Universität für Bodenkultur Wien

DNA-Barcoding of *Dreyfusia nordmannianae*

Master thesis to obtain the academic title

Dipl.-Ing.

Done at the

Institute of Forest Entomology, Forest Pathology and Forest Protection,

Department of Forest and Soil Sciences

Submitted at the BOKU, University of Natural Sciences and Life Sciences,

Vienna

by

GERHARD BLABENSTEINER

Supervisor: Ao. Univ. Prof. Dipl.-Ing. Dr. Christian Stauffer

Vienna, February 2015

Danksagung

An erster Stelle möchte ich mich bei Herrn Prof. Dipl.-Ing. Dr. Christian Stauffer für die tolle Betreuung und Unterstützung bedanken. Seine Auffassung über die Betreuung eines Diplomanden sucht seinesgleichen. Die familiäre Behandlung und Eingliederung in das Team, wie Sie von ihm betrieben wird, ist, so denke ich, einzigartig. Er stand mir jederzeit mit Rat und Tat zur Seite und hat mich auch immer wieder mit tollen Gesprächen motiviert. Die prompten und klärenden Antworten sowie das konstruktive und rasche Feedback seinerseits haben diese Arbeit in der Art und Weise möglich gemacht. Danke dir Christian für alles!

Weiterst möchte ich mich beim gesamten Team des Instituts für Forstentomologie, Forstpathologie und Forstschutz bedanken, das es ermöglichte in einem tollen Umfeld, diese Diplomarbeit zu erarbeiten. Ein besonderer Dank gilt hier Susanne Krumböck für die stetige Unterstützung und tolle Einschulung in die praktischen Arbeiten im Labor. Sie stand mir jederzeit geduldig zur Seite.

Ich möchte mich an der Stelle bei allen meinen Studienkollegen bedanken, die mich auf diesem Weg begleitet und unterstützt haben und mir viele tolle Momente während meiner Studienzeit ermöglicht haben.

Vor allem bei meinen Diplomarbeitskollegen, Fisnik Batusha und Robert Müller möchte ich mich für die tolle Zeit miteinander, die hilfreichen sowie interessanten Gespräche und die Unterstützung bedanken.

Frau Dr. Martina Žurovcová und Herrn Dr. Jan Havelka von der Universität South Bohemia, České Budějovice, Czech Republic gilt mein Dank für die zur Verfügung Stellung von identifizierten Proben. Herrn OFR J. Reisenberger gilt ebenfalls mein Dank für die Proben, die er mir zur Verfügung gestellt hat.

Zum Schluss möchte ich mich noch ganz besonders bei meinen Eltern, die mich jederzeit unterstützt haben und vor allem meiner Lebensgefährtin Jennifer bedanken, ohne ihnen wäre es nicht möglich gewesen diesen Schritt in meinem Leben zu erreichen, vielen Dank dafür!

Abstract

Dreyfusia nordmannianae belongs systematically to the family of the Adelgidae and is native to Anatolia and Caucasus whereas in Europe this species is an invasive neobiota. In its native area *D. nordmannianae* has a two years cycle being holocyclic on the primary host, *Picea orientalis* and the secondary host, *Abies nordmanniana*. In middle Europe *D. nordmannianae* is para cyclic with only asexual reproduction on *A. nordmanniana* and *A. alba*. The complex life cycle and morphological similarity of the genus *Dreyfusia* makes the identification of *D. nordmannianae*, *D. piceae* and *D. prelli* quite difficult and different systematic taxonomic systems exist between the Anglican and the Central European entomologists.

D. nordmannianae infestations results in a species characteristic gall like damage of the conifer needles and this damage can lead to an increased mortality of the tree. *D. nordmannianae* is one of the most important forest pests in Austria on *Abies alba*.

DNA barcoding, the molecular genetic identification of species, gives often good solutions for such cryptic species. Unfortunately the first studies showed that DNA barcoding with the commonly used barcode primers results in no differentiation of the three *Dreyfusia* species. Austrian samples of *D. nordmannianae* were sampled and analysed using a diverse array of mitochondrial as well as nuclear primer pairs. Although many PCR assays, cloning and sequencing were done, this master thesis did not succeed to differentiate among the three species i.e. *D. nordmannianae, D. piceae* and *D. prelli*.

Kurzfassung

Bei *Dreyfusia nordmannianae* handelt es sich um eine aus dem Kaukasus nach Europa eingeschleppte Art aus der Familie der Adelgidae (Fichtengallenläuse*). D. nordmannianae* lebt in ihrer Heimat eine zweijährigen Holozyklus am Primärwirt *Picea orientalis* und dem Sekundärwirt *Abies nordmanniana*. Der Primärwirt *Picea orientalis* ist in Mitteleuropa nicht vorhanden, *D. nordmannianae* lebt deshalb im sogenannten Parazyklus mit ausschließlich parthogenetischer Fortpflanzung auf den beiden Sekundärwirten *Abies nordmanniana* und *Abies alba*. Die Taxonomie der Adelgidae ist nicht eindeutig geklärt. Die komplexe Lebensweise und hohe morphologische Ähnlichkeit der einzelnen Gattungen und Arten stellt die genaue Identifizierung als besonders schwierig dar, was dazu führte, dass sich zwei verschiedene Systeme zur taxonomischen Systematik von der Familie der Adelgidae etabliert haben. Die genaue Identifikation einer Art ist nur wenigen Experten auf dieser Welt möglich.

Ein *D. nordmannianae* Befall führt zu charakteristischen Schäden an den beiden Tannenarten, die teilweise zum Absterben des Baumes führen können. *D. nordmannianae* gehört zu den bedeutendsten Forstschädlingen an *Abies alba* in Österreich.

DNA Barcoding, der molekular genetische Abgleich von Arten, könnte in der Taxonomie Aufschluss bringen. Allerdings zeigen erste Untersuchungen, dass auch DNA Barcoding, unter Verwendung von den bei Insekten üblichen Markern, zu keinem konkreten Ergebnis führt. Es wurden österreichische Individuen von *D. nordmanniane* mittels DNA Barcoding analysiert. Leider waren diese wie auch eine Reihe anderer mitochondrialer als auch nuklearer Marker nicht geeignet, die Arten der Gattung klar zu unterscheiden.

Table of Contents

5. References

6. Appendix

Appendix I DNA extraction

Appendix II Cloning

Appendix III PCR programmes of diverse primers

Appendix IV Sequences retrieved from the Genbank

Appendix V Index of abbreviations

Appendix VI Curriculum vitae

Appendix VII Poster

1. Introduction

1.1 Biology of *Dreyfusia nordmannianae* (Eckst. 1890)

The silver fir woolly adelgid, *Dreyfusia nordmannianae* (Eckstein, 1890), also referred *Dreyfusia nüsslini* (Börner, 1908) and *Adelges nordmannianae*, belongs systematically to the hemipteran family of the Adelgidae. The adelgids are a small group of plant sap feeding insects with about 70 species (Blackman and Eastop, 1994). *D. nordmannianae* is native to Anatolia and Caucasus and is invasive in Europe and North America. Imported Nordmann fir trees*, Abies nordmanniana*, introduced *D. nordmannianae* to Austria in the second half of the 19th century (Kitzberger, 1995). In it's native area *D. nordmannianae* has a two years cycle being holocyclic on the primary host, *Picea orentalis* and the secondary host, *Abies nordmanniana* (Steffan, 1968). On the primary host *D. nordmannianae* has its sexual generation and forms a gall (Fig. 1.1).

Figure 1.1 Gall on *Picea orientalis* built by the sexual generation of *Dreyfusiua nordmannianae* http://www.plantengallen.com/gb-gb/luizen.html.

On the secondary host only asexual i.e. parthenogenetic reproduction is done. Furthermore, *D. nordmannianae* develop into winged and wingless adult females. The winged forms are able to fly to the primary host to finalize the holocycle life cycle, the wingless stay on the same position and produce another generation on the secondary

host (Ravn et al., 2012). The winged form overwinter as a fundatrix on the base of the buds and form the species characteristic galls in spring. In these galls another winged form can develop and these are able to migrate back to the secondary host to complete the cycle (Kitzberger, 1995).

In Central Europe, where *D. nordmannianae* is invasive, the primary host *Picea orentalis* does not exist and here, only infests in a so-called para cycle on *A. nordmanniana* it's secondary host (Alles, 1994). Additionally in this area *D. nordmannianae* infests *Abies alba* Mill. (Ravn et al., 2012). The species overwinters on the secondary host mostly as a nymph on the bark of the stem and on the underside of branches (Kitzberger, 1995). The nymphs develop to mature females and females lay their eggs in wax-covered clusters during April and May (Steffan, 1972). The wax serves as protection against predators and likely is responsible that no parasitoids are able to attack these species. Further, the way supports the migration via wild deers (Kitzberger, 1995). The eggs soon develop into two different forms i.e. the bark and the branch feeding individuals. During summer the bark feeding forms lives as nymph on the bark of the stems and branches without development, reproduction and skinning. The branch feeding form develops quicker and are moving to the May sprouts sucking on the underside of the branches, which, results in the characteristic damage (Fig. 1.2) (Kitzberger, 1995).

Figure 1.2 Characteristic damage from *D. nordmannianae* on the May sprouts of the secondary host *Abies alba*. http://www.forestryimages.org/browse/detail.cfm?imgnum=5377649.

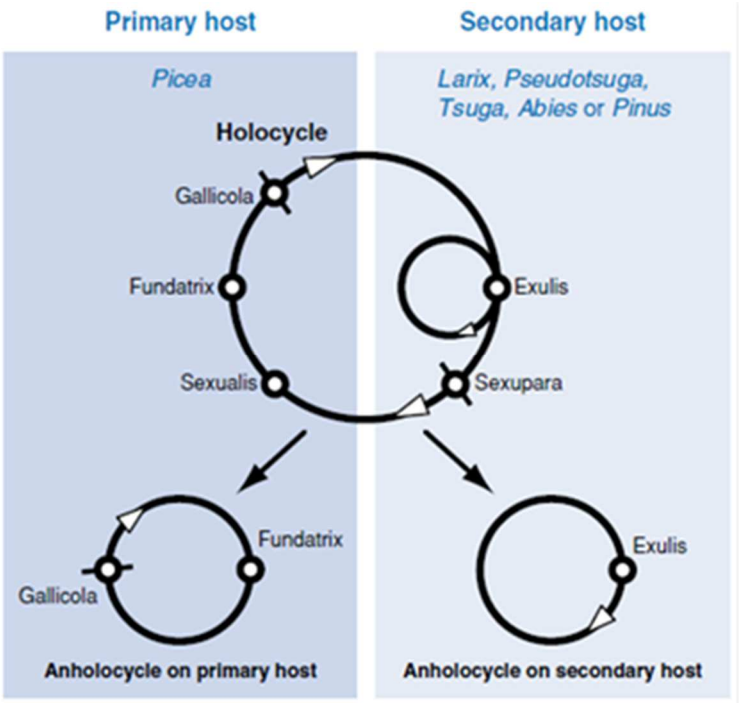


Figure 1.3 The general adelgid life cycle. Center: host-rotating holocycle; lower left: para cycle on primary host; lower right: para cycle on secondary host (Havill and Foottit, 2007).

The number of progeny per one female in the para cycle, does not achieve the whole potential but it is anyway very high. In three years one female have about 37 millions of knot feeding and 378 millions of bark feeding offspring considering 30 % mortality (Schimitschek, 1952; Steffan, 1972). Migration by wind is the main factor how this species is dispersed but also wild deer is passively transporting *Dreyfusia* species over long distances (Kitzberger, 1995).

