals in sub-clade E.

3.5. PRINCIPAL COORDINATES ANALYSIS (PCOA)

The initial outcome from the AFLP fragment analyser software, after manual scoring, yielded 94 and 102 unambiguous polymorphic loci for *Mse*I + CAT/*Eco*RI +ACC and *Mse*I + CTA/*Eco*RI + ACC, respectively. Thus, a total of 196 AFLP loci were produced for *Tamarix* species (239 specimens) comprising 44 *Tamarix chinensis* and 56 *T. ramosissima* samples from Asia (also used in Gaskin et al. 2011), while southern African samples (139 specimens) included 10 specimens identified as *T. chinensis*, 10 as *T. ramosissima*, 81 as *T. usneoides*, and 38 as possible hybrids, these species identities were confirmed based on AFLP Structure analysis.

Results of the principal coordinates analysis based on the AFLP data using the Dice similarity coefficients are shown in Fig 3.4. The first two principal coordinates account for 31.9% and 5% of the total variance among the specimens respectively. The 2D plot (Fig 3.4) shows distinct clusters separating the various species (*T. chinensis*, *T. ramosissima* and *T. usneoides*) with a continuum of genetically identified hybrids in between them. *Tamarix chinensis* specimens from China are grouped in cluster A together with some South African *T. chinensis*. Cluster B is composed mainly of the *T. ramosissima* from Asia (their native range) but there are also some South African *T. ramosissima* specimens grouped in this cluster. Most of the specimens that were identified as pure *T. usneoides* are grouped in two different clusters that are next to each other. Cluster C groups all *T. usneoides* specimens with 99.9% assignment value and cluster D groups all the individuals that have 99.8% assignment values and less. However, specimen GM043 which is identified as introgressed *T. usneoides* x *T. chinensis* falls under this cluster as it has a posterior probability of 88.9% of being *T. usneoides* and 10.9% of being *T. chinensis* with the other 0.03% belonging to *T. ramosissima*. The principal coordinates analysis indicates that southern African *Tamarix usneoides* specimens are genetically very distinct from *T. ramosissima* and *T. chinensis*. The analysis also clearly shows that *T. ramosissima* and *T. chinensis* plants from Asia are genetically distinct from each other. In addition, most of the South African specimens of *T. ramosissima* and *T. chinensis* are genetically similar to the Asian plants, but there is a continuum of genetic material from South Africa that lies between these two clusters signifying that there are many *T. chinensis* × *T. ramosissima* hybrids in South Africa.

3.6. BAYESIAN ANALYSIS

The average mean posterior probabilities of 239 *Tamarix* specimens indicated that *T. chinensis*, *T. ramosissima* and *T. usneoides* formed three clusters K=3, K= number of clusters. The average assignment values (mean posterior probabilities) of each individual plant to the three parental species are shown in Fig 3.5, and the individuals are sorted by species and grouped by regions. It was assumed that K=3 ancestral source populations in each case.

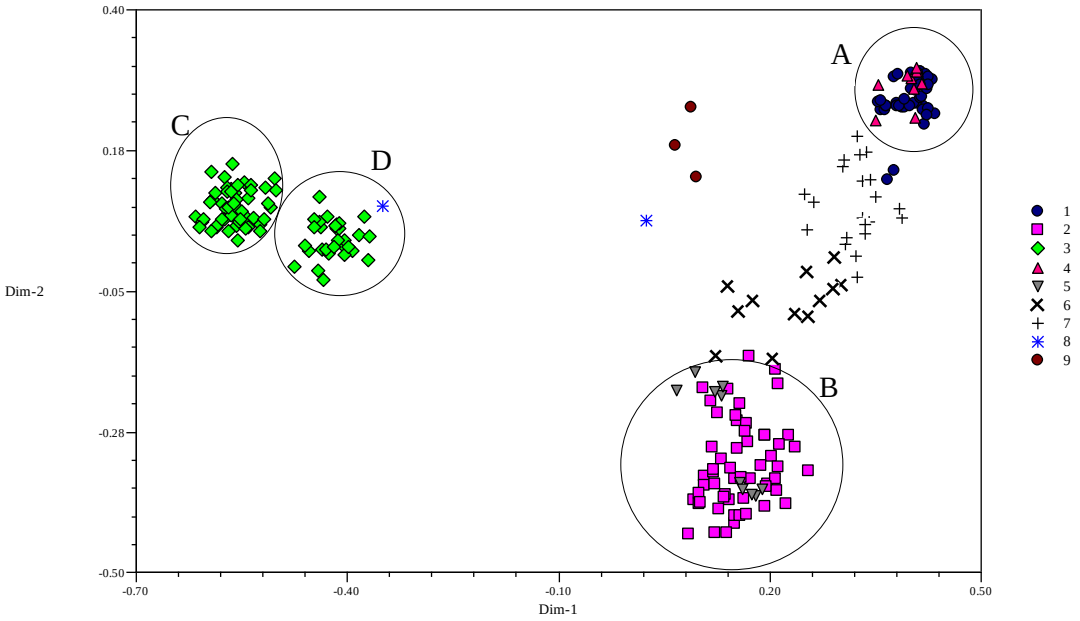

FIGURE 3. 4: 2D plot of the native South Africa *Tamarix usneoides* and alien *T. ramosissima*, *T. chinensis* and their putative hybrids resulting from Principal Coordinates Analysis using the Dice similarity coefficient. The numbers in the key are: 1= *T. chinensis* (Asia); 2= *T. ramosissima* (Asia); 3= *T. usneoides* (southern Africa); 4= *T. chinensis* (South Africa); 5= *T. ramosissima* (South Africa); 6= *T. ramosissima* x *T. chinensis*, 7= *T. chinensis* x *T. ramosissima*; 8=*T. usneoides* x *T. chinensis*; 9= *T. chinensis* x *T. usneoides*). Clusters are formed as A) *T. chinensis*, B) *T. ramosissima*, and C and D) *T. usneoides*. Results of Asian samples were obtained from Gaskin et al. 2011.

It was found that individuals identified as *T. chinensis* corresponded to one cluster (Fig 3.5) with an average posterior probability (PP) of 0.995 and those identified as *T. ramosissima* had an average assignment value of 0.985. The highest average assignment value was for the third cluster (*T. usneoides*) with a posterior probability of 0.997 (Fig 3.5). The putative hybrids of *Tamarix* from South Africa were found to be genetically intermediate with their mean posterior

probabilities corresponding to the *T. chinensis* cluster (0.415), the *T. ramosissima* cluster (0.512) and the *T. usneoides* cluster (0.074).

Tamarix usneoides populations contributed the least genetic material to the South African putative hybrid population structure, which is unexpected given the high number of hybrids found in South Africa (Mayonde 2010). Each individual was identified as any of the three parental species if it was assigned with >90% PP to one of the three clusters, e.g. specimen GM082 has 99.9% assignment value corresponding with *T. usneoides*, 0.001% *T. ramosissima* and 0% *T. chinensis*, therefore this specimen was concluded to be *T. usneoides* (Appendix IV). However, it was assumed that if a plant assigns at less than 90% to any one of the three species, it is genetically admixed (Pritchard et al. 2000; Blair and Hufbaur 2009). Based on the posterior probabilities, it was found that 10 (7.2%) specimens of southern African samples were identified as pure parental *T. chinensis*, 10 samples as pure *T. ramosissima*, and 81 (58.3%) specimens were identified as pure *T. usneoides*. Thirty-eight (27.34%) individuals were identified by the structure assignment values as hybrids with only five individuals (GM043, GM133, GM134, GM142 and GM149) being *T. usneoides* hybrids (Appendix IV). Only 13% of hybrids are *T. usneoiedes*, contrary to the 43% found in the previous study conducted based on only the ITS and morphological characters (Mayonde 2010)

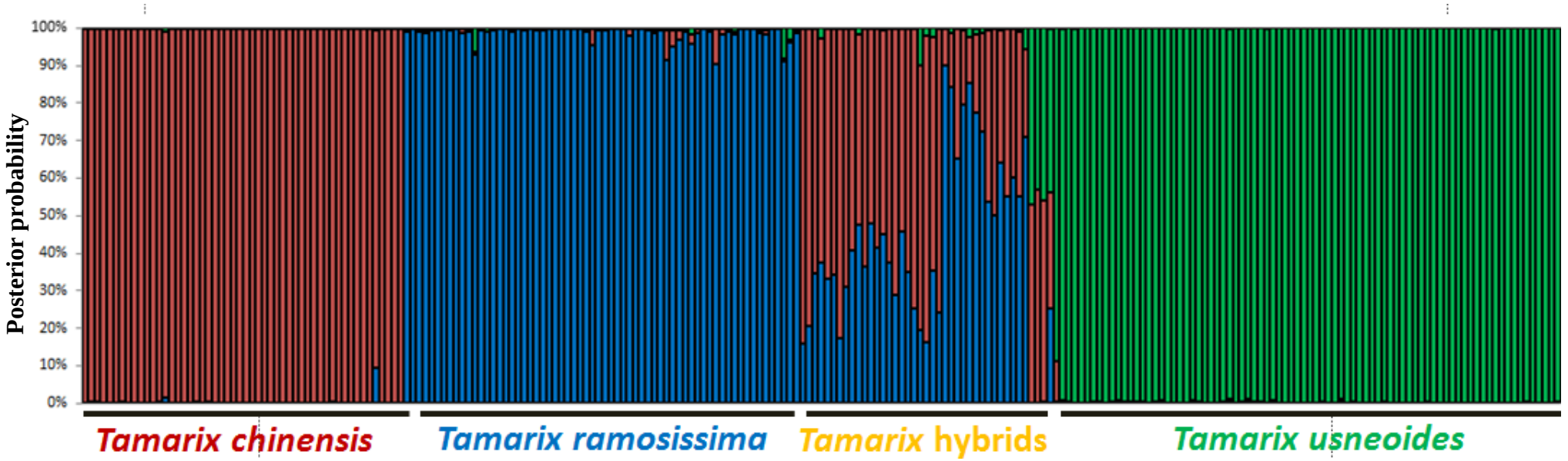

FIGURE 3. 5: Graphical summary of a Bayesian model-based clustering analysis (Structure 2.3.4) for 239 samples of *Tamarix* (100 Asian and 139 southern African plants). Each vertical bar represents one individual and individuals are grouped by regions based on their identities. Percentages on the x-axis correspond with the assignment values that an individual is associated with any of the three clusters.

3.7. GEOGRAPHIC DISTRIBUTION OF SOUTHERN AFRICAN *TAMARIX* POPULATIONS BASED ON AFLP POPULATION STRUCTURE ANALYSIS

During plant data collection, field identification based on morphological features found the three major *Tamarix* species (*T. chinensis, T. ramosissima* and *T. usneoides*) and their putative hybrids across Gauteng, Northern Cape, Free State, Western Cape and Eastern Cape provinces (Appendix IV). Based on the AFLP Structure analysis, it is evident that the indigenous species *Tamarix usneoides* predominantly occurs in the proposed native ranges of the Northern Cape, Eastern Cape and Western Cape provinces (Fig 3.6; Table 3.2), with a few cultivated specimens occurring in gardens (Gauteng). Most of the *T. chinensis* populations occur in the Western Cape where they have been widely planted in gardens as ornamentals. However, it should be noted that a garden, stand-alone, (±40 years old, specimen number GM112) tree was observed in Wallekraal in the Northern Cape, on an old abandoned farm. This specimen was initially thought to be a *T. ramosissima* (Appendix III) but AFLP diagnosis revealed that it is a *T. chinensis* with a 99.8% assignment value (Appendix IV). It is the only *T. chinensis* in the province and looks like it may have been one of the first introductions in to the country (Fig 3.6). *Tamarix ramosissima* individuals are observed in the Northern Gauteng, Free State, Northern Cape and Eastern Cape. Most of these specimens are garden planted or nursery cultivated trees for or planted on the mines.

The different types of *Tamarix* hybrids identified are scattered all over the provinces of South Africa. All the parent *Tamarix* species in South Africa co-occur with each other, where *T. chinensis* and *T. usneoides* populations are found in the same locations in the Northern Cape and in the Western Cape, *T. chinensis* and *T. ramosissima* co-occur in the Free State and *T. ramosissima* and *T. usneoides* co-occur in the Northern Cape. However, it is surprising to see that the putative hybrids do not appear in the habitats where the parental types co-occur (Fig. 3.6; Table 3.2), despite the hybrids occurring all over the country. This observation suggests that most introduced specimens are of hybrid origin, or that interbreeding is taking place in a population which was not sampled in this study. Despite some parental species co-occurring in certain populations, there is the exception of a *T. usneoides* population along the western border with Namibia, which has only the indigenous species (Fig. 3.6; Table 3.2). There are some populations of 'pure' *T. usneoides* populations across the different provinces of investigation, but

these populations are not considered remote as they are somewhat surrounded by the exotic species and their putative hybrids (Fig. 3.6).