In Europe *A. nordmannianae* are primary grown as Christmas trees (Ravn et al., 2012). In Christmas plantations often chemical insecticides are used to control *D. nordmannianae* but they are banned because of their negative impact on the environment (Perny, 2005; Ravn et al., 2012). In the fifties and sixties, the Austrian national forests tried to control *D. nordmannianae* with a large-scale output of chemical insecticides like DDT (Kotschy, 1960, Jahn and Sinreich, 1961). Kotschy (1991) and witness foresters report a low effect of the trial (Kitzberger, 1995).

D. nordmannianae has a small range of enemies in Europe because it is an invasive or alien species. A classical biological control program including the introduction of the natural enemy complex from the region of origin could help to combat *D. nordmannianae* (Ravn et al., 2012). Many polyphagous predators of sternorrhynchous Hemiptera but also several specialist predators of adelgids or *Dreyfusia* are known (Ravn et al., 2012). The family of the adelgids is an insect group without parasitoids (Ravn et al., 2012). Casually pathogens have been mentioned as natural enemies of the adelgids (e.g. Schimitschek 1952; Gouli et al., 1997).

D. nordmannianae needs warm temperature and sunny exposition for its development. Mechanical options for the combating carries mostly converse effects, they lead more solar irradiation for single trees. Different forest strategy provisions can combat the silver fir wolly adelgid, longer rotations and late meshes for example can be used to reduce the pest. This forestry strategy provisions in combination with biological control activities give a promising perspective (Kitzberger, 1995).

1.2 Taxonomy/systematic of adelgids

1.2.1 Morphology of *Dreyfusia nordmannianae*

The hemipteran superfamily Aphidoidea, a group of insects with some of the most complex life cycles of all Metazoa, comprise three families Adelgidae, Phylloxeridae and Aphididae (Havill et al., 2007). Phylloxeridae and Adelgidae can be distinguished from Aphididae, Adelgidae and Phylloxeridae are in contrast to Aphididae oviparous in all generations, and they have no siphunculi (Heie, 1987). Adelgidae and Phylloxeridae have on the dorsum of the sixth abdominal segmet structures, which are called siphunculi and these are missing by Aphididae (http://aphid.aphidnet.org/siphunculus.php). Gymnosperms were the dominant plants and the likely hosts of Aphidoidea in the Jurassic (Heie, 1987). Adelgidae are associated to gymnosperms while Phylloxeridae and Aphididae infest angiosperms hosts (Havill et al., 2007). Today adelgids and phylloxerids are less diverse than aphidids e.g. adelgids include about 70 species (Havill et al., 2007). There are some

groups of the adelgid species, which are morphologically barely differentiable, but by examining, the host cautious and the reproductive biology they could obvious be distinguished (Havill et al., 2007). Host plant information, the profile and allocation of dorsal sclerites, wax plates and wax glands of the first instar larvae are the most important information's for the classification of adelgids (Börner and Heinze, 1957; Blackman and Eastop, 1994).

Currently are two different systems used to systemize adelgids into genera. Western and Eastern Palearctic taxonomists use mainly the older system, developed primarily on characteristics of the morphology of the first instar larvae and bionomical characteristics and morphology of the galls. This system distinguishes adelgids species between eight genera and was developed primarily by Börner (1908) and Cholodkovsky (1915) and presented by Börner and Heinze (1957).

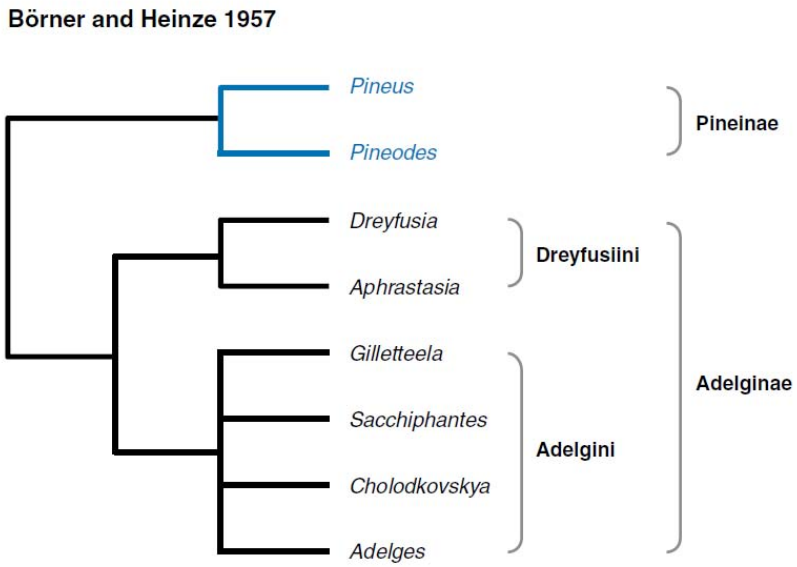


Figure 1.4 Adelgid genera classification (Havill and Foottit, 2007).

The morphology of first instar larvae is the main base for this system (Börner, 1908, 1930, 1952; Inouye, 1945; Börner & Heinze, 1957; Bodenheimer & Swirski, 1957; Steffan, 1961, 1968, 1972; Heinze, 1962; Binazzi & Covassi, 1991). Additionally adducted bionomical characteristics and the morphology of the galls (Cholodkovsky, 1896; Börner & Heinze, 1957; Lampel, 1968; Steffan, 1972).

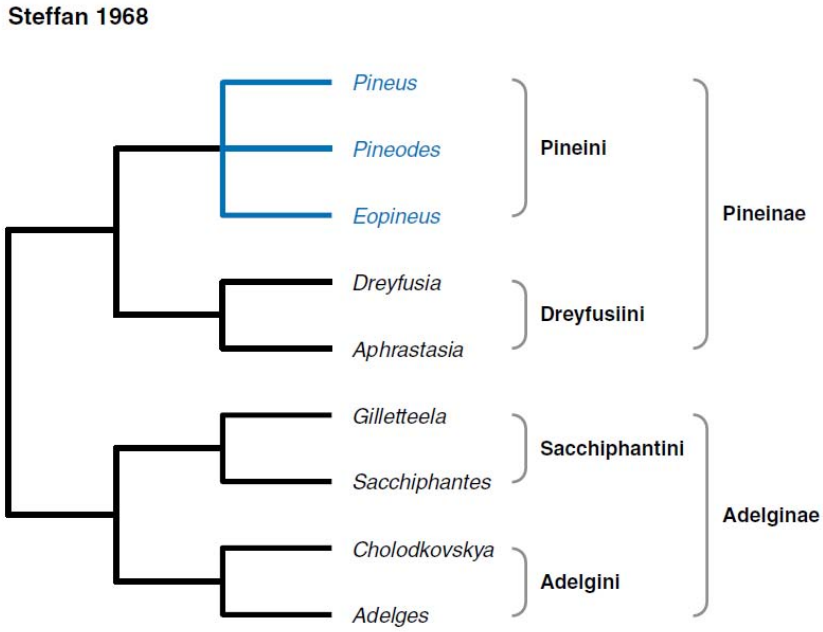


Figure 1.5 Adelgid genera classification (Havill and Foottit, 2007).

Steffan (1968, 1976) refers to the use of the number of chromosomes, the location of endosymbiotic bacteria and on the order of wax gland pores in the first instars larvae (Steffan, 1968, 1976). He constructed a new genus, *Eopineus* including the Pineinae, transferred *Aphrastasia* from Adelginae to Pineinae and constructed a new clade, *Sacchiphantini* within *Gilletteela* and *Sacchiphantes* (Havill et al., 2007). Steffan described the most accurate assumption for the relatedness of adelgids (Havill and Foottit, 2007).

USA and UK taxonomists currently use a different system, which only distinguish between two genera Pineus and Adelges, this system correspond to the subfamilies Pineinae and Adelginae of Börner and Heinze (1957) (Fig. 1.4) (Havill et al., 2007). The identification of the genera in this system is based on the number of abdominal spiracles, Börner (1908) and Annand (1928) both thought that four pairs of abdominal spiracles are ancestral and four are derived (Havill and Foottit, 2007).

Annand (1928) distinguished between four or five pairs of the abdominal spiracles, species with four belong to Pineus and species with five pairs belong to Adelges (Annand, 1928; Carter,1971; Blackman & Eastop, 1994). “This system is preferred because the differences used to distinguish genera in the other system are considered too slight for this level of classification” (Havill and Foottit, 2007). The distinction of two genera does not consider any live cycle and biological characteristics, this makes the procedure much easier but even the adelgids are a complex group where this characteristic plays an important role for the exact identification. The taxonomy and systematics of adelgids are not clear, the complex life cycle and biology makes species identification very extensive and time-consuming. Probably the system with only two genera is easier to distinguish but equal which approach is elected, only high skilled professional are able to distinguish adelgids with certainty by classical methods. The description on the genera level is very doubtful, so it is clear that the identification on the species level offers even more challenges.

1.2.2 DNA – barcoding of *Dreyfusia nordmannianae*

DNA barcoding is a molecular method for species identification and taxonomy as it can be very efficient for species and genera determination (Hebert et al., 2003; Moore 1995). Moore (1995) showed that the average sequence divergence between congeneric species of moths is about 6.5% in contrast the average sequence divergence between conspecific moths is only about 0,25%. Most of these analyses is done by the use of the mitochondrial gene cytochrome c oxidase I (COI). The concept assumes the certainty that every species have a unique genetic barcode. Once the barcode is known it is simple to identify this species by sequence analysis. The general barcode primers developed by Folmer et al. (1994) amplifies the initial 650 bp of the mitochondrial COI gene.

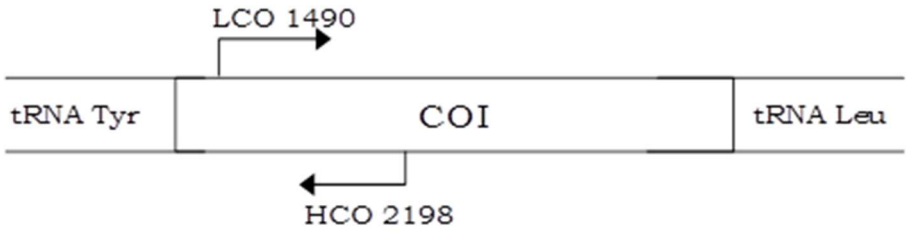


Figure 1.6 Area on the cytochrome oxidase subunit I COI amplified by barcode primers LCO 1490 and HCO 2198 (Folmers et al., 1994).

The method supposed that this segment is adequate and enough variable for the identification or at least delimitation of species (Hebert et al., 2004; Tautz et al., 2003). Hebert et al. (2004) combined DNA barcodes with food plants data, ecological distributions, caterpillar color patterns and adult facies to claridy the tropical *Astraptes fulgerator* consisting of ten cryptic species. Advanced researches and investigation's showed that DNA-barcoding is not a general solution for the whole fauna and has some limitations for example the identification of some taxa were speciation events have not left any ruts on their mitochondrial genomes by very quick radiation will only be possible by using multiple nuclear markers (Vences et al., 2005; Shearer & Coffroth, 2008).

Foottit et al. (2009) showed by his research that DNA barcoding has potential for the identification of adelgid species but find also limitations especially by life cycle characteristic defined species. Žurovcová et al. (2010) applied DNA barcode primers to the adelgids and by sequencing 97 individuals of 16 adelgid species they revealed interesting data (Fig. 1.7 and 1.8).