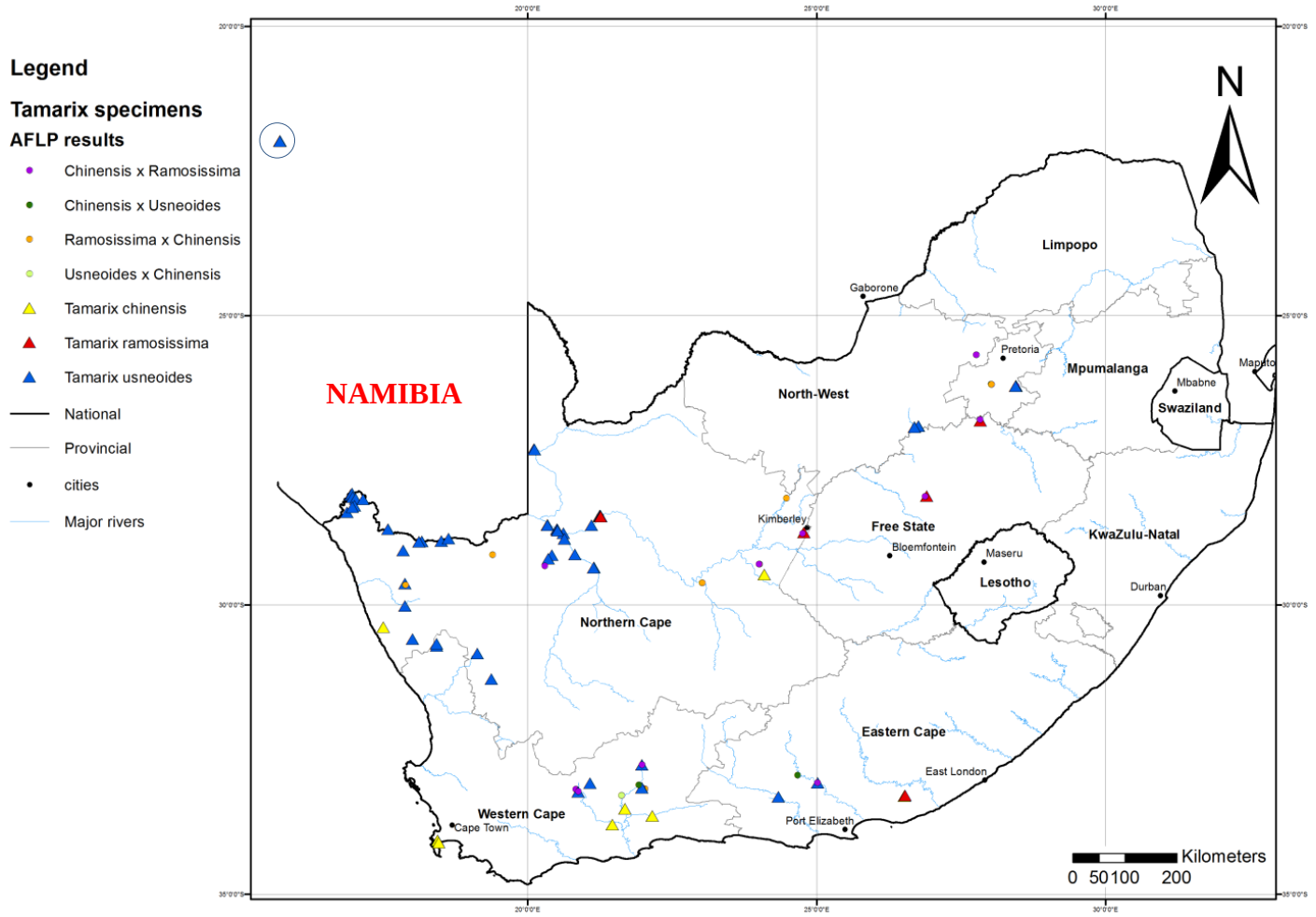

FIGURE 3. 6: Distribution map of the 139 southern African *Tamarix* specimens examined using AFLP markers. Locations are based on latitude/longitude coordinates (Table 3.2). All identities displayed were verified using AFLP markers, and some populations are close to each other and may appear as one on the map due to overlap. Namibian rivers are not shown on the map.

TABLE 3. 2: *Tamarix* species composition grouped by Provinces/South Africa. Species confirmations are based on the AFLP analysis (*Tamarix usneoides* are colored in green, *T. ramosissima* in blue, *T. chinensis* in orange and *Tamarix* hybrids in red).

Collecting Number	species composition	Latitude	Longitude
North West Province			
GM001	*Tamarix usneoides*	26°55.952"S	26°41.575"E
GM003	*Tamarix usneoides*	26°55.952"S	26°41.590"E
GM004	*Tamarix usneoides*	26°55.956"S	26°41.582"E
GM005	*Tamarix usneoides*	26°55.949"S	26°41.581"E
GM007	*Tamarix usneoides*	N/A	N/A
GM008	*Tamarix usneoides*	N/A	N/A
GM018	*Tamarix usneoides*	26°54.54"S	26°45.80"E
GM021	*Tamarix usneoides*	26°54.51"S	26°45.84"E
GM024	*Tamarix usneoides*	26°54.58"S	26°45.86"E
GM028	*Tamarix usneoides*	26°55.93"S	26°41.64"E
GM054	*Tamarix usneoides*	26°13.059"S	28°26.760"E
GM055	*Tamarix usneoides*	26°13.058"S	28°26.772"E
GM056	*Tamarix usneoides*	26°13.019"S	28°26.761"E
GM057	*Tamarix usneoides*	26°12.981"S	28°26.912"E
GM059	*Tamarix usneoides*	26°13.076"S	28°26.948"E
GM062	*Tamarix usneoides*	26°13.125"S	28°26.799"E
GM116	*Tamarix usneoides*	S30°50.701′	E19°07.810′
GM117	*Tamarix usneoides*	S31°17.119′	E19°22.576′
GM118	*Tamarix usneoides*	S29°21.720′	E21°08.706′
GM119	*Tamarix usneoides*	S29°07.832′	E20°49.202′
GM120	*Tamarix usneoides*	S28°37.382′	E21°06.568′
GM121	*Tamarix usneoides*	S27°18.766′	E20°06.561′
GM122	*Tamarix usneoides*	S27°18.981′	E20°07.296′
GM011	*T. ramosissima* x *T. chinensis*	N/A	N/A
GM012	*T. chinensis* x *T. ramosissima*	N/A	N/A
GM060	*T. chinensis* x *T. ramosissima*	26°13.133"S	28°26.801"E
GM061	*T. chinensis* x *T. ramosissima*	26°13.134"S	28°26.882"E
GM123	*T. chinensis* x *T. ramosissima*	S25°40′45.4"	E27°45′27.1"
GM124	*T. chinensis* x *T. ramosissima*	S25°40′42.8"	E27°45′24.0"
Gauteng Province			
GM014	*Tamarix ramosissima*	N/A	N/A
GM063	*T. ramosissima* x *T. chinensis*	S26°11′27.54"	E28°01′12.03"
GM064	*T. ramosissima* x *T. chinensis*	S26°11′04.55"	E28°01′12.44"
GM065	*T. ramosissima* x *T. chinensis*	S26°11′35.56"	E28°01′12.58"
Free State Province			
GM066	*Tamarix ramosissima*	S26°48.997′	E27°49.988′

Collecting Number	species composition	Latitude	Longitude
GM068	*Tamarix ramosissima*	S28°07.189′	E26°54.564′
MM156	*Tamarix ramosissima*	N/A	N/A
GM067	*T. chinensis* x *T. ramosissima*	S26°47.816′	E27°49.837′
GM069	*T. chinensis* x *T. ramosissima*	S28°07.612′	E26°52.424′
GM070	*T. chinensis* x *T. ramosissima*	S28°07.604′	E26°52.436′
MM155	*Tamarix chinensis*	N/A	N/A
Northern Cape Province			
GM031	*Tamarix usneoides*	28°27.782"s	21°15.193"E
GM032	*Tamarix usneoides*	28°27.761"S	21°15.209"E
GM033	*Tamarix usneoides*	S28°27.829"	21°15.233"E
GM035	*Tamarix usneoides*	28°27.851"S	21°15.910"E
GM037	*Tamarix usneoides*	28°28.090"S	21°14.791"E
GM038	*Tamarix usneoides*	28°27.496"S	21°15.113"E
GM039	*Tamarix usneoides*	N/A	N/A
GM040	*Tamarix usneoides*	N/A	N/A
GM042	*Tamarix usneoides*	28°27.975"S	21°15.863"E
GM045	*Tamarix usneoides*	28°27.856"S	21°15.778"E
GM046	*Tamarix usneoides*	28°28.680"E	21°16.244"E
GM047	*Tamarix usneoides*	28°28.683"S	21°16.285"E
GM050	*Tamarix usneoides*	29°21.292"S	21°08.862"E
GM051	*Tamarix usneoides*	28°45.511"S	20°37.272"E
GM052	*Tamarix usneoides*	28°37.263"S	20°20.883"E
GM053	*Tamarix usneoides*	28°37.246"S	20°20.864"E
GM080	*Tamarix usneoides*	S28°41.627′	E20°30.466′
GM081	*Tamarix usneoides*	S28°41.602′	E20°30.504′
GM082	*Tamarix usneoides*	S28°42.301′	E20°31.130′
GM083	*Tamarix usneoides*	S28°43.228′	E20°31.312′
GM084	*Tamarix usneoides*	S28°37.381′	E20°20.849′
GM085	*Tamarix usneoides*	S28°51.486′	E20°38.485′
GM086	*Tamarix usneoides*	S29°08.319′	E20°25.392′
GM087	*Tamarix usneoides*	S29°12.217′	E20°21.732′
GM090	*Tamarix usneoides*	S28°53.757′	E18°30.724′
GM091	*Tamarix usneoides*	S28°53.759′	E18°30.729′
GM092	*Tamarix usneoides*	S28°51.263′	E18°38.125′
GM093	*Tamarix usneoides*	S28°54.413′	E18°10.454′
GM094	*Tamarix usneoides*	S28°54.359′	E18°10.368′
GM095	*Tamarix usneoides*	S28°54.546′	E18°07.444′
GM096	*Tamarix usneoides*	S28°54.616′	E18°07.446′
GM097	*Tamarix usneoides*	S28°41.417′	E17°35.231′
GM098	*Tamarix usneoides*	S28°41.414′	E17°35.183′
GM099	*Tamarix usneoides*	S29°03.709′	E17°50.786′
GM100	*Tamarix usneoides*	S28°24.107′	E16°52.632′
GM101	*Tamarix usneoides*	S28°06.085′	E16°56.571′

Collecting Number	species composition	Latitude	Longitude
GM102	*Tamarix usneoides*	S28°04.499′	E16°57.776′
GM103	*Tamarix usneoides*	S28°04.424′	E16°57.822′
GM104	*Tamarix usneoides*	S28°08.442′	E16°58.727′
GM105	*Tamarix usneoides*	S28°10.107′	E17°01.547′
GM106	*Tamarix usneoides*	S28°10.469′	E17°09.507′
GM107	*Tamarix usneoides*	S28°17.378′	E17°00.346′
GM108	*Tamarix usneoides*	S28°18.655′	E16°58.290′
GM109	*Tamarix usneoides*	S29°38.190′	E17°52.855′
GM111	*Tamarix usneoides*	S30°01.095′	E17°52.815′
GM113	*Tamarix usneoides*	S30°35.671′	E18°00.606′
GM044	*Tamarix ramosissima*	28°27.918"S	21°15.856"E
GM048	*Tamarix ramosissima*	28°28379"S	21°15.709"E
GM049	*Tamarix ramosissima*	28°28.379"S	21°15.709"E
GM071	*Tamarix ramosissima*	S28°45.018′	E24°46.777′
GM036	*T. ramosissima* x *T. chinensis*	28°28.078"S	21°14.761"E
GM043	*T. usneoides* x *T. chinensis*	28°27.946"S	21°15.863"E
GM072	*T. chinensis* x *T. ramosissima*	S28°45.915′	E24°45.596′
GM073	*T. ramosissima* x *T. chinensis*	S28°09.573′	E24°28.800′
GM074	*T. ramosissima* x *T. chinensis*	S29°17.732′	E24°00.589′
GM075	*T. ramosissima* x *T. chinensis*	S29°17.881′	E24°00.293′
GM076	*T. chinensis* x *T. ramosissima*	S29°17.901′	E24°00.284′
GM078	*T. ramosissima* x *T. chinensis*	S29°37.366′	E23°00.984′
GM079	*T. chinensis* x *T. ramosissima*	S29°37.363′	E23°00.988′
GM088	*T. chinensis* x *T. ramosissima*	S29°19.781′	E20°17.945′
GM089	*T. ramosissima* x *T. chinensis*	S29°07.966′	E19°23.547′
GM110	*T. ramosissima* x *T. chinensis*	S29°39.569′	E17°53.368′
GM077	*Tamarix chinensis*	S29°28.812′	E24°05.929′
GM112	*Tamarix chinensis*	S30°23.324′	E17°30.515′
Eastern Cape			
GM148	*Tamarix usneoides*	S33°19.350′	E24°20.410′
GM151	*Tamarix usneoides*	S33°04.678′	E25°00.962′
GM147	*Tamarix ramosissima*	S33°19.330′	E24°20.410′
GM154	*Tamarix ramosissima*	S33°17.873′	E26°32.001′
GM149	*T. chinensis* x *T.usneoides*	S32°56.990′	E24°40.184′
GM150	*T. chinensis* x *T. ramosissima*	S33°04.604′	E25°00.927′
GM152	*T. chinensis* x *T. ramosissima*	S33°04.705′	E25°00.947′
GM153	*Tamarix chinensis*	S33°18.491′	E26°31.368′
Western Cape			
GM114	*Tamarix usneoides*	S30°42.559′	E18°25.655′
GM115	*Tamarix usneoides*	S30°40.458′	E18°25.727′
GM130	*Tamarix usneoides*	S33°13.889′	E20°52.587′
GM141	*Tamarix usneoides*	S33°09.896′	E21°58.884′
GM143	*Tamarix usneoides*	S33°05.217′	E21°04.783′