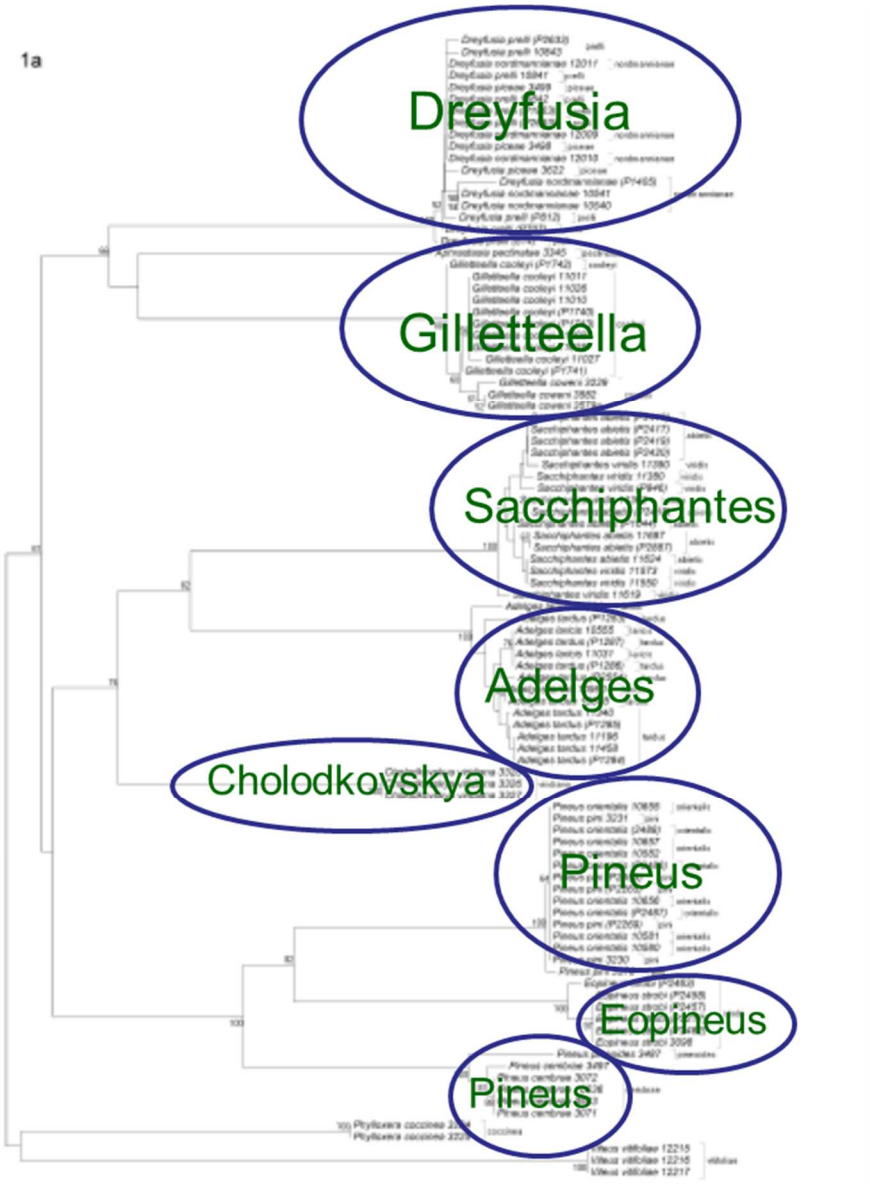


Figure 1.7 Neighbour-Joining tree showing the evolutionary relationships of 97 specimens representing 16 species of adelgids and 2 phylloxerids based on analyses with the barcode primer LCO1490 and HCO 2198 (Žurovcová et al., 2010).

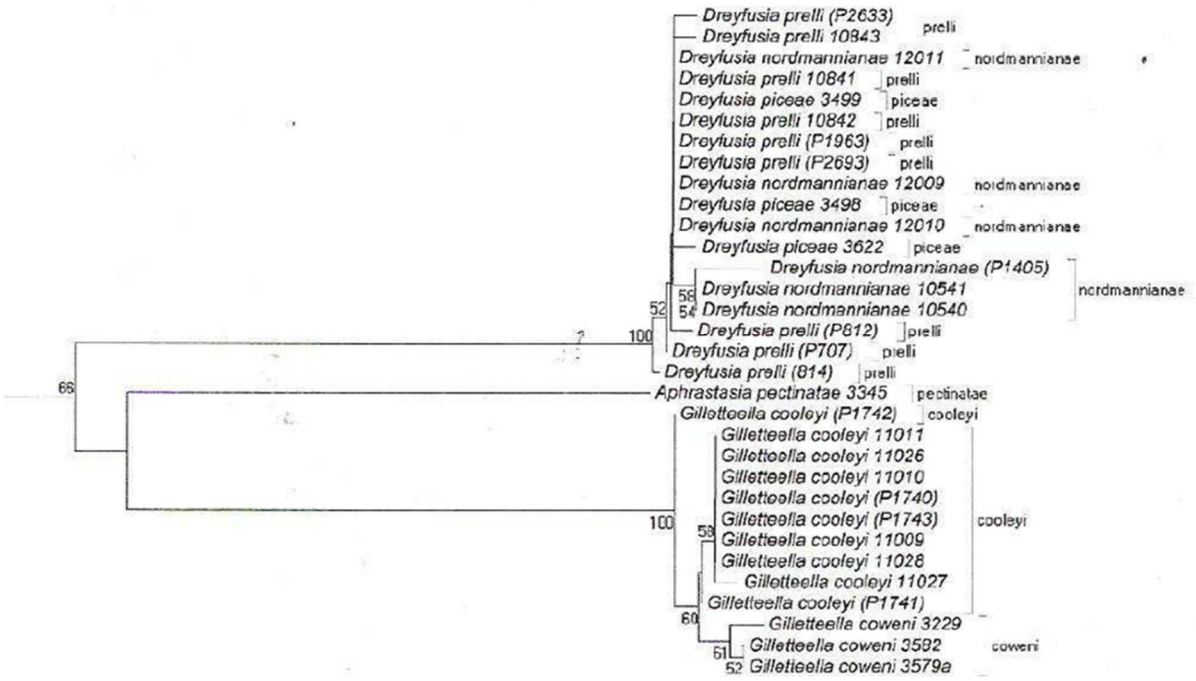


Figure 1.8 Part of the Neighbour-Joining tree (Fig 1.7) showing the evolutionary relationships of the genera *Dreyfusia*, *Gilletteella* and *Aphrastasia* (Žurovcová et al., 2010).

Eight different genera were defined supporting the morphological findings of Börner and Heinze (1957). On the other hand the barcode primer LCO 1490 and HCO 2198 of the COI gene was not polymorphic enough to distinguish between three *Dreyfusia* speices (Žurovcová et al., 2010). Ravn et al. (2012) investigated only the genera *Dreyfusia* and confirmed the data of Žurovcová et al. (2010). They were not able to distinguish the three *Dreyfusia* species and individuals from Europe, North America and also the Caucasus had the same haplotype of the three species (Ravn et al., 2010). This makes it necessary to analyse other regions.

2. Aims

The aim of this master thesis was to study the official barcode primer (Folmer et al., 1994) plus some additional ones to distinguish not only the closely related *Dreyfusia* species but also to compare Austrian locations with other European ones. Comparing data of Austrian *D. nordmannianae* populations with previous investigations (Žurovcová et al., 2010; Ravn et al., 2012) is one of the aims. These former studies showed that “DNA-Barcoding” can support traditional morphology but has limitations i.e. the genera *Dreyfusia* seemed to be the most doubtful group of the adeglids. In this taxonomic complex and partial unsettled group of insects was it possible to identify the genera with DNA barcoding but primary by the genera *Dreyfusia* the identification of the species was not possible (Ravn et al., 2012; Žurovcová et al., 2010).

Furthermore, other primer pairs from mitochondria as well as nuclear genome were selected to test if they are more appropriate i.e. polymorphic for the genus *Dreyfusia*. For that literature as well as the Genbank was searched for potential sequences. Samples were collected in Austria and morphologically defined species (*D. piceas, D. prelli and D. nordmannianae*) were received from Dr. Martina Žurovcová (University of South Bohemia, České Budějovice, Czech Republic) and Dr. Jan Havelka (Biology Centre ASCR, České Budějovice, Czech Republic).

3. Materials and methods

3.1 Collection of insects

Dreyfusia nordmanninanae nymphs and eggs were collected from *Abies alba* and *Abies nordmannianae* from locations in north west of lower Austria and from locations in Upper Austria and Styria (Table 3.1). The samples were collected during the spring season 2013 and mostly nymphs and eggs from *Abies* spp. branches were taken. Often there were no individuals available, then a smear from the stem was taken - this method provides more material but bears the risk of contamination with other insect or arthropods. The samples were immediately put into plastic vials filled with absolute ethanol and stored at -20°C at the Institute of Forest Entomology, Forest Pathology and Forest Protection, Boku, Vienna. DNA from 24 different adelgids were provided by Dr. Martina Žurovcová (University of South Bohemia, České Budějovice, Czech Republic) and Dr. Jan Havelka (Biology Centre ASCR, České Budějovice, Czech Republic). These samples were collected between 2005 and 2008 in the Czech Republic (Table 3.2).

Table 3.1 Samples collected in Austria in the year 2003 and partly genetically screened in this thesis.

Sample	Host plant	Locality
1Bl	*Abies alba*	Schoenbach
2BL	*Abies alba*	Schoenbach
3Bl	*Abies alba*	Schoenbach
4Bl	*Abies alba*	Schoenbach
5Bl	*Abies alba*	Schoenbach
6Bl	*Abies alba*	Schoenbach
7Bl	*Abies alba*	Schoenbach
8Bl	*Abies alba*	Schoenbach
9Bl	*Abies alba*	Gmunden
10Bl	*Abies alba*	Gmunden
11Bl	*Abies alba*	Gmunden
12Bl	*Abies alba*	Gmunden
13Bl	*Abies alba*	Gmunden
14Bl	*Abies alba*	Gmunden
15Bl	*Abies nordmanniana*	Ottenschlag
16Bl	*Abies nordmanniana*	Ottenschlag
17Bl	*Abies nordmanniana*	Ottenschlag
18Bl	*Abies nordmanniana*	Ottenschlag
19Bl	*Abies alba*	Waidenegg
20Bl	*Abies alba*	Waidenegg
21Bl	*Abies alba*	Waidenegg
22Bl	*Abies alba*	Waidenegg
23Bl	*Abies alba*	Waidenegg
24Bl	*Abies alba*	Waidenegg
25Bl	*Abies alba*	Waidenegg
26Bl	*Abies alba*	Waidenegg
27Bl	*Abies alba*	Dorfstadt
28Bl	*Abies alba*	Dorfstadt
29Bl	*Abies alba*	Dorfstadt
30Bl	*Abies nordmanniana*	Schoenbach
31 Bl	*Abies alba*	Ysperklamm
32 Bl	*Abies alba*	Ysperklamm
33 Bl	*Abies alba*	Ysperklamm
34 Bl	*Abies alba*	Maria Lankowitz
35 Bl	*Abies alba*	Maria Lankowitz
36 Bl	*Abies alba*	Maria Lankowitz

Table 3.2 Morphologically defined adelgid species provided by Dr. Martina Žurovcová (University of South Bohemia, České Budějovice, Czech Republic) and Dr. Jan Havelka (Biology Centre ASCR, České Budějovice, Czech Republic)for the screening of mitochondrial as well as nuclear markers.

Species	**Host plant**	**Locality**
Adelges laricis	*Picea abies*	Buchlovice
Adelges laricis	*Picea abies*	České Budějovice, Borek
D. nordmannianae	*Abies alba*	Chvalčov
D. nordmannianae	*Abies alba*	Libín
D. nordmannianae	*Abies alba*	Libín
D. nordmannianae	*Picea orientalis*	Buchlovice
D. nordmannianae	*Picea orientalis*	Buchlovice
D. nordmannianae	*Picea orientalis*	Buchlovice
D. piceae	*Abies alba*	Bystřice pod Hostýnem
D. piceae	*Abies alba*	Bystřice pod Hostýnem
D. piceae	*Abies alba*	Branisov
D. prelli	*Picea orientalis*	Buchlovice
D. prelli	*Picea orientalis*	Buchlovice
D. prelli	*Picea orientalis*	Nový Dvůr
D. prelli	*Picea orientalis*	Buchlovice
D. prelli	*Picea orientalis*	Buchlovice
D. prelli	*Picea orientalis*	Buchlovice
D. prelli	*Picea orientalis*	Buchlovice
Eopineus strobi	*Pinus strobus*	Nový Dvůr
Gillettella cooleyi	*Picea pungens*	České Budějovice
Gillettella cooleyi	*Picea pungens*	Praha
Sacchiphantes viridis	*Picea abies*	České Budějovice, Haklovy Dvory
Pineus cembrae	*Pinus cembra*	Těchobuz u Pacova
Pineus pini	*Pinus sylvestris*	Praha, Břevnov

3.2 DNA extraction

DNA extraction of individual *Dreyfusia* species was done with a DNA Miniprep SIGMA kit (Appendix I). Four to six individuals were used from each species. The insects were put in Eppendorf tubes and 180 µl lysis solutions was added. For homogenizing a small sterile metal ball and a ball mill was used. 20 µl SIGMA proteinase K was added and the tubes were put in the heating block at 55°C for 10 min. Subsequently 20 µl of RNase was added, the tubes were incubated for 2 min and 200 µl lysis solution was added. After incubation at 70°C for 10 min, 200 µl absolute ethanol were added to the samples. The whole solution was then transferred to binding columns. Afterwards two washing steps with the SIGMA washing solution were done. Finally the DNA was eluted with 100 µl of the elution buffer from the Sigma Kit.

3.3 PCR with barcode and other primers

The barcode primers LCO 1490 5´-GGTCAACAAATCATAAAGATATTGG-3´ forward and HCO2198 5´-TAAACTTCAGGGTGACCAAAAAATCA-3´ reverse developed by Folmer et al. (1994) were used for the amplification of about 700 bp of the forward region of the mitochondrial gene region cytochrome oxidase subunit I COI. PCR reactions were done in volumes of 20 µl in Eppendorf tubes containing 9,8 µl H_2O, 1 µl Bovine Serum Albumin BSA, 2 µl PCR buffer, 2 µl $MgCl_2$, 0,8 µl dNTPs, 1 µl of each primer, 0,4 µl Taq polymerase and 2 µl of template DNA. The PCR reaction consisted of 35 cycles with an annealing temperature T_M of 47°C. For detection a 1,5 µl to 2 µl aliquot of the PCR product mixed with 5 µl of a loading buffer were loaded in a submarine horizontal agarose gel system. The agarose concentration was 1,5 % and 5 µl GelRed® was added to a 100 ml solution. Visualizing and pictures were done with a UV transilluminator and pictures were taken with a Sony MVC-FD83 digital camera. For purification and sequencing the PCR product were sent to Eurofins (Ebersberg, Germany).

Furthermore, 19 primer pairs amplifying mitochondrial genes and one primer pair amplifying the nuclear gene ITS2 were applied in this thesis (Table 3.4). For finding the optimal T_M and optimal BSA the temperature and concentrations were varied. Furthermore, an enhancer solution P - triggers a modification of the denaturation properties - was added. Using different reaction buffers and changing the template DNA amount were also done. For the PCR with these primers the peqGOLDTaq-DNA-Polymerase was used. The PCR reactions were done in volumes of 20 µl containing 12,2 µl H_2O, 2 µl BSA, 2 µl Y or S puffer, 0,4 µl dNTPs, 0,6 µl of each primer, 0,2 µl Taq polymerase and 2 µl of template DNA. If the PCR did not results in positive amplicons the amount of the primers was decreased to 0,4 µl, 2 µl of the enhancer solution P was added and the amount of template DNA was increased. The reaction puffer Y contains 200 mMTris-HCI (pH 8.55), 160 nM $(NH_4)_2SO_4$, 0,1 % Tween 20 and 20 mM $MgCl_2$ and the reaction puffer S contains 100mM Tris-HCI (pH 8.8), 500 mM KCI, 0,1% Tween 20 and 15 mM $MgCl_2$.

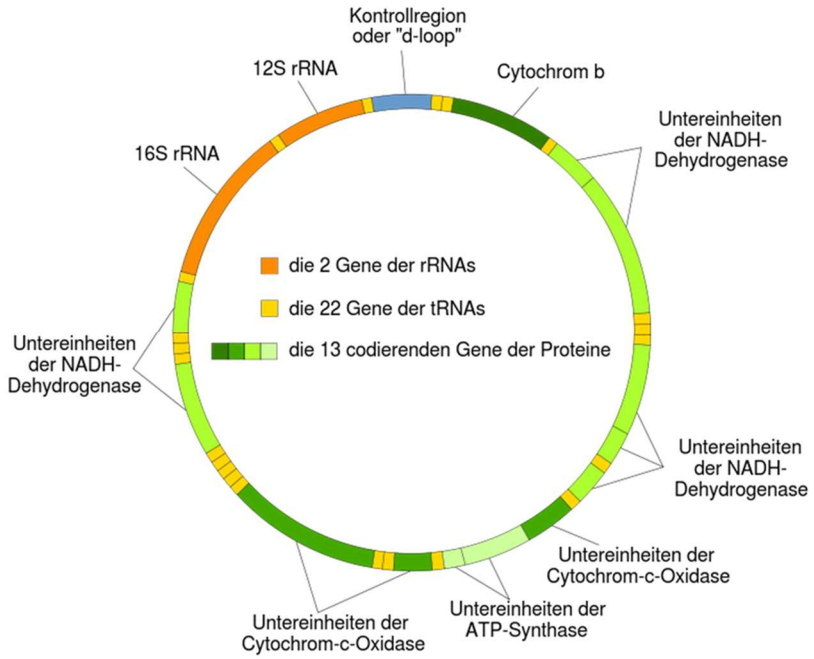


Figure 3.3 Schematic description of the regions and the mitochondrial DNA (http://de.wikipedia.org/wiki/Mitochondriale_DNA). In Table 3.4 primers and mitochondrial regions screened during this thesis are listed.

Table 3.4 Primers used for mitochondrial regions and for the nuclear gene ITS2.

Gen	Primer	T	Sequences (5´ - 3´)
COI	UEA 3[1)]	F	TATAGCATTCCCACGAATAAATAA
	UEA 4[1)]	R	AATTTCGGTCAGTTAATAATATAG
	UEA 5[1)]	F	AGTTTTAGCAGGAGCAATTACTAT
	UEA 6[1)]	R	TTAATWCCWGTWGGNACNGCAATRATTA
	UEA 7[1)]	F	TACAGTTGGAATAGACGTTGATAC
	UEA 8[1)]	R	AAAAATGTTGAGGGAAAAATGTTA
	UEA 9[1)]	F	GTAAACCTAACATTTTTTCCTCAACA
	C1-J-2183[2)]	F	TTGATTTTTTGGTCATCCAGAAGT
	PAT[3)]	F	
	DICK[3)]	R	
tRNA	UEA 10[1)]	R	TCCAATGCACTAATCTGCCATATTA
COII	mt2993[4)]	F	CATTCATATTCAGAATTACC
	A3773[5)]	R	GAGACCATTACTTGCTTTCAGTCATCT
Cytochrom b 3´-end	CP1[6)]	F	GATGATGAAATTTTGGATC
	CP2[6)]	R	CTAATGCAATAACTCCTCC
	CB2[7)]	R	ATTACACCTCCTAATTTATTAGGAAT
ATP	AphidCO2f1[8)]	F	CAA TGC TCA GAA ATT TGT GG
	AphidCO3rev[8)]	R	TTG GTG GTC AAT TTA ATC CTA
	C3-N-5460[2)]	R	TCA ACA AAG TGT CAG TAT CA
AT-rich	SR-J-14612[2)]	F	AGG GTA TCT AAT CCT AGT TT
	TIN-24[2)]	R	ATT TAC CCT ATC AAG GTA A
	TM-N-193[2)]	R	TGG GGT ATG AAC CCA GTA GC
	AdelATfor1[8)]	F	CAA TTA TAG AAC AGA TTC CTC
	AdelATrev1[8)]	R	TTG AAG TAT GAA TCC AAA AGC
ITS2	CAS5p8sFc[9)]	F	TGAACATCGACATTTYGAACGCACAT
	CAS28sB1d[9)]	R	TTCTTTTCCTCCSCTTAYTRATATGCTTAA

1) Lunt et al. 1996;
2) Simon et al. 1994
3) Juan et al. 1995
4) Stern 1994
5) Normak 1996
6) Harry et al. 1998
7) Jermiin& Crozier 1994
8) Zurovcova et al. 2010
9) Ji et al. 2003

For the UEA 3 – 10 primers two different PCR programs and for optimization of the PCR T_M between 45 °C and 55 °C were used.

Table 3.5 PCR programs for the amplification of UEA 3 – UEA 10,C1-J-21832 and A3772 primers.

Three PCR programs for UEA 3 – UEA 10, C1-J-21832 and A3772			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	45 °C – 55 °C	45 sec	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
Denaturation	94°C	5 min	1
Denaturation	95°C	40 sec	35
Annealing	45 °C – 55 °C	45 sec	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
Denaturation	94°C	5 min	1
Denaturation	94°C	40 sec	35
Annealing	45 °C – 55 °C	1 min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1

Table 3.6 PCR programs for the amplification

PAT and DICK			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	50 °C	40 sec	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
Mt 2993 and A3772			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	48 °C	1min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
CP1, CP2 and CB2			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	48 °C	1min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
AdelATfor1 and AdelATrev1			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	54 °C	45 min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1

AphidCO2f1, AphidCO3 rev and C3-N-5460			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	56 °C	45 min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
SR-J-14612, TIN-24 and TM-N-193			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	35
Annealing	50 °C/55 °C	45 min	35
Elongation	72 °C	1 min	35
Final Elongation	72 °C	10 min	1
CAS5p8sFc and CAS28sB1d			
PCR step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	4 min	1
Denaturation	95°C	20 sec	35
Annealing	50 °C	40 min	35
Elongation	72 °C	20 min	35
Final Elongation	72 °C	10 min	1

3.4 Cloning

The exact procedures are listed in Appendix II. For cloning a 10 µl PCR product was amplified and 0,8 µl of the amplicon was mixed with 1,6 µl distilled water, 0,2 µl pTZ57R (Instar Clone PCR, Fermentas), 0,3 µl of T4 buffer and 0,1 µl T4 ligase, the tubes with this mix was incubated at 4°C overnight.