Collecting Number	species composition	Latitude	Longitude
GM145	*Tamarix usneoides*	S32°46.067′	E21°58.780′
GM125	*Tamarix chinensis*	S34°04.345′	E18°26.828′
GM126	*Tamarix chinensis*	S34°04.432′	E18°26.808′
GM127	*Tamarix chinensis*	S34°06.829′	E18°27.914′
GM140	*Tamarix ramosissima*	S33°09.897′	E21°58.877′
GM128	*T. chinensis* x *T. ramosissima*	S33°11.544′	E20°49.877′
GM129	*T. chinensis* x *T. ramosissima*	S33°11.552′	E20°49.863′
GM131	*T. chinensis* x *T. ramosissima*	S33°13.830′	E20°52.517′
GM133	*T. chinensis* x *T.usneoides*	S33°17.618′	E21°37.386′
GM134	*T. usneoides* x *T. chinensis*	S33°17.611′	E21°37.405′
GM137	*T. chinensis* x *T. ramosissima*	S33°10.923′	E22°01.648′
GM138	*T. ramosissima* x *T. chinensis*	S33°10.945′	E22°01.662′
GM139	*T. chinensis* x *T. ramosissima*	S33°09.968′	E21°58.813′
GM142	*T. chinensis* x *T.usneoides*	S33°07.100′	E21°55.490′
GM144	*T. chinensis* x *T. ramosissima*	S32°46.051′	E21°58.770′
GM146	*T. chinensis* x *T. ramosissima*	S32°46.070′	E21°58.842′
GM132	*Tamarix chinensis*	S33°31.749′	E21°41.151′
GM135	*Tamarix chinensis*	S33°47.952′	E21°28.258′
GM136	*Tamarix chinensis*	S33°39.004′	E22°09.410′
Namibia			
MS003	*Tamarix usneoides*	S21°58′45.26"	E15°43′14.60"
MS004	*Tamarix usneoides*	S21°58′45.33"	E15°43′14.24"
MS005	*Tamarix usneoides*	S21°59′09.78"	E15°43′10.96"

CHAPTER IV DISCUSSION

4.1. CHARACTERIZATION OF TAMARIX SPECIES AND THEIR PUTATIVE HYBRIDS IN SOUTHERN AFRICAN BASED ON MOLECULAR MARKERS (ITS AND *trn*S-*trn*G REGIONS).

Phylogenetic analysis of the relationship of the southern African *Tamarix* species and hybrids using nuclear DNA (ITS) sequence data resulted in a consensus tree with two strongly supported clades that clearly separate the native *T. usneoides* from the two alien species (Fig 3.1). Clade A groups all South African exotic *Tamarix chinensis* and *T. ramosissima* together with some of their hybrids. It is apparent that *T. chinensis* and *T. ramosissima* are closely related as evidenced by both morphological (Baum, 1978) and DNA molecular data (Fig 3.1; Gaskin and Schaal, 2003; Mayonde, 2010). Baum's 1978 morphological differentiation documents that the slight differences between these two taxa lie in their floral features (sepal margins, petal shape, and filament insertion). *Tamarix chinensis* and *T. ramosissima*, both from Asia, are exotic and invasive in South Africa (Henderson, 2001). They appear to be very closely related species, as they are unresolved (form a polytomy) in the consensus tree resulting from parsimony analysis based on the ITS regions (Clade A, Fig 3.1) and are mixed in a fairly well supported clade in the consensus from the *trn*S-*trn*G analysis (Fig 3.2). This result corresponds with the investigation conducted in the United States of America where these same species are equally exotic and invasive (Gaskin and Schaal, 2003). The phylogenies of the USA invasive species based on both the nuclear ITS and plastid regions also have unresolved clades that fail to separate the two taxa (Gaskin and Schaal, 2002–2003). However, in the phylogenetic analysis of ITS data from the South African samples, some specimens of *T. ramosissima* (GM066, GM068, GM154) have two synapomorphic point mutations that group them together and separate them from others. Another strongly supported sub-clade within clade A (Fig 3.1) of mainly *T. ramosissima*, *T. ramosissima* hybrids and *T. chinensis* hybrids appears to comprise mainly garden cultivated specimens, with the exception of one sample of *T. ramosissima* hybrid (GM073), from Barkley in the Northern Cape Province.

Tamarix chinensis and *Tamarix ramosissima* were synonymized as *T. ramosissima* (Crins, 1989). However, Baum (1978) in his revision of the Genus *Tamarix* showed some distinct differences between these two taxa and placed them in different sections. He placed *T.*

ramosissima in section Tamarix, series Gallicae, while *T. chinensis* was put in section Oligandenia, series Laxae. The two species can be distinguished by eroded-denticulate sepals, obovate petals, 3–4 mm raceme width, hypodiscal filaments insertion in *T. ramosissima*, as opposed to entire sepals, elliptic-ovate petals, 5–7 mm raceme width and hypo-peridiscal filament insertion in *T. chinensis* (Baum 1978). Despite the morphological differences of *T. chinensis* and *T. ramosissima*, it is evident that there is not enough signal at the ITS regions to separate these two closely related species given that they consistently remain unresolved in the consensus tree (Fig 3.1) and the presence of hybrids confounds the analysis. They are nonetheless shown to be distinct species based on the AFLP analyses (Figs. 3.4 and 3.5; Gaskin and Kazmer 2009; Gaskin et al. 2011)

The southern African indigenous *T. usneoides* appears to be genetically distantly related to the exotic species (*T. ramosissima*, and *T. chinensis*) as evidenced by the phylogenies presented here. This fact explains the reason why there is little hybridization between the indigenous *Tamarix usneoides* and the exotic *Tamarix* species. It has been proven that closely related species are more likely to interbreed than distantely related species (Harper, 1961, Mallet, 2005). Clade B (Fig 3.1) consists of all specimens identified as pure bred *Tamarix usneoides* and a 'morphologically identified' *T. usneoides* hybrid, and it is subdivided into sub-clades C and D. Most of *Tamarix* specimens from the mines group together in sub-clade C with the exception of six specimens (GM031, GM035, GM037, GM041, GM043 and GM120) from the wild. These 'non-mine' specimens are all from one population at Upington (Northern Cape) except for GM120 which is from Kanoneiland c. 20 km from Upington en route to Keimoes and that particular tree grows along the Orange River which flows from Upington to Kanonieland. There is a geographic correlation and a history associated with specimens grouped in sub-clade C as the wild *Tamarix* populations from Upington and the surrounding areas have been propagated for planting on the mines (I. Weiersbye pers com). Thus individuals in sub-clade C might be clonal. The fact that specimens in sub-clade C (Fig 3.1) group together in cluster D on the Principal Coordinate Analysis plot (Fig. 3.4), further suggests that they are genetically slightly different from specimens in sub-clade D. Sub-clade D (Fig 3.1) contains most of the pure-breeding *T. usneoides* with ≥99.8% PP values for *T. usneoides* (Appendix IV), and these are grouped together in cluster C (Fig 3.4). *Tamarix* specimens in sub-clade D are from remote wild populations in the Northern Cape, Western Cape, Eastern Cape and as far north as Namibia (Appendix III).

Analysis of the ITS sequence data of revealed differences in the number of polymorphisms present in the specimens of *Tamarix usneoides* grouped in clade B (Fig. 3.1; and see Table 3, Mayonde 2010). It was found that specimens grouped in sub-clade C (Fig. 3.1) have considerably more clear polymorphisms that correspond with both putative parents (viz. *T. usneoides* and either *T. chinensis* or *T. ramosissima*) (Mayonde 2010). Double base readings (polymorphisms) reflecting alleles from both parents were considered informative and used to assist in the recognition of hybrids. This form of polymorphism occurs due to heterozygosity at a locus (sequence base combined into the same genome through hybridization) and they appear to be a good indicator of hybridization (Nickrent and Soltis, 1995). However, if polymorphisms reflected only one allele from either of the parents, they were not used to recognise hybrids and separate them from pure-breeding specimens (Mayonde 2010) because they can also occur in plants due to incomplete concerted evolution (Bailey et al., 2003). Although, polymorphisms can be a good indicator of hybridization, they do not contribute toward phylogenetic reconstruction (Nickrent and Soltis 1995) and therefore did not play a role in the branching of specimens in clade B (Fig 3.1), as they may carry information from two sources (maternal and paternal alleles). Nonetheless, the presence of many distinct polymorphisms (apparently indicating hybrid parentage) amongst the specimens in sub-clade C, together with some morphological intermediacy, puts some doubt on their status as entirely pure-breeding *T. usneoides*.

The lack of informative synapomorphies encountered in the ITS nuclear region to distinguish the closely related species *T. chinensis* and *T. ramosissima* (clade A, Fig. 3.1) suggests that there is a high level of hybridization among *Tamarix* species in southern Africa. This is evidenced by the lack of resolution in the ITS consensus tree (Fig. 3.1), which could be due to homoplasy. Since there are many hybrids carrying information from both parents present in the clade, it may result in branches collapsing. The high number of polymorphisms (not phylogenetically informative) due to both hybridization and incomplete concerted evolution also contributes to the collapse of branches that are not well supported.

The Partition Homogeneity test shows that there is lack of congruency ($P = 0.01$) between the data sets of the plastid (*trn*S-*trn*G) and nuclear (ITS) regions. When nuclear DNA sequences from distantly related taxa are used to infer higher level phylogenetic relationships, the phylogenetic tree reconstruction will result in gene-tree species-tree conflicts due to phylogenies generated with other data sources (Nickrent and Soltis 1995). Contrary to the full ITS

phylogenetic analysis (Fig 3.1) and the restricted ITS phylogeny (Fig. 3.3), there is considerable resolution among *Tamarix chinensis* and *T. ramosissima* specimens in the phylogenetic analysis of the plastid region. However, *T. chinensis*, *T. ramosissima* are not separated into species-specific groups, except that sub-clade F groups *T. ramosissima* individuals with its hybrids and sub-clade J groups *T. chinensis* together with its hybrids (Fig 3.2). The mixed clades indicate that there is hybridization occurring between *T. chinensis* and *T. ramosissima* and this is confirmed by the AFLP analyses. There is however a deletion of two bases (TA) at positions 189—190 in all *T. ramosissima* (Table 3.1) that indicates a distinct evolutionary difference between *T. chinensis* (and some members of hybrids) and *T. ramosissima*. Nevertheless, the majority of the indels (deletions or insertions) observed in Table 3.1 distinguish between *T. usneoides* and the other taxa. The high level of hybridization in South African *Tamarix* populations is causing branches to collapse in the phylogenies causing the lack of congruence in trees generated from the analysis of the maternally inherited (*trn*S-*trn*G) vs. the biparental (ITS) data sets.

The phylogenetic relationships of southern African *Tamarix* species and their putative hybrids based on the nuclear ITS sequence data (Fig 3.1, 3.3) and plastid sequence (*trn*S-*trn*G; Fig 3.2) indicate that there are three *Tamarix* species in South Africa with the indigenous species being distinct from the two exotic ones. However, closely related species such as *T. chinensis*, *T. ramosissima* and their putative hybrids are not separated due to lack of resolution in the phylogenies; a finding that corresponds with the results obtained by Gaskin and Schaal (2002, 2003). *Tamarix usneoides* specimens also cannot be readily distinguished from their putative hybrids by the phylogenetic analyses. The lack of resolution in the phylogenies is very likely partly due to the high incidence of hybridization among these *Tamarix* species (Whitney et al., 2010). The distinct lineages that should be resolved using a phylogenetic approach are confounded by individuals of hybrids origin. Another possible explanation could be that there has been introgression amongst the hybrid and the parent populations (Gaskin and Kazmer, 2009).

4.2. POPULATION STRUCTURE OF *TAMARIX* SPECIES IN SOUTHERN AFRICA USING THE DOMINANT MOLECULAR MARKER (AFLP)

As evidenced by the lack of congruence between the nuclear and plastid sequence data sets, it is clear that hybridization is occurring among the various *Tamarix* species in South Africa. However, the phylogenetic analyses have not been able to clearly distinguish the exotic *Tamarix*

species present in South Africa as the clades containing *Tamarix chinensis* and *T. ramosissima* remain unresolved or are mixed. To further investigate the *Tamarix* species composition, hybrid status, invasiveness and show the genetic population structure of *Tamarix* species in South Africa, we used AFLP markers (i.e. quickly evolving markers) which can distinguish closely related species and identify cryptic species. When evaluating hybridization between taxa that are closely related, it is important to determine species genetic boundaries (Nicole et al., 2007) which is critical for conservation purposes; and molecular markers have been useful for clarifying the relationships of closely related species (Gaskin and Kazmer, 2009, Moody and Les, 2007, Safer, 2011).

AFLP markers have clearly shown that there are three genetically distinct *Tamarix* species in South Africa as evidenced by their separation in the plots of the Principal Coordinates Analysis (Fig 3.4) and the Structure analysis (Fig 3.5). Both these analyses show that individuals are largely sorted into three distinct genetic clusters and these clusters correspond to species identified using morphological features. This study included some *Tamarix chinensis* and *T. ramosissima* individuals examined by Gaskin et al. (2011) from their places of origin in Asia. The Principal Coordinates analysis and the population structure analysis both indicate that there is a continuum of hybrids in the South African *Tamarix* populations. AFLP markers have been able to identify more hybrids individuals than the ITS sequence data (27.34% as compared to 16%). This finding corresponds with the results by Gaskin and Kazmer (2009) where the AFLP markers identified more than 80% hybrids in the US invasion compared to the 23% observed using sequence data (Gaskin and Schaal, 2002, 2003). The identification of southern African hybrids based on nuclear ITS sequence data appear to be higher as compared to the analysis done on the US individuals, and this could be because analysis of polymorphisms was used to assist in the identification of the southern African hybrids (Mayonde, 2010).

The AFLP data showed that most of South Africa *Tamarix* hybrids are between *T. chinensis* × *T. ramosissima* with only five specimens (13%) of the hybrids being between *T. chinensis* (exotic) and *T. usneoides* (indigenous). In recent years, ITS characters (markers) have improved the understanding of phylogeny in several aspects e.g. improving resolution of species relationships; resolving phylogenetic conflicts between closely related species (Baldwin et al. 1995). However, despite high copy numbers of ITS1 and ITS2 spacers, the near uniformity of ITS paralogues is attributed to concerted evolution, which in most cases will require direct sequencing to construct

phylogeny trees. But in case of divergent paralogues, the ITS spacers may require cloning to reveal the genetic differences that may improve phylogenetic resolution in closely related species (Baldwin, 1995). This might be the case with our *Tamarix* sequence data in order to distinguish *T. chinensis* and *T. ramosissima* and their putative hybrids. Since the divergent paralogue hypothesis applies mostly within a species, the presence of hybrids in our data set could be the reason for branches to collapse. On the other hand, AFLP analysis is one of the three most common techniques for multilocus genomic fingerprinting (Meudt and Clark, 2007). Moreover, AFLPs have reproducibility, robustness and rapidly generate hundreds of highly informative replicable markers from DNA; thus, they allow high-resolution genotyping by fingerprinting (Mueller and Wolfenbarger 1999; Meudt and Clark 2007). AFLPs have been able to produce high polymorphic markers in the South African *Tamarix* populations which have enabled discrimination of the three parental species, and proven useful in the identification of their putative hybrids (Appendix IV) using Principal Coordinates Analysis and Structure analyses.

Excluding the indigenous species *Tamarix usneoides*, there are 58 (42% of the total data set) individuals representing the *Tamarix* invasion in South Africa. Of the 58 individuals, the AFLP data reveal that this *Tamarix* invasion is dominated by hybrids (65.5%, Appendix IV). These results correspond with those of Gaskin and Kazmer, (2009) who showed that *Tamarix* infestations in the western U.S.A are pre-dominantly hybrids. The high proportion of the *Tamarix* hybrids may have facilitated the success of *Tamarix* invasion in South Africa. It is not surprising that the posterior probability or genetic assignment values (Appendix IV) indicate that *Tamarix* hybrids in South Africa are dominated by interbreeding between *T. chinensis* and *T. ramosissima* as they are the most closely related species. In invasion biology studies closely related exotic species have histories of hybridizing, thereby producing admixed progenies that are highly invasive (e.g. watermilfoil '*Myriophyllum*' populations, Moody and Les 2002; the invasion of *Casuarina* species as Florida Sheoak, USA, Gaskin et al. 2009). Of all the hybrids in South Africa, 33 (87%) are a combination between either *T. chinensis* × *T. ramosissima* or vice-versa with the remainder (13%) being the result of interbreeding between *T. chinensis* and *T. usneoides*. This result is unexpected as observation of morphological intermediates (mainly in cultivation on the mines) had led to the hypothesis that *T. usneoides* was hybridizing with *T. ramosissima* (Weiersbye et al. 2006). This hypothesis was tested and not rejected by Bredenkamp and Phepo (2008), Mayonde (2010). However, difficulties in distinguishing the species and hybrids using morphological features and the lack of clear signal in the nuclear ITS

sequence might have been misleading, especially with the recognition of the role played by *T. ramosissima* and *T. chinensis* in hybrid presence in South Africa.

Hybridization has been linked to aggressive and invasive traits in plants (Ellstrand and Schierenbeck, 2000) where there are 28 examples in which invasiveness was proceeded by hybridization and attributed in many cased to hybrid vigour. (Anderson, 1949) suggested that hybridity can also produce novel genotypes with ecological tolerances that differ from those of the parents (Abbott 1992). This is evidently the case with *Tamarix* invasion in South Africa (Mayonde, 2010) and in the United States of America (Gaskin and Schaal, 2003; Gaskin and Kazmer, 2009). However, neither of the two studies has tested the *Tamarix* hybrids for selective advantages over their parental types regarding characters that may enhance their invasiveness abilities. Some recent studies have shown that three *Helianthus* species of hybrid origin survived in extreme habitats not suitable for their parental taxa as they possess unique combinations of genes acquired in linkage groups representing each of their parental species (Rieseberg and Ellstrand, 1993, Rieseberg et al., 1990).

Even though hybridization may have been proven to provide an advantage to a plant invasion, hybridization is not the only factor that promotes the invasiveness of a plant species because many plants are known to hybridize naturally within their native ranges but do not necessarily become invasive there. The success of a hybrid to become invasive in the introduced ranged, maybe because it suffers less biotic (e.g. herbivors, pests, pathogens) and abiotic pressures (e.g. environmental limiting conditions) (Blair and Huffbaur, 2009). Although this suggests that hybridization might confer some advantage to an invasive species, the evidence can not be definitive, as other aspects need to be taken into consideration (Blair and Huffbaur, 2009). To better understand the mechanism behind the invasion of exotic plant species, one needs to look as well at the enemy release hypothesis (ERH). The ERH states that plant species, on introduction into an exotic habitat, experience a decrease in overall herbivore attack (Cappuccino and Carpenter, 2005, Muller-sharer and Schaffner, 2008), or are attacked fewer by herbivorous invertebrate (Kean and Crawley, 2002) and fungal or viral pathogens (Mitchell and Power, 2003). The reduction in top-down regulation by natural enemies allows exotic plants to out-compete the native plants in the introduced range and increase in abundance (Muller-sharer and Schaffner, 2008). In a biological control management programme of alien invasive plants, hybridization has the potential to change interactions between potential bio-control agents and

the target weed through evolutionary changes within populations of the weeds (Fritz, 1999). However, in some instances, hybrid plants could exploit an ecological resource different from either parents, or even occupy a niche that is not found to either of the parents (Spencer et al., 2011). In such case, hybrid plants might have their own entofauna interactions which are different from their parent species.

Hybrids might be more or less, or equally resistant to enemy attack than their parental species (Fritz, 1994). A study by (Roley and Newman, 2006) found the highest survival of a native weevil on an introduced watermilfoil, with the lowest survival on the native species, and an intermediate survival on the hybrids between the two. This scenario will have a positive implication for effectiveness of biological control programme of alien invasive plants. In contrast, (Whitham, 1989) observed that hybrid cotton woods were more susceptible to aphid attack than either pure parental species, causing these hybrid individuals acts as sinks for the aphids preventing aphid adaptation to either of the parents. This scenario will be of concern if one of the parents is the targeted plant in a biocontrol programme. Hybridization and introgression between alien invasive plant species and closely related native species may create a genetic pool that may serve as 'bridge' allowing host specific insects to expand their host ranges from one parental species to another (Floate and Whitham, 1993). It has been shown here by the AFLP data that only 5.26% (2/38) of the hybrids analysed are *Tamarix usneoides* hybrids, i.e. individuals with a relatively high proportion of *T. usneoides* genetic make-up and some other parental species contributing less (Appendix IV). This outcome should be of great advantage to the management programme of alien invasive *Tamarix* species in South Africa, especially when considering classical biological control. This is because evolutionary biologists have documented that hybridization and introgression involving native and non-native species creates difficulties in determining native status of future generations of the indigenous plant (Petit, 2004, Saltonstall, 2002). The presence of putative hybrids involving the exotic and indigenous species can somehow indicate a lack of co-evolution between the target plant and potential biological control agents (Gaskin et al., 2009) although in some instances co-evolution might still have occurred in the past.

Although the *Tamarix* invasion in South Africa does seem to have been dominated by hybrid individuals, it is evident through the assignment values from the Structure analyses that there are different degrees of hybrids. These different degrees of hybrids suggest that there are F_1, F_2

hybrids and introgressed (back-crossed) individuals (Appendix IV) in the *Tamarix* invasion in South Africa. This indicates that *Tamarix* species have been interbreeding for more than two generations since the introduction of the exotic species in to South Africa. Whether novel hybrids are created after an alien weed's introduction (e.g. Gaskin and Schaal, 2002; Gaskin et al., 2009), or if they represent a significant portion of the invasion (as in the case of *Tamarix* in South Africa), they should be considered for inclusion in host specificity tests of a potential biological control agent (Gaskin et al., 2011). This should be applicable even if the hybrids are as a result of interbreeding between or among invasive, alien species that are closely related (Gaskin and Kazmer, 2009); or when an invasive alien taxon hybridizes with a closely-related native species (Moody and Les, 2007).

4.3. GEOGRAPHIC DISTRIBUTION OF DIFFERENT *TAMARIX* SPECIES ACROSS SOUTH AFRICA

It is understandably important to document the geographic pattern of the different *Tamarix* species in South Africa in order to observe their distribution patterns. Revealing the geographic sources of invasive *Tamarix* species introduced into South Africa will help to determine the possible place of origin of potential biological control agents if biocontrol is envisaged as the management measure. It is seen in the 2D plot of PCoA that some of the parental exotic species (*Tamarix chinensis* and *T. ramosissima*) that are invasive in South Africa are overlapping with their counterparts from Asia, their place of origin (clusters A and B, Fig. 3.4). A genetic comparison of these specimens based on the assignment values from Structure analysis show that sample numbers GM077, GM112, GM125, GM126, GM136, GM153 and GM155 have 99.8% *T. chinensis* assignment values (Appendix IV). These specimens are genetically identical to 47.6% of the *T. chinensis* from Asia. In the same manner, *T. ramosissima* specimens GM066, GM068, GM071, GM140, GM154 and GM156 have over 95% *T. ramosissima* posterior probability values (Appendix IV) and are similar to more than 50% of the plants from their place of origin. These observations suggest that in the South African *Tamarix* invasion there are some populations of pure breeding species from their place of origin in Asia. The respective places of origin in Asia could provide the sources of potential biological control agents. It should also be noted that the biological control programme of alien invasive *Tamarix* species (*T. chinensis*, *T. ramosissima* and their putative hybrids) in the United States, using introduced insect species (Milbrath and DeLoach, 2006), have generally not attacked *T. aphylla* which is a closely related

species to the southern African indigenous *T. usneoides* (Baum 1978; Gaskin and Schaal 2003). This suggests that host specific biological control agents (from Asia) for the alien invasive *Tamarix* in South Africa are unlikely to attack *T. usneoides*. Nevertheless, in places such as Texas and Mexico it has been evidenced that *Diorhabda* beetles have defoliated *Tamarix aphylla* as much as the invasive species although the former seem to recover from the damage. This observation could still prove that indigenous *Tamarix usneoides* might suffer damage by the potential biological control agents during host specificity testing. Buckham (2011) found that native insects are more abundant and common on the indigenous species (*T. usneoides*) than on the exotic alien invasive species (*T. ramosissima*) and their putative hybrids. Despite, the presence of these insects on the invasive *Tamarix* there are doing little damage to suppresse the population of the plants. Therefore, they are unlikely to be used as biological control agents.