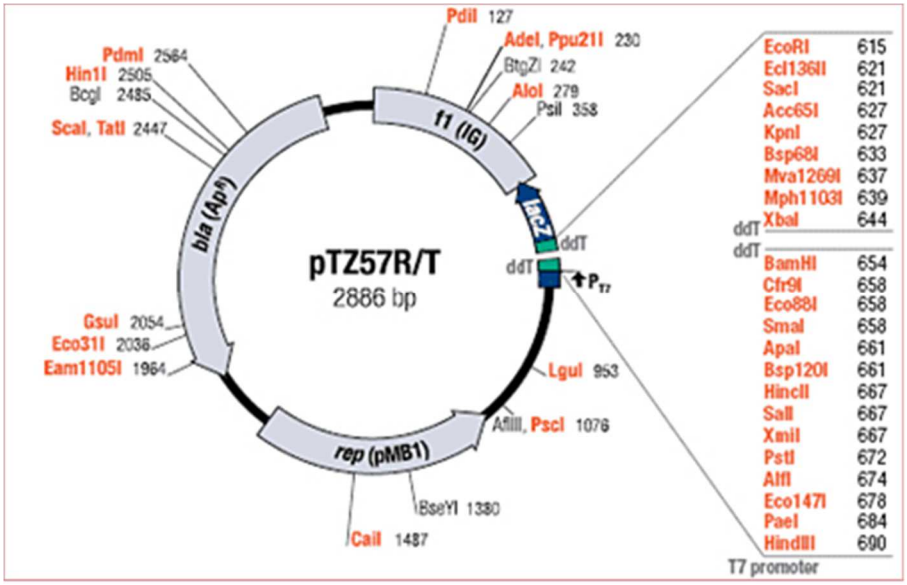


Figure 3.7 Restriction map of vector pTZ57R/T used in this study. Being an AT cloning vector the PCR product was inserted in the coloured region (http://www.thermoscientificbio.com/molecular-cloning/instaclone-pcr-cloning-kit/).

Competent JM109 E. coli cells stored at -80°C were used for the transformation. Before 50 µl of the *Eschericha. coli* cells were pipetted to each sample the cells were placed on ice for 20 min. After adding the *E. coli* cells the new mix were carefully vortexed and again placed on ice for 20 min. Subsequently the samples were heated to 42°C for 50 sec. 950 µl of a SOC-media elution was added and the tubes were placed at 37°C for 60 min. The samples were centrifuged for 5 min at 4°C by 2.700 rpm. The supernatant with the bacteria was deposited in an autoclaved vial. To select the positive bacteria, the Blue White Cloning Screening was used. Therefore Petri dishes with agar were taken (agar contained 100 µg/ml ampicillin, 160 µg/ml xGal and 48 µg/ml IPTG). The remaining substrate on the agar plates, after that the plates were stored at 37 °C over night. On the following day the plates were controlled for white bacteria colonies, these

colonies were marked and transformed with a toothpick to a test tube containing a lysogeny broth (LB) medium (Sambrook et al. 1989). After that a PCR with the M13 primers was done. A gel electrophoresis showed which bacteria contained plasmids with PCR products and which ones did not. The plasmid purification was performed by an alkaline lysis mini prep procedure. For sequencing the purified DNA was sent to the Cancer Research Centre DNA Sequencing & Genotyping Facility in Chicago (IL, USA) or to Eurofins (Ebersberg, Germany).

3.5 Phylogenetic analysis of the sequence data

The carried sequences were edited with Chromas 2.1 http://www.technelysium.com.au/chromas.html9 and GeneRunner (GeneRunner 4.0.9.4 Beta). The alignment of the sequences was done with ClustalX (Thompson et al., 1997). For finding most similar sequences in Genbank (NCBI) the Basic Local Alignment Search Tool BLAST http://www.ncbi.nlm.nih.gov was used. BLAST not only finds most similar sequences but moreover identifies sequences relating the query sequence above a defined threshold (Altschul et al. 1990). For the Phylogenetic analyses and tree construction MEGA6 (Tamura et al., 2013) was used. Neighbor Joining Trees (Saitou and Nei, 1987), Maximum Parsimony Trees and Maximum Likelihood ML Trees (Tamura and Nei, 1993) were mostly constructed. For the Neighbor Joining Trees a Kimura-2-parameter distance (Kimura 1980) algorithm was applied. Bootstrap analysis was done with 500 replicates.

4. Results & Discussion

The aims of my thesis were to analyse Austrian populations of *Dreyfusia nordmannianae* with the barcode primers LCO 1490 and HCO 2198 described by Folmer et al.(1994) and to compare the results with Genbank entries of Žurovcová et al. (2010) and Ravn et al. (2012). Furthermore, other markers were searched with higher polymorphism to detect differences between the cryptic *Dreyfusia* species i.e. *D. nordmannianae*, *D. piceae* and *D. prelli.*

4.1 DNA – barcoding of Dreyfusia nordmannianae

Twenty nine individuals *of D. nordmannianae* collected in Austria (Table 4.1) were analysed with the barcode primers using the PCR programme developed by Žurovcová et al., 2010.

Table 4.1 Screened and analysed samples with barcode primers LCO 1490 and HCO 2198

Sample	Host plant	Locality
1Bl	*Abies alba*	Schoenbach
2BL	*Abies alba*	Schoenbach
3Bl	*Abies alba*	Schoenbach
4Bl	*Abies alba*	Schoenbach
5Bl	*Abies alba*	Schoenbach
6Bl	*Abies alba*	Schoenbach
7Bl	*Abies alba*	Schoenbach
8Bl	*Abies alba*	Schoenbach
9Bl	*Abies alba*	Gmunden
10Bl	*Abies alba*	Gmunden
11Bl	*Abies alba*	Gmunden
12Bl	*Abies alba*	Gmunden
13Bl	*Abies alba*	Gmunden
14Bl	*Abies alba*	Gmunden
15Bl	*Abiesnordmanniana*	Ottenschlag

16Bl	*Abiesnordmanniana*	Ottenschlag
17Bl	*Abiesnordmanniana*	Ottenschlag
18Bl	*Abiesnordmanniana*	Ottenschlag
19Bl	*Abies alba*	Waidenegg
20Bl	*Abies alba*	Waidenegg
21Bl	*Abies alba*	Waidenegg
22Bl	*Abies alba*	Waidenegg
23Bl	*Abies alba*	Waidenegg
24Bl	*Abies alba*	Waidenegg
25Bl	*Abies alba*	Waidenegg
26Bl	*Abies alba*	Waidenegg
27Bl	*Abies alba*	Dorfstadt
29Bl	*Abies alba*	Dorfstadt
30Bl	*Abies nordmanniana*	Schoenbach

Most reactions showed positive amplicons on the agarose gel electrophoresis (Fig. 4.2). Some individuals failed to show amplicons and had to be repeated, after repeating also this showed positive amplicons on the agarose gel electrophoresis and could be used for the analyse.

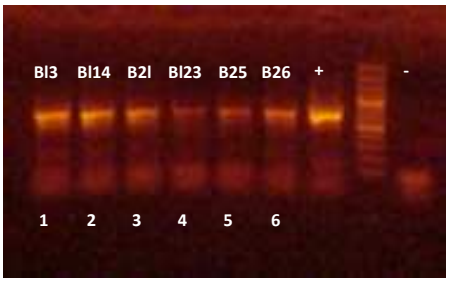


Figure 4.2 Agarose gel electrophoresis of the PCR with LCO 1490 and HCO 2198 (Folmer et al., 1994) primers.

Amplicons were sequenced from all positive samples and quality of chromatograms strongly varied from very high quality (Fig. 4.3) to bad quality sequences (Fig. 4.5).

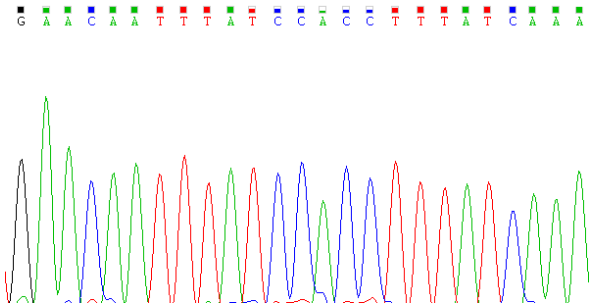


Figure 4.3 A small fragment of the sequence chromatogram of the amplicon polymerized with LCO 1490 – HCO 2198 (Folmer et al., 1994) having high quality.

High quality sequences were edited. For the phylogenetic analysing BLAST (NCBI; http://www.ncbi.nlm.nih.gov) were used to get additional sequences, which are showing high similarity, from the Genbank. Retrieved and own sequences were aligned and thereafter the phylogenetic tree construction was done with MEGA6 (Tamura et al., 2013). Neighbor Joining (NJ) (Saitou and Nei, 1987), Maximum Likelihood ML (Tamura and Nei, 1993) and Maximum Parsimony (MP) trees were constructed. The different methods resulted in equivalent topologies and thus only NJ is presented (Figure 4.4).

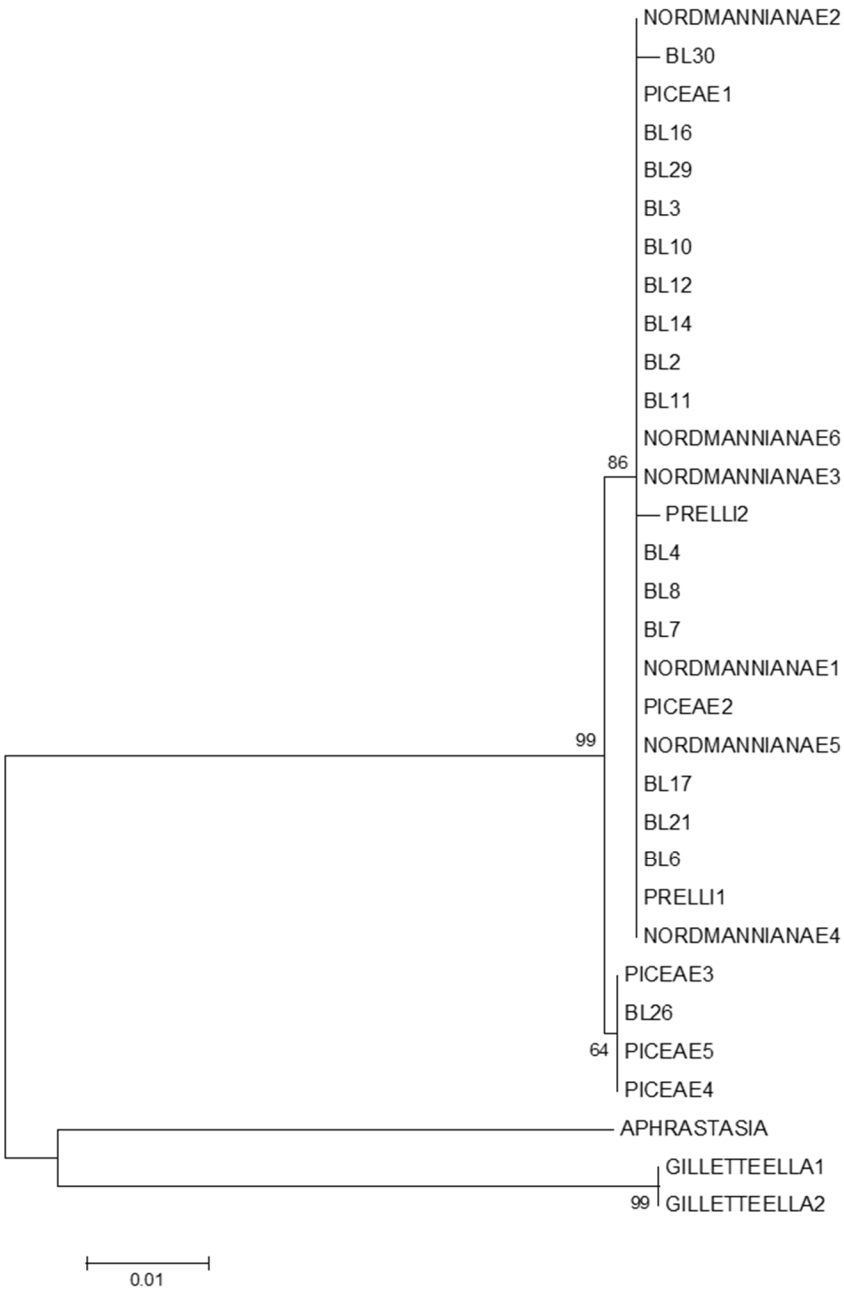


Figure 4.4 Sixteeen of the Austrian individuals of *D. nordmannianae* were used for the phylogenetic analyses by NJ method (Saitou and Nei, 1987). Additionally six individuals of *D. nordmannianae*, five individuals of *D. piceae*, two individuals of *D. prelli*, two individuals of the genera *Gilletteella* and one individual of the genera *Aphrastasia* were retrieved from the Genbank and used for the performance of the NJ analyses (Saitou and Nei, 1987). The Genebank numbers of the sequences are in the appendix. *Aphrastasia spp.* and *Gilletteella* spp. were used as outgroup species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Aphrastasia spp. and *Gilletteella* spp. were taken as outgroups as genetically they are close to the genus *Dreyfusia* (Žurovcová et al., 2010). Especially the identification of the species level of the genera *Dreyfusia* by DNA barcoding were often described as problematic (Mantovani et al., 2001; Žurovcová et al., 2010, Ravn et al., 2013). The phylogenetic analyses of the sequences amplified with the barcode supports the results of these studies. *D. nordmannianae* could not were identified on the species level as *D. nordmannianae*, *D. piceae* and *D. prelli* formed a species complex. The amplified sequences of the three species had partly the same haplotypes and formed one significant clade. Two haplotypes of *D. nordmannianae* in Lower Austria were detected. The phylogenetic analyses showed that the Austrian sequences have some point mutations. The sequences of the samples BL30 and BL26 showed mutations, all others are completely similar. Thus, DNA methods used here can not clarify the taxonomic problems occurring in the genus *Dreyfusia*.

4.2 Cloning of amplicons with unambigous sequences

Some of the sequences amplified by using the DNA barcode primers showed ambiguous chromatograms i.e. the peaks of the single basepairs were not clear (Fig. 4.5).

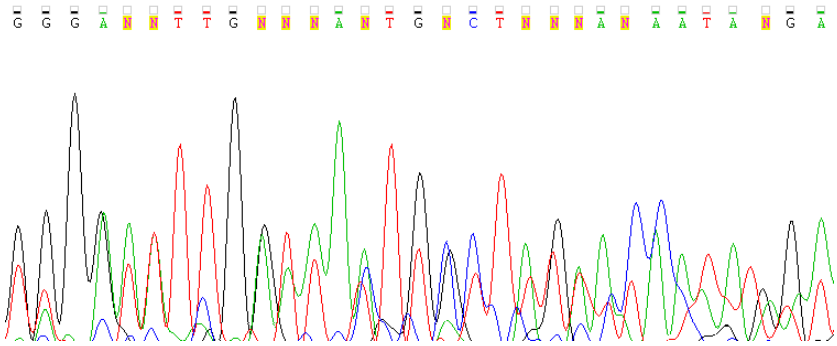


Figure 4.5 A fragment of the sequence chromatogram of the amplicon polymerized with LCO 1490 – HCO 2198 (Folmer et al., 1994) showing ambiguous peaks.

Therefore, cloning of fresh PCR products was done. Here five individuals with bad sequence results after direct sequencing of the PCR products were ligated into a TA-plasmid. The recombinant plasmid was transformed into competent JM109 *Escherichia coli* bacteria and transformed bacteria were plated on an agar plate. Selection was done via blue-white screening technique.

Figure 4.6 Agar plate showing the result of the selection via blue-white screening technique (http://en.wikipedia.org/wiki/Blue_white_screen)

White colonies were picked and a PCR was done with M13 primers, products revealed partly positive amplicons. Finally 15 positive plasmids were sent for sequencing. Most of those resulted in high quality sequence chromatograms. For phylogenetic analyses only the good sequences of the *D. nordmannianae* and *D. piceae* were used. The consensus tree of NJ and optimizing method are shown in Fig. 4.7 using the closest related genera *Aphrastasia spp.* and *Gilletteella spp.* as outgroups.

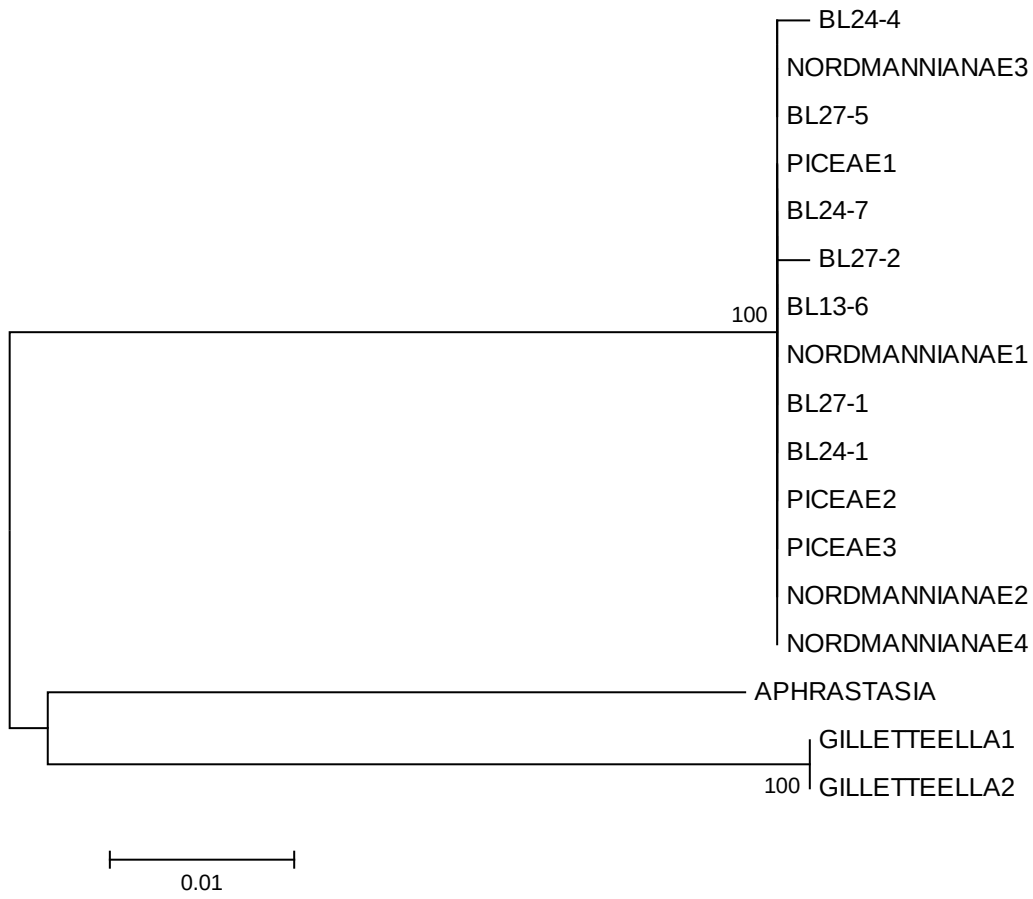


Figure 4.7 Seven sequences of three samples from the Austrian individuals were used after cloning for analyse by NJ method (Saitou and Nei, 1987). Additionally four individuals of *D. nordmannianae*, three individuals of *D. piceae*, two individuals of the genera *Gilletteella* and one individual of the genera *Aphrastasia* was retrieved from the Genbank and used for the performance of the NJ analyses (Saitou and Nei, 1987). The Genebank numbers of the sequences are in the appendix. All 17 nucleotide sequences are based on the "DNA Barcode" primers LCO 1490 and HCO 2198 (Folmer et al., 1994). *Aphrastasia spp.* and *Gilletteella* spp. were used as outgroup species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

The tree analysis resulted in a similar topology as the one shown in Fig.4.4. The DNA barcode primer was not polymorphic enough to distinguish *D. nordmannianae* from *D. piceae*. Only the sequences BL24-4 and BL27-4 showed mutations, all others are completely similar. They all form one clade and ranks on the same level with the sequences from the Genbank, which includes sequences from *D. nordmannianae* and *D. piceae*.

The sequences obtained by the plasmids also resulted in seven sequences which had a different sequence then *D. nordmannianae* and one sequence had no result which is depending on the bad quality (Table 4.8).

Table 4.8 Results of BLAST searches of the 15 cloned sequences

Sample	**BLAST search reveals**
BL13-5	no significant similarity found
BL13-6	*Adelges nordmannianae*
BL13-8	*Achipteria coleoptrata*
BL15-1	*Achipteria coleoptrata*
BL15-2	*Achipteria coleoptrata*
BL15-3	*Achipteria coleoptrata*
BL23-1	*Adineta Vaga, Habrotrocha Constricta, Uncultured bdelloid rotifer…..*
BL23-4	*Adineta Vaga, Habrotrocha Constricta, Uncultured bdelloid rotifer…..*
BL23-7	*Adineta Vaga, Habrotrocha Constricta, Uncultured bdelloid rotifer…..*
BL24-1	*Adelges nordmannianae*
BL24-4	*Adelges nordmannianae*
BL24-7	*Adelges nordmannianae*
BL27-1	*Adelges nordmannianae*
BL27-2	*Adelges nordmannianae*
BL27-5	*Adelges nordmannianae*

BLAST search revealed that four sequences had most similarity to *Achipteria coleoptrata* (acari) and three sequences had most similarity to different rotifer genera, they all are not feeding on *D. nordmannianae* and are herbivorous. Achipteriidae species are usually living in the greenery and the soil of forests but also on foliose lichen and on the bark of trees (Lindo et al., 2008). *Achipteria coleoptrata* frequent in European deciduous forest is mainly feeding on the alga *Desmococcus vulgaris* or on grass or other plant material (makrophytophag) (Schatz H., 1979).

Particularly the acari sequence shows that the samples were contaminated with other organisms. This contamination is the reason for the ambiguous bands in the sequences. The *D. nordmannianae* species is minor and mostly wax covered on the trees, so the collecting of the samples from the trees is difficult. It were done with a molecular twizzer to preserve the individual as effectively as possible. However, it is hard to collect one single individual without contamination.

4.3 Other mitochondrial and nuclear primers for barcoding

In this study the universal barcode primers were not sufficiently polymorphic to distinguish the three *Dreyfusia species i.e. D. nordmannianae* , *D. prelli and D. piceae.* Therefore 19 primer pairs, amplifying other mitochondrial regions and one primer pair amplifying a nuclear gene were tested here. Primers from the 5' end of the COI region was used as it is known to be more polymorphic then the 3' start of that gene (Lunt et al., 1996).

The amplicons of the primers tested (Table 3.4) were directly loaded onto the agarose gel electrophoresis (Fig. 4.9) and consequently sequenced.

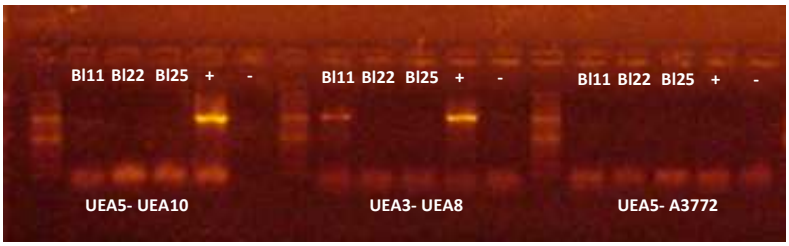


Figure 4.9 Agarose gel electrophoresis of the PCR with UEA5 – UEA10[1], UEA3 – UEA8[1] and UEA5[1] – A3772[2]primer pairs. 1) Lunt et al., 1996, 2) Normak, 1996

The primer pair UEA3 (Lunt et al, 1996) and A3772 (Normak, 1996) were used as they are at the 5´ end of the COI gene. Nevertheless, they failed and even several assays with different T_M and other modifications (BSA, buffers etc.) did only partly improve the PCR results. As shown in (Fig. 4.10) the amplicons did result in faint bands and repeating the PCR with the same conditions resulted mostly in no amplicons. Thus no sequences were obtained from the primer pair UEA3 and A3772.