Molecular diagnostic methods (ITS and *trn*S-*trn*G sequences, and AFLP markers) for identification of *Tamarix* species and their putative hybrids in South Africa have been a far better indicator of hybrids as compared to the morphological diagnosis (Appendix IV). However, there is no difference in identifying pure-bred specimens. In particular, distinguishing pure *T. chinensis* and *T. ramosissima* is difficult as the reproductive morphological features that separate them are better seen under a compound microscope, because of the size of the flower. The morphological identification between *T. usneoides* and *T. ramosissima* was less of a problem due to the fact that the eight morphological characters (Table 1.1) used in the diagnosis were chosen to discriminate them. However, the identification of hybrids was based on the observation of intermediate characters (Mayonde 2010). Therefore the identification of hybrids based on molecular DNA markers is much more reliable than using morphological features. However, the eight morphological features and keys used in this study can be used to separate the indigenous and the exotic species, during field collection for phytoremediation samples. Their identity could then be confirmed using the molecular tools prior to the propagation for cultivation on the mines.

CHAPTER V. CONCLUSIONS

Analysis using molecular markers such as AFLPs, ITS and *trn*S-*trn*G clearly confirm that we have three distinct *Tamarix* species in South Africa with a large number of their putative hybrids. Nuclear and plastid DNA sequence data indicated that *Tamarix chinensis* is genetically very closely related to *T. ramosissima* as they are grouped in the same clade and are distinct from the indigenous *T. usneoides*. However, there is not enough resolution in the phylogenetic signal to reveal the relationships or to separate closely related species from each other or even distinguish the different species from their putative hybrids. Among the three molecular diagnostic measures, AFLPs appear to discriminate between *Tamarix chinensis*, *T. ramosissima* and *T. usneoides* and assign the different types of hybrids than the DNA sequence data from ITS and *trn*S-*trn*G where branches are collapsing due to high levels of hybridization.

In this study, we have conclusively shown that the *Tamarix* infestation in South Africa is predominantly hybrids and introgressed individuals. *Tamarix* hybrid populations in South Africa are apparently dominated by interbreeding between *T. chinensis* and *T. ramosissima*. The low hybridization incidence between the native *Tamarix usneoides* and the exotic species is encouraging news for a potential biological control program. The low number of hybrids means the lesser the chance for the potential biological agents to host shift and cause damage to the indigenous species. And this comes as good news for conservation. A phylogeographic investigation of *Tamarix* species in South Africa has shown that *Tamarix* hybrids co-occur with their parental types. In this case, they mostly occur in places where *Tamarix chinensis* co-occurs with *T. ramosissima*. However, *Tamarix* hybrids are found in some populations together with *T. usneoides* without the presence of their exotic alien invasive parents. This prompts us to suggest that these plants were introduced as hybrids. Some pure breeding exotic *Tamarix* species that have become invasive species have been matched genetically to samples from Asia (their places of origin). These places of origin in Asia should be used to collect the *Tamarix* indigenous specialist insects that can be used as potential biological control agents in a host specificity test. Finally, there a co-relation between the use of molecular markers and morphological characters in identifying pure-bred *Tamarix* species, therefore, characters described in Table 1.1 can be used to discriminate between the indigenous and the exotic species in a field collection, although more characters are needed to further separate the closely related exotic species *T. chinensis* and *T. ramosissima* and their hybrids.

The use of *Tamarix usneoides* for phytoremediation in land mine rehabilitation is of great importance to South African mines. The fact that the indigenous *Tamarix usneoides* has been proven to be more effective when it comes to heavy metal uptake, coupled with legislations on planting and spread of exotic plant species. It is important to propagate pure *Tamarix usneoides* for planting on the mines. Based on this study, it is found that pure *Tamarix usneoides* can be found in some remote areas on the western coast of the country (e.g. Witbank, Goodhouse and Henkries in the Northern Cape/South Africa, APPENDIX IV).

APPENDICES

APPENDIX I. DNA Purification

The PCR product was purified using a Zymo Clean and Concentrator Kit following the standard protocol yielding 20 µl in nuclease free water, which was stored at −20°C until sequencing. 100 µl (5x the PCR product) of the DNA Binding Buffer was added to the DNA sample in a 2 ml centrifuge tube and mixed by vortexing. This was loaded into a Zymo-Spin Column, placed in a 2 ml collection tube, and then incubated for 5 min at room temperature. Thereafter it was centrifuged at 14,000 rpm for 10 s and the flow-through was discarded. 200 µl of Wash Buffer was added to the column and centrifuged at 14,000 rpm for 10 s, then the flow through was discarded and this step was repeated but with spinning for 30 s. The Zymo-Spin column was placed into a new 1.5 ml tube and 20 µl of nuclease free water was added directly to the column matrix; this was incubated at room temperature for 5 min and centrifuged for 1 minute at 8,000 rpm to elute the DNA.

The Gel DNA recovery method was used to extract the desired band of the *trn*S-*trn*G regions using the ZymoClean Gel DNA Recovery Kit following the protocol in the user manual: the DNA fragment was excised from the agarose gel using a razor blade and was placed into a clean 1.5ml microcentrifuge tube (N.B: each sample was excised using a new and clean razor blade). Three volumes of ADB (Agarose Dissolving Buffer) was added to each volume of agarose excised from the gel (e.g. for 100 µl (mg) of agarose gel slice 300 µl of ADB was added). This was incubated at 55°C for 10 min until the gel slice was completely dissolved. Then the melted agarose solution was transferred into a Zymo-Spin™ I Column in a 2ml collection tube and was centrifuged for 60 s at ≥10 000. The flow-through was discarded thereafter. 200 µl of Wash Buffer was added to the column and centrifuged for 30 s at ≥10 000. The flow-through was discarded and this step was repeated. Then the column matrix was placed into a new collection tube and 10 µl was added directly into it. This was incubated at room temperature for 5 min and then was centrifuged for 1 min at ≥10 000 to elute the DNA. The total final yield was improved by eluting the DNA with nuclease free water at 60°C. The ultra-pure DNA in water was ready for sequencing.

All the final products obtained from the following stages of DNA extraction, PCR amplification and DNA purification were visualized by 1% agarose gel electrophoresis for results. See Appendix II for preparation of Agarose gel and loading of products.

APPENDIX II: Preparation of Agarose gel and loading of products

A 1% agarose gel was made by weighing 0.3g of agarose powder and poured it into a conical flask. Then 30 ml of 1 × TAE Buffer was added and swirled to mix. The mixture was microwaved for 23–26 s at 1000W. The mixture was not allowed to boil. After complete dissolution it was left to cool to 50–55°C at room temperature then 3µl of GelRed (intercalating nucleic acid stain) was added and mixed by swirling, poured into a gel rig with cleaned comb in place. The 6X loading dye (bromophenol blue) was used to load the product into the gel at the proportions of 3µl of the loading dye and 3 µl of the DNA or PCR product.

APPENDIX III: List of samples collected for identification of *Tamarix* species and their hybrids in southern Africa including their localities, sites and GPS coordinates.

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
GM001	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River	26°55.952"S	26°41.575"E
GM002	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River	26°55.951"S	26°41.578"E
GM003	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River	26°55.952"S	26°41.590"E
GM004	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River	26°55.956"S	26°41.582"E
GM005	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River	26°55.949"S	26°41.581"E
GM006	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River		
GM007	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River		
GM008	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River		
GM009	Mayonde, Finnies	2010/02/19	*Tamarix usneoides*	North West	Vaal River		
GM010	Mayonde, Finnies	2010/02/19	*Tamarix ramosissima*	North West	Vaal River		
GM011	Mayonde, Finnies	2010/02/19	*Tamarix ramosissima*	North West	Vaal River		
GM012	Mayonde, Finnies	2010/02/19	*Tamarix ramosissima*	North West	Vaal River		
GM013	Mayonde, Finnies	2010/02/19	*Tamarix ramosissima*	North West	Vaal River		
GM014	Byrne	2010/03/10	*Tamarix chinensis*	Gauteng	Johannesburg		
GM015	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.55"S	26 ^{0}45.81"E
GM016	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26 ^{0}54.53"S	26 ^{0}45.79"E
GM017	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26 ^{0}54.52"S	26 ^{0}45.82"E
GM018	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26 ^{0}54.54"S	26 ^{0}45.80"E
GM019	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	S26 ^{0}54.58"	26 ^{0}45.86"E
GM020	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26 ^{0}54.53"S	26 ^{0}45.80"E
GM021	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.51"S	26^{0}45.84"E
GM022	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.55"S	26^{0}45.80"S
GM023	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.55"S	26^{0}45.81"E
GM024	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.58"S	26^{0}45.86"E
GM025	Mayonde, Finnies, Botha	2010/03/12	Possible hybrid	North West	Vaal River	26^{0}54.58"S	26^{0}4587"E
GM026	Mayonde, Finnies, Botha	2010/03/12	*Tamarix usneoides*	North West	Vaal River	26^{0}55.90"S	26^{0}41.64"E
GM027	Mayonde, Finnies, Botha	2010/03/12	*Tamarix usneoides*	North West	Vaal River	26^{0}55.93"S	26^{0}41.64"E
GM028	Mayonde, Finnies, Botha	2010/03/12	*Tamarix usneoides*	North West	Vaal River	26^{0}55.93"S	26^{0}41.64"E
GM029	Mayonde, Finnies, Botha	2010/03/12	*Tamarix usneoides*	North West	Vaal River	26^{0}55.96"S	26^{0}41.70"E
GM030	Mayonde, Finnies, Botha	2010/03/12	*Tamarix usneoides*	North West	Vaal River	26^{0}55.97"S	26^{0}41.69"E

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
GM031	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°27.782"s	21°15.193"E
GM032	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°27.761"S	21°15.209"E
GM033	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	S28^{o}27.829"	21°15.233"E
GM034	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°27.927"S	21°15.109"E
GM035	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°27.851"S	21°15.910"E
GM036	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°28.078"S	21°14.761"E
GM037	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°28.090"S	21°14.791"E
GM038	Mayonde, Cron, Botha, Wilson	2010/03/19	*Tamarix usneoides*	Northern Cape	Upington	28°27.496"S	21°15.113"E
GM039	Mayonde, Cron, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington		
GM040	Mayonde, Cron, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington		
GM041	Mayonde, Cron, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington		
GM042	Mayonde, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington	28°27.975"S	21°15.863"E
GM043	Mayonde, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington	28°27.946"S	21°15.863"E
GM044	Mayonde, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington	28°27.918"S	21°15.856"E
GM045	Mayonde, Botha, Wilson	2010/03/20	*Tamarix usneoides*	Northern Cape	Upington	28°27.856"S	21°15.778"E
GM046	Mayonde, Botha, Wilson	2010/03/20	*Tamarix chinensis*	Northern Cape	Upington	28°28.680"E	21°16.244"E
GM047	Mayonde, Botha, Wilson	2010/03/20	*Tamarix chinensis*	Northern Cape	Upington	28°28.683"S	21°16.285"E
GM048	Mayonde, Botha, Wilson	2010/03/20	*Tamarix ramosissima*	Northern	Upington	28°28379"S	21°15.709"E

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
				Cape			
GM049	Mayonde, Botha, Wilson	2010/03/20	*Tamarix ramosissima*	Northern Cape	Upington	28^{0}28.379"S	21^{0}15.709"E
GM050	Mayonde, Botha, Wilson	2010/03/21	*Tamarix usneoides*	Northern Cape	Kenhardt	29^{0}21.292"S	21^{0}08.862"E
GM051	Mayonde, Botha, Wilson	2010/03/21	*Tamarix usneoides*	Northern Cape	Kenhardt	28^{0}45.511"S	20^{0}37.272"E
GM052	Mayonde, Botha, Wilson	2010/03/21	*Tamarix usneoides*	Northern Cape	Augrabies	28^{0}37.263"S	20^{0}20.883"E
GM053	Mayonde, Botha, Wilson	2010/03/21	*Tamarix usneoides*	Northern Cape	Augrabies	28^{0}37.246"S	20^{0}20.864"E
GM054	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.059"S	28^{0}26.760"E
GM055	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.058"S	28^{0}26.772"E
GM056	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.019"S	28^{0}26.761"E
GM057	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}12.981"S	28^{0}26.912"E
GM058	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}12.986"S	28^{0}26.886"E
GM059	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.076"S	28^{0}26.948"E
GM060	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.133"S	28^{0}26.801"E
GM061	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.134"S	28^{0}26.882"E
GM062	Mayonde, Botha, Finnies	2010/04/15	Possible hybrid	North West	Impala Platinum	26^{0}13.125"S	28^{0}26.799"E
MS001	Sutton, Rentel, Rentel	2010/06/16	*Tamarix usneoides*	Namibia	Karibid	S21°58′52.51"	E15°43′13.52"
MS002	Sutton, Rentel, Rentel	2010/06/16	*Tamarix usneoides*	Namibia	Karibid	S21°58′46.03"	E15°43′14.30"
MS003	Sutton, Rentel, Rentel	2010/06/16	*Tamarix usneoides*	Namibia	Karibid	S21°58′45.26"	E15°43′14.60"
MS004	Sutton, Rentel, Rentel	2010/06/16	*Tamarix usneoides*	Namibia	Karibid	S21°58′45.33"	E15°43′14.24"
MS005	Sutton, Rentel, Rentel	2010/06/16	*Tamarix usneoides*	Namibia	Karibid	S21°59′09.78"	E15°43′10.96"
GM063	Mayonde, Wilson, Muller		*Tamarix usneoides*	Gauteng	Johannesburg	S26°11′27.54"	E28°01′12.03"
GM064	Mayonde, Wilson, Muller		*Tamarix usneoides*	Gauteng	Johannesburg	S26°11′04.55"	E28°01′12.44"
GM065	Mayonde, Wilson, Muller		*Tamarix usneoides*	Gauteng	Johannesburg	S26°11′35.56"	E28°01′12.58"
GM066	Mayonde, Wilson, Muller	2011/05/17	*Tamarix chinensis*	Free State	Sasolburg	S26°48.997′	E27°49.988′
GM067	Mayonde, Wilson, Muller	2011/05/17	*Tamarix chinensis*	Free State	Sasolburg	S26°47.816′	E27°49.837′
GM068	Mayonde, Wilson, Muller	2011/05/17	*Tamarix ramosissima*	Free State	Virginia (outskirts)	S28°07.189′	E26°54.564′
GM069	Mayonde, Wilson, Muller	2011/05/17	*Tamarix ramosissima*	Free State	Virginia	S28°07.612′	E26°52.424′
GM070	Mayonde, Wilson, Muller	2011/05/17	*Tamarix ramosissima*	Free State	Virginia	S28°07.604′	E26°52.436′
GM071	Mayonde, Wilson, Muller	2011/05/18	*Tamarix ramosissima*	Northern	Kimberley	S28°45.018′	E24°46.777′

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
				Cape			
GM072	Mayonde, Wilson, Muller	2011/05/18	*Tamarix ramosissima*	Northern Cape	Kimberley	S28°45.915′	E24°45.596′
GM073	Mayonde, Wilson, Muller	2011/05/18	*Tamarix ramosissima*	Northern Cape	Barkley	S28°09.573′	E24°28.800′
GM074	Mayonde, Wilson, Muller	2011/05/19	*Tamarix ramosissima*	Northern Cape	Salt Lake	S29°17.732′	E24°00.589′
GM075	Mayonde, Wilson, Muller	2011/05/19	*Tamarix ramosissima*	Northern Cape	Salt Lake	S29°17.881′	E24°00.293′
GM076	Mayonde, Wilson, Muller	2011/05/19	*Tamarix ramosissima*	Northern Cape	Salt Lake	S29°17.901′	E24°00.284′
GM077	Mayonde, Wilson, Muller	2011/05/19	*Tamarix ramosissima*	Northern Cape	Hopetown	S29°28.812′	E24°05.929′
GM078	Mayonde, Wilson, Muller	2011/05/19	Possible hybrid	Northern Cape	Prieska	S29°37.366′	E23°00.984′
GM079	Mayonde, Wilson, Muller	2011/05/19	Possible hybrid	Northern Cape	Prieska	S29°37.363′	E23°00.988′
GM080	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Marchand	S28°41.627′	E20°30.466′
GM081	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Marchand	S28°41.602′	E20°30.504′
GM082	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Marchand	S28°42.301′	E20°31.130′
GM083	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Marchand	S28°43.228′	E20°31.312′
GM084	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Augrabies	S28°37.381′	E20°20.849′
GM085	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Kakamas—Kenhardt	S28°51.486′	E20°38.485′
GM086	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Kakamas—Kenhardt	S29°08.319′	E20°25.392′
GM087	Mayonde, Wilson, Muller	2011/05/20	*Tamarix usneoides*	Northern Cape	Kakamas—Kenhardt	S29°12.217′	E20°21.732′
GM088	Mayonde, Wilson, Muller	2011/05/20	Possible hybrid	Northern Cape	Bossiekom	S29°19.781′	E20°17.945′

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
GM089	Mayonde, Wilson, Muller	2011/05/20	Possible hybrid	Northern Cape	Pofadder	S29°07.966′	E19°23.547′
GM090	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Witbank	S28°53.757′	E18°30.724′
GM091	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Witbank	S28°53.759′	E18°30.729′
GM092	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Goodhouse	S28°51.263′	E18°38.125′
GM093	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Goodhouse	S28°54.413′	E18°10.454′
GM094	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Goodhouse	S28°54.359′	E18°10.368′
GM095	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Henkries	S28°54.546′	E18°07.444′
GM096	Mayonde, Wilson, Muller	2011/05/21	*Tamarix usneoides*	Northern Cape	Henkries	S28°54.616′	E18°07.446′
GM097	Mayonde, Wilson, Muller	2011/05/22	*Tamarix usneoides*	Northern Cape	Vioolsdrif	S28°41.417′	E17°35.231′
GM098	Mayonde, Wilson, Muller	2011/05/22	*Tamarix usneoides*	Northern Cape	Vioolsdrif	S28°41.414′	E17°35.183′
GM099	Mayonde, Wilson, Muller	2011/05/23	*Tamarix usneoides*	Northern Cape	Steinkopf	S29°03.709′	E17°50.786′
GM100	Mayonde, Wilson, Muller	2011/05/23	*Tamarix usneoides*	Northern Cape	Kuboes	S28°24.107′	E16°52.632′
GM101	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern Cape	Richtersveld	S28°06.085′	E16°56.571′
GM102	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern Cape	Richtersveld	S28°04.499′	E16°57.776′
GM103	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern Cape	Richtersveld	S28°04.424′	E16°57.822′
GM104	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern Cape	Richtersveld	S28°08.442′	E16°58.727′
GM105	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern Cape	Richtersveld	S28°10.107′	E17°01.547′
GM106	Mayonde, Wilson, Muller	2011/05/24	*Tamarix usneoides*	Northern	Richtersveld	S28°10.469′	E17°09.507′

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
				Cape			
GM107	Mayonde, Wilson, Muller	2011/05/25	Possible hybrid	Northern Cape	Richtersveld	S28°17.378′	E17°00.346′
GM108	Mayonde, Wilson, Muller	2011/05/25	Possible hybrid	Northern Cape	Richtersveld	S28°18.655′	E16°58.290′
GM109	Mayonde, Wilson, Muller	2011/05/26	*Tamarix usneoides*	Northern Cape	Springbok	S29°38.190′	E17°52.855′
GM110	Mayonde, Wilson, Muller	2011/05/26	*Tamarix ramosissima*	Northern Cape	Springbok	S29°39.569′	E17°53.368′
GM111	Mayonde, Wilson, Muller	2011/05/26	*Tamarix usneoides*	Northern Cape	Kamieskroon	S30°01.095′	E17°52.815′
GM112	Mayonde, Wilson, Muller	2011/05/26	*Tamarix ramosissima*	Northern Cape	Wallekraal	S30°23.324′	E17°30.515′
GM113	Mayonde, Wilson, Muller	2011/05/27	*Tamarix usneoides*	Northern Cape	Garies	S30°35.671′	E18°00.606′
GM114	Mayonde, Wilson, Muller	2011/05/27	*Tamarix usneoides*	Western Cape	Kliprand	S30°42.559′	E18°25.655′
GM115	Mayonde, Wilson, Muller	2011/05/27	*Tamarix usneoides*	Western Cape	Kliprand	S30°40.458′	E18°25.727′
GM116	Mayonde, Wilson, Muller	2011/05/27	*Tamarix usneoides*	North West	Loeriefontein	S30°50.701′	E19°07.810′
GM117	Mayonde, Wilson, Muller	2011/05/27	*Tamarix usneoides*	North West	Brandkop	S31°17.119′	E19°22.576′
GM118	Mayonde, Wilson, Muller	2011/05/28	*Tamarix usneoides*	North West	Kenhardt	S29°21.720′	E21°08.706′
GM119	Mayonde, Wilson, Muller	2011/05/28	*Tamarix usneoides*	North West	Kakamas─Kenhardt	S29°07.832′	E20°49.202′
GM120	Mayonde, Wilson, Muller	2011/05/29	Possible hybrid	North West	Kanoneiland	S28°37.382′	E21°06.568′
GM121	Mayonde, Wilson, Muller	2011/05/29	*Tamarix usneoides*	North West	Noenieput	S27°18.766′	E20°06.561′
GM122	Mayonde, Wilson, Muller	2011/05/29	*Tamarix usneoides*	North West	Noenieput	S27°18.981′	E20°07.296′
GM123	Mayonde, Wilson, Muller	2011/07/03	*Tamarix ramosissima*	North West		S25°40′45.4"	E27°45′27.1"
GM124	Mayonde, Wilson, Muller	2011/07/03	*Tamarix ramosissima*	North West		S25°40′42.8"	E27°45′24.0"
GM125	Mayonde, Mugwedi	2011/11/07	*Tamarix ramosissima*	Western Cape	Cape Town	S34°04.345′	E18°26.828′
GM126	Mayonde, Mugwedi	2011/11/07	*Tamarix ramosissima*	Western Cape	Cape Town	S34°04.432′	E18°26.808′
GM127	Mayonde, Mugwedi	2011/11/07	*Tamarix ramosissima*	Western Cape	Cape of Good Hope	S34°06.829′	E18°27.914′
GM128	Mayonde, Mugwedi	2011/11/07	Possible hybrid	Western Cape	Laingsburg	S33°11.544′	E20°49.877′
GM129	Mayonde, Mugwedi	2011/11/07	*Tamarix ramosissima*	Western Cape	Laingsburg	S33°11.552′	E20°49.863′
GM130	Mayonde, Mugwedi	2011/11/07	*Tamarix usneoides*	Western Cape	Laingsburg	S33°13.889′	E20°52.587′
GM131	Mayonde, Mugwedi	2011/11/07	*Tamarix ramosissima*	Western Cape	Laingsburg	S33°13.830′	E20°52.517′
GM132	Mayonde, Mugwedi	2011/11/08	*Tamarix ramosissima*	Western Cape	Calitzdorp	S33°31.749′	E21°41.151′
GM133	Mayonde, Mugwedi	2011/11/08	Possible hybrid	Western Cape	Gamkapoort Dam	S33°17.618′	E21°37.386′