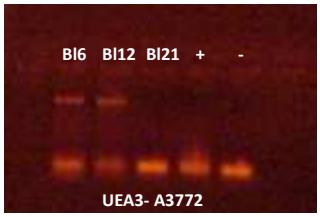


Figure 4.10 Agarose gel electrophoresis of the PCR with UEA3 (Lunt et al., 1996) – A3772 (Normak, 1996) primer pairs.

Finally only five 5 of the 20 tested primer pairs resulted in positive amplicons and could be sequenced (Fig. 4.11).

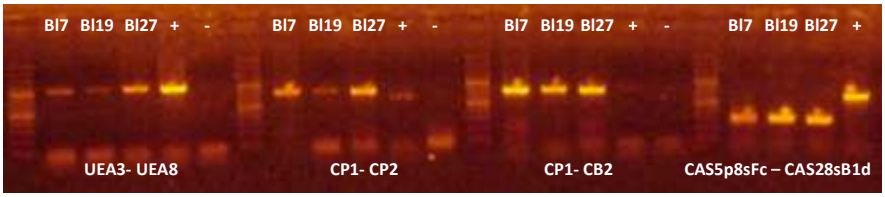


Figure 4.11 Agarose gel electrophoresis of the PCR with UEA3 – UEA8[1], CP1 – CP2[2], CP1[2] – CB2[3] and CAS5p8sFc[4] - CAS28sB1d[4] primer pairs. 1) Lunt et al., 1996, 2) Harry et al., 1998 3) Jermiin& Crozier, 1994 4) Ji et al., 2003

These were following primer pairs: UEA3 – UEA8 (Lunt et al., 1996) and PAT – DICK (Juan, et al., 1995) amplifying on the COI region. Furthermore CP1 – CP2 (Harry et al., 1998) and CP1 – CB2 (Harry et al., 1998; Jermiin & Crozier, 1994) amplifying on the cytochrom b 3´-end region and the nuclear ITS2 primer pair: CAS5p8sFc – CAS28sB1d (Ji et al., 2003).

The Coleoptera specific primer pair Pat and Dick provided only one positive amplicon and was sequenced. Sequence was blasted in the Genbank and showed most similarity with *Laricobius erichsoni* (Coleoptera, Derotondidae). *L. erichsoni* is one of four genera in the family of the Derotondidae, feeding on adelgids (Montgomery et al., 2011). The other three genera feed on fungi. *L. erichsoni* could be used to control the silver fir woolly adelgid similar to the close relative *L. nigrinus* being successfully used to control of the hemlock wolly adelgid (Zilahi-Balogh et al., 2005).

The other four primer pairs were UEA3 – UEA8 (Lunt et al., 1996) amplifying a large part of the COI region; CP1 – CP2 (Harry et al., 1998) and CP1 – CB2 (Harry et al., 1998; Jermiin& Crozier, 1994) amplifying on the cytochrom b 3´-end region; CAS5p8sFc – CAS28sB1d (Ji et al., 2003) amplifying the nuclear ITS2 region. These four primers resulted in positive amplicons and were sequenced. However, editing and aligning them with most similar sequences from Genbank resulted in no differences between the *D. nordmannianae* populations and *D. piceae* and *D. prelli*. (Fig. 4.12).

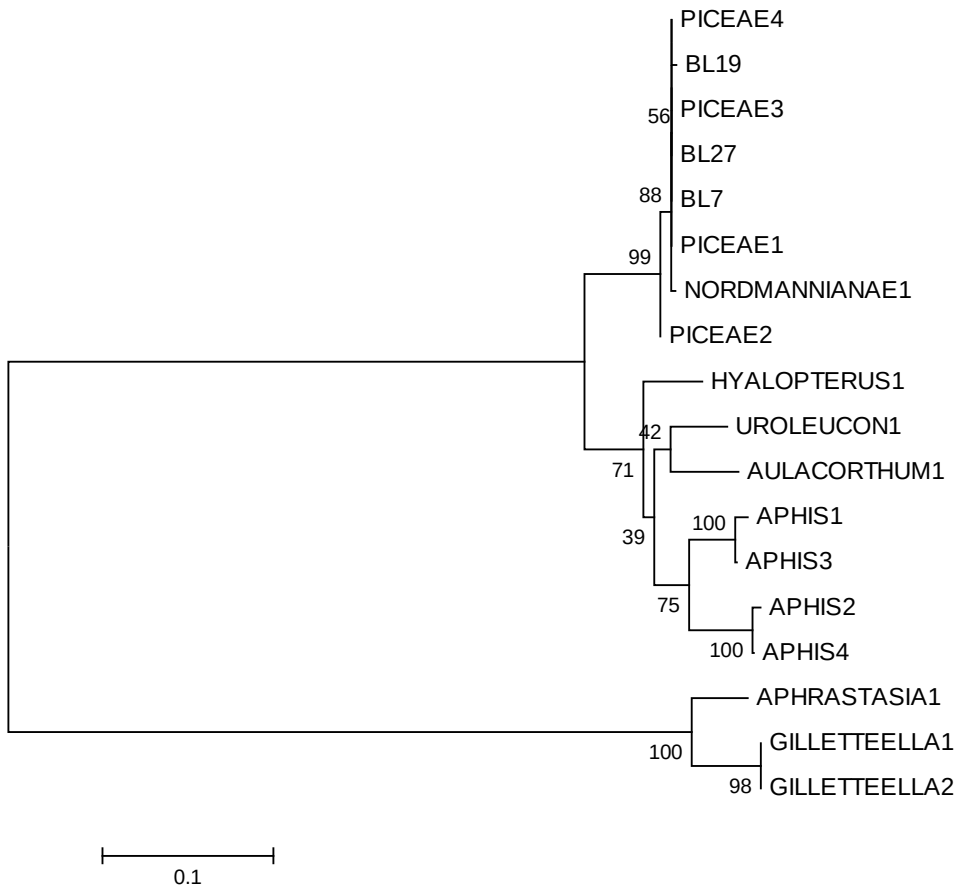


Figure 4.12 Three of the Austrian individuals of D. nordmannianae were used for the phylogenetic analyses by NJ method (Saitou and Nei, 1987). Additionally one individual of *D. nordmannianae*, four individuals of *D. piceae*, four individuals of the genera *Aphis*, one individual of *Aulacorthum cirsicola*, one individual of *Hyalopterus pruni*, one individual of *Uroleucon leonardi*, two individual of the genera *Gilletteella* and one individual of the genera *Aphrastasia* was retrieved from the Genbank and used for the performance of the Neighbor Joining Tree (Saitou and Nei, 1987). The used sequences descend to analyses with the primer pair CP1-CP2 (Harry et al., 1998). The Genebank numbers of the sequences are in the appendix. *Aphrastasia* spp. and *Gilletteella* spp. was used as outgroup. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Similar to the results with the barcode primers other DNA markers do not provide a sufficient polymorphism to distinguish *D. nordmannianae* from the other morphologically similar species i.e. *D. prelli* and *D. piceae*. Thus also a phylogeographic identification of the *D. nordmannianae* populations was not possible in this study. The adelgids with their difficult life cycles and their morphological challenges expose the limitations of DNA barcoding. DNA barcoding supported traditional methods and were able to identify the adelgids on the genera level, but the

identification on the species level of the genera *Dreyfusia* is more than uncertain. In the future maybe whole genome sequencing will give some indication of the molecular uncertainties of *D. nordmannianae* and will clarify the taxonomical situation of the adelgids.

5. References

Alles, D. (1994) Study to the generation cycle of *Dreyfusia nordmannianae* (Eckstein, 1890) (Hom; Adelgidae) in the middle of Europe. Z. ang. Ent. 117, 234-242.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

Annand, P.N. (1928) A Contribution toward a Monograph of the Adelginae (Phylloxeridae) of North America. Palo Alto, CA: Stanford Univ. Press. 146 pp.

Blackman, R.L. & Eastop, V.F. (1994) Aphids of the world's trees: an identification and information guide. CAB Int. 987 pp.

Binazzi, A. & Covassi, M. (1991) Contributions to the knowledge of the conifer aphid fauna XII, a review of the species of *Dreyfusia* Boerner occurring in Italy with description of *Dreyfusia nebrodensis* n. sp. (Homoptera: Adelgidae). Redia 74, 233–288.

Bodenheimer, F.S. & Swirski, E. (1957) Aphidoidea of the Middle East. The Weizmann Science Press of Israel, Jerusalem, 378 pp.

Börner, C.V. (1908) Eine monographische Studie über die Chermiden. Arb. Kais. Biol. Anst. Land Forstw. 6, 81–318.

Börner, C.V. (1930) Beiträge zur einem neuen System der Blattläuse. Arch. Klassif. Phylogen. Entomol. 50, 115–194.

Börner, C.V. (1952) Europae centralis Aphides, die Blattläuse Mitteleuropas. Mitt. Thurin. Bot. Ges. 3, 1–484.

Börner, C.V. & Heinze, K. (1957) Aphidina – Aphidoidea. In Blunck H. (ed.): Tierische Schädlinge an Nutzpflanzen. Homoptera. 2. Teil. Paul Parey, Berlin, pp. 323–355.

Carter, C.I. (1971) Conifer Woolly Aphids (Adelgidae) in Britain. Forestry Commission Bulletin No. 42, London, 51 pp.

Ccholodkovsky, N. (1896) Zur Biologie der Lärchen – Chermes - Arten. Zool. Anz. 19, 37–40.

Cholodkovsky, N.A. (1915) *Chermes Injurious to Conifers*. Petrograd: Dep. Agric. Central Board Land Admin. Agric. 89 pp.

Eckstein, Kl. (1890) Zur Biologie der Gattung Chermes L. Zeitschrift für Forst-und Jagdwesen, XXII Jhg.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783-791.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrikenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Marine Biol. Biotechnol. 3, 294–299.

Foottit, R.G., Maw, E.L., Havill, N.P., Ahern, R.G. & Montgomery, M.E. (2009) DNA barcodes to identify species and explore diversity in the Adelgidae (Insecta: Hemiptera: Aphidoidea). Mol. Ecol. Res. 9, 188–195.

Gouli, S., Parker, B.L. & Skinner, M., (1997) Isolation of fungi associated with hemlock woolly adelgid (Homoptera: Adelgidae). J. Invertebr. Pathol. 70, 76–77.

Harry, M., Solignac, M. & Lachaise, D. (1998) Molecular evidence for parallel evolution of adaptative syndromes in fig-breeding *Lissocephala* (Drosophilidae): Mol. Phylogen. Evol. 9, 542-551.

Havill, N.P., Foottit, R.G. & von Dohlen, C.D. (2007) Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. Mol. Phyogenet. Evol. 44, 357-370.

Havill, N.P. & Foottit, R.G. (2007) Biology and evolution of Adelgidae. Annu. Rev. Entomol. 52, 325-349.

Heie, O.E. (1987) Paleontology and phylogeny. In *Aphids: Their Biology, Natural Enemies and Control*, ed. Minks A.K. & Harrewijn P., pp. 367–91. Amsterdam: Elsevier.