Collecting Number	Collectors	Date	Species Name	Region	Locality	Latitude	Longitude
GM134	Mayonde, Mugwedi	2011/11/08	Possible hybrid	Western Cape	Gamkapoort Dam	S33°17.611′	E21°37.405′
GM135	Mayonde, Mugwedi	2011/11/08	Possible hybrid	Western Cape	Van Wyksdorp	S33°47.952′	E21°28.258′
GM136	Mayonde, Mugwedi	2011/11/08	Possible hybrid	Western Cape	Oudtshoorn	S33°39.004′	E22°09.410′
GM137	Mayonde, Mugwedi	2011/11/09	*Tamarix ramosissima*	Western Cape	Prince Albert	S33°10.923′	E22°01.648′
GM138	Mayonde, Mugwedi	2011/11/09	*Tamarix ramosissima*	Western Cape	Prince Albert	S33°10.945′	E22°01.662′
GM139	Mayonde, Mugwedi	2011/11/09	*Tamarix chinensis*	Western Cape	Prince Albert	S33°09.968′	E21°58.813′
GM140	Mayonde, Mugwedi	2011/11/09	*Tamarix chinensis*	Western Cape	Swart river	S33°09.897′	E21°58.877′
GM141	Mayonde, Mugwedi	2011/11/09	Possible hybrid	Western Cape	Swart river	S33°09.896′	E21°58.884′
GM142	Mayonde, Mugwedi	2011/11/09	*Tamarix chinensis*	Western Cape	Gamk's river	S33°07.100′	E21°55.490′
GM143	Mayonde, Mugwedi	2011/11/09	*Tamarix usneoides*	Western Cape	Dwyka	S33°05.217′	E21°04.783′
GM144	Mayonde, Mugwedi	2011/11/09	*Tamarix chinensis*	Western Cape	Leeu-Gamka	S32°46.051′	E21°58.770′
GM145	Mayonde, Mugwedi	2011/11/09	*Tamarix usneoides*	Western Cape	Leeu-Gamka	S32°46.067′	E21°58.780′
GM146	Mayonde, Mugwedi	2011/11/09	*Tamarix chinensis*	Western Cape	Leeu-Gamka	S32°46.070′	E21°58.842′
GM147	Mayonde, Mugwedi	2011/11/10	*Tamarix chinensis*	Eastern Cape	Steytlerville	S33°19.330′	E24°20.410′
GM148	Mayonde, Mugwedi	2011/11/10	*Tamarix usneoides*	Eastern Cape	Steytlerville	S33°19.350′	E24°20.410′
GM149	Mayonde, Mugwedi	2011/11/11	*Tamarix chinensis*	Eastern Cape	Jansenville	S32°56.990′	E24°40.184′
GM150	Mayonde, Mugwedi	2011/11/11	*Tamarix chinensis*	Eastern Cape	Waterford	S33°04.604′	E25°00.927′
GM151	Mayonde, Mugwedi	2011/11/11	*Tamarix usneoides*	Eastern Cape	Waterford	S33°04.678′	E25°00.962′
GM152	Mayonde, Mugwedi	2011/11/11	*Tamarix chinensis*	Eastern Cape	Waterford	S33°04.705′	E25°00.947′
GM153	Mayonde, Mugwedi	2011/11/12	*Tamarix chinensis*	Eastern Cape	Grahamstown	S33°18.491′	E26°31.368′
GM154	Mayonde, Mugwedi	2011/11/12	*Tamarix chinensis*	Eastern Cape	Grahamstown	S33°17.873′	E26°32.001′
MM155	Moroka	2012/05/28	*Tamarix usneoides*	Free State	Thaba Nchu		
MM156	Moroka	2012/05/28	*Tamarix usneoides*	Free State	Thaba Nchu		

APPENDIX IV: *Tamarix* Structure analysis results of the AFLP data and species composition based on Posterior probability values including the morphological identities and the localities (sites).

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
0.13	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
140	*T. chinensis*	Asia	0.003	0.996	0.001	*T. chinensis*
2011	*T. chinensis*	Asia	0.003	0.993	0.004	*T. chinensis*
2027	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
2028	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
2029	*T. chinensis*	Asia	0.002	0.997	0.001	*T. chinensis*
23	*T. chinensis*	Asia	0.006	0.993	0.001	*T. chinensis*
3144.1	*T. chinensis*	Asia	0.002	0.997	0.002	*T. chinensis*
3144.2	*T. chinensis*	Asia	0.002	0.997	0.002	*T. chinensis*
3144.3	*T. chinensis*	Asia	0.001	0.997	0.001	*T. chinensis*
3145.1	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3145.2	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3145.3	*T. chinensis*	Asia	0.004	0.994	0.002	*T. chinensis*
3145.4	*T. chinensis*	Asia	0.016	0.976	0.008	*T. chinensis*
3145.5	*T. chinensis*	Asia	0.002	0.997	0.001	*T. chinensis*
3145.6	*T. chinensis*	Asia	0.002	0.997	0.001	*T. chinensis*
3148	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3149	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3150	*T. chinensis*	Asia	0.003	0.995	0.002	*T. chinensis*
3151	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3152	*T. chinensis*	Asia	0.004	0.995	0.001	*T. chinensis*
3153	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3154	*T. chinensis*	Asia	0.001	0.997	0.001	*T. chinensis*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
3156	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3157	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3158	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3159	*T. chinensis*	Asia	0.002	0.996	0.002	*T. chinensis*
3160	*T. chinensis*	Asia	0.002	0.996	0.002	*T. chinensis*
3162	*T. chinensis*	Asia	0.002	0.996	0.002	*T. chinensis*
3163	*T. chinensis*	Asia	0.002	0.996	0.003	*T. chinensis*
3164	*T. chinensis*	Asia	0.002	0.996	0.002	*T. chinensis*
3165	*T. chinensis*	Asia	0.002	0.996	0.003	*T. chinensis*
3167	*T. chinensis*	Asia	0.002	0.995	0.003	*T. chinensis*
3168	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3169	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3170	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3171	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3172	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3174	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3176	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
3178	*T. chinensis*	Asia	0.004	0.995	0.001	*T. chinensis*
3182	*T. chinensis*	Asia	0.001	0.998	0.001	*T. chinensis*
GM077	*T. ramosissima*	Hopetown	0.001	0.998	0.001	*T. chinensis*
GM112	*T. ramosissima*	Wallekraal	0.001	0.998	0.001	*T. chinensis*
GM125	*T. ramosissima*	Cape Town	0.001	0.998	0.001	*T. chinensis*
GM126	*T. ramosissima*	Cape Town	0.001	0.998	0.001	*T. chinensis*
GM127	*T. ramosissima*	Cape of Good Hope	0.002	0.997	0.001	*T. chinensis*
GM132	*T. ramosissima*	Calitzdorp	0.094	0.901	0.005	*T. chinensis*
GM135	Possible hybrid	Van Wyksdorp	0.001	0.997	0.002	*T. chinensis*
GM136	Possible hybrid	Oudtshoorn	0.001	0.998	0.001	*T. chinensis*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
GM155	*T. usneoides*	Thaba Nchu	0.001	0.998	0.001	*T. chinensis*
GM153	*T. chinensis*	Grahamstown	0.001	0.998	0.001	*T. chinensis*
1106.7	*T. ramosissima*	Asia	0.989	0.006	0.004	*T. ramosissima*
1122.1	*T. ramosissima*	Asia	0.996	0.001	0.002	*T. ramosissima*
1122.2	*T. ramosissima*	Asia	0.991	0.008	0.002	*T. ramosissima*
1122.3	*T. ramosissima*	Asia	0.986	0.006	0.007	*T. ramosissima*
1129.1	*T. ramosissima*	Asia	0.995	0.001	0.004	*T. ramosissima*
1129.7	*T. ramosissima*	Asia	0.993	0.002	0.005	*T. ramosissima*
1145.3	*T. ramosissima*	Asia	0.996	0.002	0.002	*T. ramosissima*
1145.4	*T. ramosissima*	Asia	0.994	0.002	0.005	*T. ramosissima*
1145.6	*T. ramosissima*	Asia	0.997	0.002	0.002	*T. ramosissima*
1147.3	*T. ramosissima*	Asia	0.985	0.014	0.002	*T. ramosissima*
1147.4	*T. ramosissima*	Asia	0.992	0.004	0.004	*T. ramosissima*
1147.6	*T. ramosissima*	Asia	0.93	0.006	0.064	*T. ramosissima*
1150.1	*T. ramosissima*	Asia	0.995	0.001	0.004	*T. ramosissima*
1150.2	*T. ramosissima*	Asia	0.99	0.002	0.008	*T. ramosissima*
1150.6	*T. ramosissima*	Asia	0.995	0.001	0.004	*T. ramosissima*
1163.2	*T. ramosissima*	Asia	0.997	0.001	0.002	*T. ramosissima*
1163.3	*T. ramosissima*	Asia	0.997	0.001	0.002	*T. ramosissima*
1163.4	*T. ramosissima*	Asia	0.992	0.002	0.006	*T. ramosissima*
1163.5	*T. ramosissima*	Asia	0.996	0.001	0.004	*T. ramosissima*
1163.6	*T. ramosissima*	Asia	0.995	0.003	0.002	*T. ramosissima*
1175.2	*T. ramosissima*	Asia	0.996	0.001	0.002	*T. ramosissima*
1175.5	*T. ramosissima*	Asia	0.995	0.002	0.002	*T. ramosissima*
1175.6	*T. ramosissima*	Asia	0.993	0.002	0.005	*T. ramosissima*
293	*T. ramosissima*	Asia	0.997	0.001	0.002	*T. ramosissima*
31	*T. ramosissima*	Asia	0.997	0.002	0.002	*T. ramosissima*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
411	*T. ramosissima*	Asia	0.997	0.002	0.002	*T. ramosissima*
412	*T. ramosissima*	Asia	0.997	0.002	0.001	*T. ramosissima*
413	*T. ramosissima*	Asia	0.996	0.001	0.002	*T. ramosissima*
416	*T. ramosissima*	Asia	0.997	0.002	0.002	*T. ramosissima*
417	*T. ramosissima*	Asia	0.989	0.01	0.001	*T. ramosissima*
418	*T. ramosissima*	Asia	0.955	0.042	0.004	*T. ramosissima*
4218	*T. ramosissima*	Asia	0.994	0.004	0.002	*T. ramosissima*
423	*T. ramosissima*	Asia	0.995	0.003	0.002	*T. ramosissima*
424	*T. ramosissima*	Asia	0.996	0.003	0.001	*T. ramosissima*
428	*T. ramosissima*	Asia	0.997	0.001	0.002	*T. ramosissima*
431	*T. ramosissima*	Asia	0.997	0.001	0.001	*T. ramosissima*
773.1	*T. ramosissima*	Asia	0.981	0.016	0.003	*T. ramosissima*
773.2	*T. ramosissima*	Asia	0.996	0.001	0.003	*T. ramosissima*
773.3	*T. ramosissima*	Asia	0.997	0.001	0.002	*T. ramosissima*
773.4	*T. ramosissima*	Asia	0.994	0.003	0.003	*T. ramosissima*
773.5	*T. ramosissima*	Asia	0.988	0.007	0.006	*T. ramosissima*
773.6	*T. ramosissima*	Asia	0.994	0.002	0.004	*T. ramosissima*
917.1	*T. ramosissima*	Asia	0.915	0.08	0.004	*T. ramosissima*
917.2	*T. ramosissima*	Asia	0.949	0.044	0.007	*T. ramosissima*
917.3	*T. ramosissima*	Asia	0.968	0.025	0.006	*T. ramosissima*
917.4	*T. ramosissima*	Asia	0.99	0.006	0.004	*T. ramosissima*
917.5	*T. ramosissima*	Asia	0.957	0.025	0.018	*T. ramosissima*
917.6	*T. ramosissima*	Asia	0.988	0.008	0.004	*T. ramosissima*
1010.1	*T. ramosissima*	Asia	0.996	0.002	0.002	*T. ramosissima*
1010.5	*T. ramosissima*	Asia	0.992	0.004	0.003	*T. ramosissima*
1010.8	*T. ramosissima*	Asia	0.904	0.092	0.003	*T. ramosissima*
1024.1	*T. ramosissima*	Asia	0.984	0.014	0.001	*T. ramosissima*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
1024.2	*T. ramosissima*	Asia	0.989	0.009	0.002	*T. ramosissima*
1024.6	*T. ramosissima*	Asia	0.983	0.008	0.009	*T. ramosissima*
GM014	*T. chinensis*	Garden	0.997	0.001	0.002	*T. ramosissima*
GM048	*T. ramosissima*	Upington	0.997	0.001	0.002	*T. ramosissima*
GM049	*T. ramosissima*	Upington	0.996	0.001	0.002	*T. ramosissima*
GM066	*T. chinensis*	Sasolburg	0.988	0.007	0.005	*T. ramosissima*
GM068	*T. ramosissima*	Virginia (outskirts)	0.984	0.01	0.006	*T. ramosissima*
GM071	*T. ramosissima*	Kimberley	0.997	0.001	0.002	*T. ramosissima*
GM140	*T. chinensis*	Swart river	0.997	0.001	0.002	*T. ramosissima*
GM147	*T. chinensis*	Steytlerville	0.911	0.007	0.082	*T. ramosissima*
GM154	*T. chinensis*	Grahamstown	0.961	0.008	0.031	*T. ramosissima*
GM156	*T. usneoides*	Thaba Nchu	0.986	0.005	0.008	*T. ramosissima*
3184	*T. chinensis*	Asia	0.161	0.836	0.002	*T. chinensisx T. ramosissima*
3186	*T. chinensis*	Asia	0.205	0.792	0.003	*T. chinensisx T. ramosissima*
GM012	*T. ramosissima*	Vaal River	0.348	0.65	0.002	*T. chinensisx T. ramosissima*
GM060	Possible hybrid	Impala Platinum	0.377	0.595	0.028	*T. chinensisx T. ramosissima*
GM061	Possible hybrid	Impala Platinum	0.331	0.667	0.003	*T. chinensisx T. ramosissima*
GM067	*T. chinensis*	Sasolburg	0.343	0.656	0.001	*T. chinensis x T. ramosissima*
GM069	*T. ramosissima*	Virginia	0.173	0.824	0.002	*T. chinensis x T. ramosissima*
GM070	*T. ramosissima*	Virginia	0.309	0.689	0.001	*T. chinensis x T. ramosissima*
GM072	*T. ramosissima*	Kimberley	0.408	0.588	0.003	*T. chinensis x T. ramosissima*
GM076	*T. ramosissima*	Salt Lake	0.477	0.505	0.018	*T. chinensis x T. ramosissima*
GM079	Possible hybrid	Prieska	0.363	0.633	0.004	*T. chinensis x T. ramosissima*
GM088	Possible hybrid	Bossiekom	0.481	0.518	0.002	*T. chinensis x T. ramosissima*
GM123	*T. ramosissima*		0.416	0.58	0.004	*T. chinensis x T. ramosissima*
GM124	*T. ramosissima*		0.452	0.542	0.006	*T. chinensis x T. ramosissima*
GM128	Possible hybrid	Laingsburg	0.374	0.624	0.001	*T. chinensis x T. ramosissima*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
GM129	*T. ramosissima*	Laingsburg	0.289	0.708	0.003	*T. chinensis x T. ramosissima*
GM131	*T. ramosissima*	Laingsburg	0.458	0.54	0.002	*T. chinensis x T. ramosissima*
GM137	*T. ramosissima*	Prince Albert	0.35	0.647	0.003	*T. chinensis x T. ramosissima*
GM139	*T. chinensis*	Prince Albert	0.254	0.743	0.004	*T. chinensis x T. ramosissima*
GM144	*T. chinensis*	Leeu-Gamka	0.197	0.704	0.099	*T. chinensis xT. ramosissima*
GM146	*T. chinensis*	Leeu-Gamka	0.164	0.815	0.021	*T. chinensis xT. ramosissima*
GM150	*T. chinensis*	Waterford	0.354	0.622	0.024	*T. chinensis x T. ramosissima*
GM152	*T. chinensis*	Waterford	0.241	0.758	0.002	*T. chinensis x T. ramosissima*
1106.2	*T. ramosissima*		0.899	0.099	0.002	*T. ramosissimax T. chinensis*
1106.3	*T. ramosissima*		0.844	0.144	0.012	*T. ramosissimax T. chinensis*
GM011	*T. ramosissima*	Vaal River	0.651	0.347	0.003	*T. ramosissimax T. chinensis*
GM036	*T. usneoides*		0.795	0.198	0.007	*T. ramosissimax T. chinensis*
GM063	*T. usneoides*	Johannesburg	0.855	0.122	0.023	*T. ramosissima x T. chinensis*
GM064	*T. usneoides*	Johannesburg	0.775	0.207	0.017	*T. ramosissima x T. chinensis*
GM065	*T. usneoides*	Johannesburg	0.723	0.263	0.014	*T. ramosissima x T. chinensis*
GM073	*T. ramosissima*	Barkley	0.536	0.457	0.007	*T. ramosissima x T. chinensis*
GM074	*T. ramosissima*	Salt Lake	0.5	0.497	0.004	*T. ramosissima x T. chinensis*
GM075	*T. ramosissima*	Salt Lake	0.642	0.352	0.006	*T. ramosissima x T. chinensis*
GM078	Possible hybrid	Prieska	0.551	0.447	0.003	*T. ramosissima x T. chinensis*
GM089	Possible hybrid	Pofadder	0.601	0.397	0.003	*T. ramosissima x T. chinensis*
GM110	*T. ramosissima*	Springbok	0.552	0.44	0.007	*T. ramosissima x T. chinensis*
GM138	*T. ramosissima*	Prince Albert	0.709	0.233	0.059	*T. ramosissima x T. chinensis*
GM133	Possible hybrid	Gamkapoort Dam	0.002	0.529	0.469	*T. chinensis x T.usneoides*
GM142	*T. chinensis*	Gamk's river	0.002	0.569	0.43	*T. chinensis x T.usneoides*
GM149	*T. chinensis*	Jansenville	0.005	0.535	0.46	*T. chinensis x T. usneoides*
GM134	Possible hybrid	Gamkapoort Dam	0.254	0.308	0.437	*T. usneoides x T. chinensis*
GM043	*T. usneoides*	Upington	0.003	0.109	0.889	*T. usneoidesx T. chinensis*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
GM001	*T. usneoides*	Vaal River	0.003	0.007	0.989	*T. usneoides*
GM003	*T. usneoides*	Vaal River	0.002	0.001	0.998	*T. usneoides*
GM004	*T. usneoides*	Vaal River	0.001	0.001	0.997	*T. usneoides*
GM005	*T. usneoides*	Vaal River	0.001	0.001	0.998	*T. usneoides*
GM007	*T. usneoides*	Vaal River	0.001	0.001	0.998	*T. usneoides*
GM008	*T. usneoides*	Vaal River	0.002	0.001	0.997	*T. usneoides*
GM018	Possible hybrid	Vaal River	0.002	0.001	0.997	*T. usneoides*
GM021	Possible hybrid	Vaal River	0.001	0.001	0.998	*T. usneoides*
GM024	Possible hybrid	Vaal River	0.002	0.001	0.997	*T. usneoides*
GM028	*T. usneoides*	Vaal River	0.005	0.003	0.992	*T. usneoides*
GM031	*T. usneoides*	Upington	0.002	0.004	0.994	*T. usneoides*
GM032	*T. usneoides*	Upington	0.002	0.001	0.997	*T. usneoides*
GM033	*T. usneoides*	Upington	0.002	0.001	0.997	*T. usneoides*
GM035	*T. usneoides*	Upington	0.002	0.001	0.997	*T. usneoides*
GM037	*T. usneoides*	Upington	0.001	0.001	0.998	*T. usneoides*
GM038	*T. usneoides*	Upington	0.004	0.002	0.994	*T. usneoides*
GM039	*T. usneoides*	Upington	0.003	0.004	0.993	*T. usneoides*
GM040	*T. usneoides*	Upington	0.001	0.001	0.998	*T. usneoides*
GM042	*T. usneoides*	Upington	0.001	0.001	0.998	*T. usneoides*
GM044	*T. usneoides*	Upington	0.001	0.001	0.998	*T. usneoides*
GM045	*T. usneoides*	Upington	0.001	0.001	0.998	*T. usneoides*
GM046	*T. usneoides*	Upington	0.005	0.003	0.992	*T. usneoides*
GM047	*T. usneoides*	Upington	0.002	0.002	0.997	*T. usneoides*
GM050	*T. usneoides*	Kenhardt	0.001	0.001	0.998	*T. usneoides*
GM051	*T. usneoides*	Kenhardt	0.001	0.001	0.998	*T. usneoides*
GM052	*T. usneoides*	Augrabies	0.001	0.001	0.998	*T. usneoides*
GM053	*T. usneoides*	Augrabies	0.002	0.001	0.997	*T. usneoides*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
GM054	Possible hybrid	Impala Platinum	0.003	0.008	0.988	*T. usneoides*
GM055	Possible hybrid	Impala Platinum	0.001	0.001	0.998	*T. usneoides*
GM056	Possible hybrid	Impala Platinum	0.002	0.001	0.997	*T. usneoides*
GM057	Possible hybrid	Impala Platinum	0.006	0.006	0.988	*T. usneoides*
GM059	Possible hybrid	Impala Platinum	0.002	0.002	0.996	*T. usneoides*
GM062	Possible hybrid	Impala Platinum	0.002	0.003	0.995	*T. usneoides*
GM080	*T. usneoides*	Marchand	0.001	0.001	0.997	*T. usneoides*
GM081	*T. usneoides*	Marchand	0.009	0.001	0.99	*T. usneoides*
GM082	*T. usneoides*	Marchand	0.001	0	0.999	*T. usneoides*
GM083	*T. usneoides*	Marchand	0.001	0.001	0.998	*T. usneoides*
GM084	*T. usneoides*	Augrabies	0.001	0.001	0.998	*T. usneoides*
GM085	*T. usneoides*	Kakamas—Kenhardt	0.001	0	0.999	*T. usneoides*
GM086	*T. usneoides*	Kakamas—Kenhardt	0.001	0	0.999	*T. usneoides*
GM087	*T. usneoides*	Kakamas—Kenhardt	0.001	0.001	0.999	*T. usneoides*
GM090	*T. usneoides*	Witbank	0.001	0.001	0.998	*T. usneoides*
GM091	*T. usneoides*	Witbank	0.003	0.001	0.997	*T. usneoides*
GM092	*T. usneoides*	Goodhouse	0.001	0	0.999	*T. usneoides*
GM093	*T. usneoides*	Goodhouse	0.001	0	0.999	*T. usneoides*
GM094	*T. usneoides*	Goodhouse	0.008	0.004	0.988	*T. usneoides*
GM095	*T. usneoides*	Henkries	0.001	0.001	0.998	*T. usneoides*
GM096	*T. usneoides*	Henkries	0.002	0.001	0.997	*T. usneoides*
GM097	*T. usneoides*	Vioolsdrif	0.001	0	0.999	*T. usneoides*
GM098	*T. usneoides*	Vioolsdrif	0.001	0	0.999	*T. usneoides*
GM099	*T. usneoides*	Steinkopf	0.001	0.001	0.998	*T. usneoides*
GM100	*T. usneoides*	Kuboes	0.001	0.001	0.999	*T. usneoides*
GM101	*T. usneoides*	Richtersveld	0.003	0.001	0.996	*T. usneoides*
GM102	*T. usneoides*	Richtersveld	0.001	0.001	0.999	*T. usneoides*

Collecting Numbers	Morphological IDs	Locality	Posterior probabilities			*Tamarix* IDs based on AFLP Structure results
			Cluster 1	Cluster 2	Cluster 3	
			T. ramosissima	*T. chinensis*	*T. usneoides*	
GM103	*T. usneoides*	Richtersveld	0.001	0	0.999	*T. usneoides*
GM104	*T. usneoides*	Richtersveld	0.001	0	0.999	*T. usneoides*
GM105	*T. usneoides*	Richtersveld	0.001	0	0.999	*T. usneoides*
GM106	*T. usneoides*	Richtersveld	0.001	0	0.999	*T. usneoides*
GM107	Possible hybrid	Richtersveld	0.001	0.001	0.998	*T. usneoides*
GM108	Possible hybrid	Richtersveld	0.004	0.001	0.996	*T. usneoides*
GM109	*T. usneoides*	Springbok	0.001	0.001	0.999	*T. usneoides*
GM111	*T. usneoides*	Kamieskroon	0.001	0.001	0.998	*T. usneoides*
GM113	*T. usneoides*	Garies	0.001	0	0.999	*T. usneoides*
GM114	*T. usneoides*	Kliprand	0.001	0.001	0.999	*T. usneoides*
GM115	*T. usneoides*	Kliprand	0.001	0	0.999	*T. usneoides*
GM116	*T. usneoides*	Loeriefontein	0.001	0.001	0.999	*T. usneoides*
GM117	*T. usneoides*	Brandkop	0.001	0	0.999	*T. usneoides*
GM118	*T. usneoides*	Kenhardt	0.001	0.001	0.998	*T. usneoides*
GM119	*T. usneoides*	Kakamas—Kenhardt	0.001	0	0.999	*T. usneoides*
GM120	Possible hybrid	Kanoneiland	0.001	0.001	0.998	*T. usneoides*
GM121	*T. usneoides*	Noenieput	0.001	0	0.998	*T. usneoides*
GM122	*T. usneoides*	Noenieput	0.001	0.001	0.998	*T. usneoides*
GM130	*T. usneoides*	Laingsburg	0.001	0.001	0.999	*T. usneoides*
GM141	Possible hybrid	Swart river	0.001	0.001	0.998	*T. usneoides*
GM143	*T. usneoides*	Dwyka	0.001	0.001	0.998	*T. usneoides*
GM145	*T. usneoides*	Leeu-Gamka	0.004	0.001	0.996	*T. usneoides*
GM148	*T. usneoides*	Steytlerville	0.001	0.001	0.999	*T. usneoides*
GM151	*T. usneoides*	Waterford	0.001	0.001	0.999	*T. usneoides*
MS003	*T. usneoides*	Karibid	0.001	0.001	0.998	*T. usneoides*
MS004	*T. usneoides*	Karibid	0.001	0.001	0.999	*T. usneoides*
MS005	*T. usneoides*	Karibid	0.002	0.001	0.997	*T. usneoides*

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