Heinze, K. (1962) Pflanzenschädliche Blattläusarten der Familien Lachnidae, Adelgidae und Phylloxeridae, eine systematisch faunistische Studie. Dt. Entomol. Z. 9, 143–227.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen D.H. & Hallwachs W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator.* Proc Nat Acad Scien US 101, 14812–14817.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lon B 270, 596–S599.

Inouye, M. (1945) Monographische Studie über die japanischen Koniferen-Gallenläuse (Adelgidae). Bull. Sapporo Bran. Gov. For. Exp. Stn. 15: 1–91.

Jahn, E. & Sinreich, A., (1961) *Verbreitung der Tannentrieblaus Dreyfusia Nüsslini C.B. in Österreich und neue Methoden der Bekämpfung.* Anz. F. Schädlingskunde 34, 97-102.

Jermiin, L. & Crozier, R. H. (1994) The cytochrome b region in the mitochondrial DNA of the ant *Teraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J. Mol. Evol., 38, 282-294.

Ji, Y.J., Zhang, D.X. & He, L.J. (2003) Evolutionary conservation and versatility of a new set of primers for amplifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. Mol Ecol 38, 282-294.

Juan, C., Oromi, P. & Hewitt, G.M. (1995) Mitochondrial DNA phylogeny and sequential colonization of Canary Islands by darkling beetles of the genus Pimelia (Tenebrionidae) Proc Royal Soc Lond B 261, 173-180.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111-20.

Kitzberger, P. (1995) Tannentrieblaus (*Dreyfusia nordmannianae* Eckst. 1890), präventiver Waldschutz auf waldbaulich- ökologischer Grundlage zur Vermeidung von Schäden an der Tannenverjüngung- Phd. Thesis, Institute of Forest Entomology, Forest Pathology and Forest Protection, University of Natural Sciences and Life Sciences, Vienna.

Kotschy, K. (1960) Untersuchung über die Tannentrieblaus (*D. nüsslini* C.B.). Cbl. Ges. Forstw. 77, 127-155.

Kotschy, K. (1991) Ist heute ein Insektizideinsatz im Wald noch vertretbar? Österr. Forstztg. 102, 64-65.

Lampel, G. (1968) *Die Biologie des Blattlaus-Generationswechsels*. Gustav Fischer, Jena, 264 pp.

Lindo, Z., Clayton, M. & Behan-Pelletier, V. M. (2008) Systematics and ecology of nachipteria geminus sp. nov. (Acari: Oribatida: Achipteriidae) from arboreal lichens in western North America. Can. Entomol. 140, 539-556.

Lunt, D. H., Zhang, D.-X., Szymura, J. M. & Hewitt, G. M. (1996) The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol. Biol. 5, 153-165.

Mantovani, B., Francardi, V., Binazzi, A. & Leccese, A. (2001) A molecular approach to differentiate the species of Dreyfusia Börner occurring in Italy (Aphidoidea, Adelgidae). Reddia 84, 151–159.

Montgomery, M. E., Shiyake, S., Havill, N.P. & Leschen, R.A.B. (2011) A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and Introduced Congeners in North America. Annal Entomol Soc Am 104, 389-401.

Moore, W. S. (1995) Inferring Phylogenies from mtDNA Variation: Mitochondrial-Gene Trees Versus Nuclear-Gene Trees. Evol 49, 718–729.

Normark, B. B. (1996) Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. Evol 50, 734-745.

Ravn, H.P., Havill, N.P., Akbulut, S., Foottit, R.G., Serin, M., Erdem, M., Mutun, S. & Kenis, M. (2012) *Dreyfusia nordmannianae* in Northern and Central Europe: potential for biological control and comments on it's taxonomy. J. Appl. Entomol. 137, 401-417.

Saitou, N., Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) *Molecular Cloning a Laboratory Manual*. 2nd edn. Cold Spring Harbour Laboratory Press,New York.

Schatz, H. (1979) Über Ernährungsbiologie von Oribatiden (Acari) im Hochgebirge (Obergurgl, Tirol). Berichte der naturwissenschaftlich-medizinischen Vereinigung Innsbruck, 66, S. 7-20

Schimitschek, E. (1952) Zum Massenauftreten der Tannentrieblaus Dreyfusia Nüsslini CB in Österreich. Der land- u. forstwirtsch. Gutsbetr 7, 113-116.

Shearer, T.L. & Cofforth, M.A. (2008) Barcoding corals: limited by interspecific divergence, not intraspecific variation. Mol. Ecol. Res. 8, 247–255.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, phylogenetic utility of mitochondrial gene sequences and compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Stefan, A.W. (1961) Die Stammes- und Siedlungsgeschichte des Artenkreises *Sacchiphantes viridis* (Ratzeburg, 1843) (Adelgidae, Aphidoidea). Zoologica 109, 1–113.

Stefan, A.W. (1968) Evolution und Systematik der Adelgidae (Homoptera: Aphidina): Eine Verwandtschaftsanalyse auf vorwiegend ethologischer, zytologischer und karyologischer Grundlage. Zoologica 115, 1–139.

Stefan, A.W. (1972) Unterordnung Aphidina, Blattläuse. In Schwenke W. (ed.): *Die Forstschädlinge Europas. Ein Handbuch in fünf Bänden.* Parey, Hamburg, pp. 162–386.

Stefan, AW. (1976) Evolution of morphological characters and of endosymbionts in the aphid family Adelgidae (Homoptera: Aphidina). Verh. Dtsch. Zool. Gesell. 69, 232.

Stern, D.L. (1994) A phylogenetic analysis of soldier evolution in the aphid family Hormaphididae. Proc Roy Soc Lon B 256, 203-209.

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10, 512-526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30, 2725-2729.

Tautz, D., Arctander, P., Minelli, A., Thomas, R.H. & Vogler, A.P. (2003) A plea for DNA taxonomy. Trends Ecol. Evol. 18, 70–74.

Thompson, J.D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res. 24, 4876-4882.

Vences, M., Thomas, M., Bonett, R.M. & Vietes, D.R. (2005) Deciphering amphibian diversity through DNA barcoding: chances and challenges. Phil. Trans. R. Soc. B 360, 1859–1868.

Perny, B. (2005) Schädlinge an Trieben von Tannen. Forstschutz Aktuell, Wien, (34), 33-34.

Zilahi-Balogh, G. M. G, Kok, L. T. & Salom, S. M. (2005) A predator case history: Laricobius nigrinus, a derodontid beetle introduced against the hemlock woolly adelgid. In: *Second International Symposium on Biological Control of Arthropods*. Davos, Switzerland. 651-64.

Žurovcova, M., Havelka, J., Vechtova, P., Chundelová, D., Jarosova, A. & Kucerova, L. (2010) DNA barcoding is of limited value for identifying adelgids (Hemiptera: Adelgidae) but supports traditional morphological taxonomy. Eur. J. Entomol. 107, 147–156.

6. Appendix

Appendix I – DNA extraction

- Extraction after SIGMA kit
  - pipette 180 µl of lysis solution T (B-6678) in a 1,5 ml Eppendorf tube
  - add the insect specimen and mince with the ball mill – put the samples on ice
  - add 20 µl of proteinase K
  - vortex (ca. 15 sec.) and put on the heating block at 55°C/450 rpm for 2 hrs
  - add 20 µl RNase and let tubes stand for 2 min at room temperature
  - add 200 µl of lysis solution C (B-8803)
  - vortex carefully and incubate at 70 °C for 10 min
  - during incubation prepare the tubes and columns - add 500 µl column preparation
  - Solution to the column and spin at 13.000 rpm for 1 minute
  - discard flow- through and put column back into the same tube
  - add 200 µl absolute ethanol to the sample
  - vortex for 15 sec
  - transfer the samples to the binding columns (approx. 650 µl)
  - spin at 8.000 rpm for 1 min
  - discard tube with flow-through and put column in a fresh tube
  - add 500 µl of wash solution
  - spin at 8.000 rpm for one minute
  - discard flow-through and put column back into the same tube
  - add 500 µl wash solution
  - spin at 13.000 rpm for 3 minutes

- discard flow-through and put column back into the same tube
- spin again for 1 min at 13.000 rpm to get rid of any remaining alcohol
- put column in a fresh tube
- add 50 µl of elution solution and let column stand for 5 minutes
- spin at 8.000 rpm for 1 min
- store DNA in the fridge

Appendix II – Cloning

- Day 1
  - 2,4 µl mix PCR product with 1,6 µl H2O, 0,3 µl T4
  - Buffer, 0,2 µl ptZ57R (Vector) and 0,1 µl T4 ligase
  - add 0,6 µl DNA
  - incubate over night

- Day 2
  - Prepare hybrid oven at 37 °C and H2O-bath at 42 °C
  - thaw competent cells on ice for 20 min and put Ligation-mixes on ice
  - add 50 µl competent cells per reaction
  - incubate on ice for 20 min
  - heat shock bacterial suspensions in a 42 °C hot water bath for 50 sec
  - put reactions back on ice for 1-2 min immediately
  - add 900 µl of SOC medium were added to each tube
  - incubate at 37 °C for 60 min in hybrid oven
  - prepare LB-Amp plates in the meantime: plate 40 µl X-Gal (20 mg/ml) and 40 µl IPTG (24 mg/ml) on each plate with a Drigalski spatula
  - centrifugation at 4 °C for 5min
  - plate transformation reactions on the plates
  - incubate upside down over night at 37 °C

- Day 3
  - 2 ml tubes containing LB-Broth and ampicillin were prepared
  - 8 colonies/sample were chosen
  - tip with a sterile toothpick
  - transfer into Eppendorf tubes (containing master mix for PCR) and tubes containing 2 ml LB broth and ampicillin.
  - vials were incubated at 37 °C overnight under vigorous shaking (180 – 200 rpm).

- Day 4
  - transfer 0.5 ml up to 2 ml of overnight E. coli cultures into 1.5 ml reaction tubes.
  - pellet cells by centrifugation: 10.000 rpm, 4 min
  - discard supernatant and re-suspend pellets in 100 µl re suspension solution
  - add 1µl RNAse
  - incubate for 2-5 min
  - add 200 µl NaOH-SDS
  - vortex at 1.400 rpm
  - add 150 µl ice cold Kac-solution, vortex 10 sec
  - put samples 5 min on ice
  - centrifuge 5 min on 4°C at 15.000 rpm
  - pipette supernatant in a new tube
  - add 900 µl EtOH and vortex carefully
  - incubate for 2 min and centrifuge for 5 min at 4°C on 15.000 rpm
  - discard flow-through and dry the tube
  - add cold 70% EtOH vortex carefully and centrifuge for 5 min at 4°C on 15.000
  - rpm
  - discard ethanol and air-dry pellets for approximately 2 hours
  - re-suspend pellet in 10 mM Tris

LB broth (Sambrook et al. 1989)

	g/l
Bacto-tryptone	10
Bacto-yeast Extract	5
NaCl	10
Agar	15

Adjust the pH to 7,0 with 5N NaOH.

Appendix III – PCR programmes of diverse primers

Appendix III.1 Fermentas Taq-Polymerase and the chemicals used.

Advanced PCR

Anzahl der Reaktionen:	5
Anzahl der Primer:	2
Template pro Reaktion (µl):	2,00
MgCl2 - Stammlösung	25 mM

Primer-Endkonzentration µM	0,5
Mg-Endkonzentration mM	2,5
dNTP-Endkonz je .. µM	100

Reaktionsmaßstab	**10 µl**		**15 µl**		**20 µl**	
	1 Rk	**Master**	**1 Rk**	**Master**	**1 Rk**	**Master**
H2O dest.	4,40	22,00	7,58	37,90	10,80	54,00
10 x PCR Buffer	1,00	5,00	1,50	7,50	2,00	10,00
MgCl2	1,00	5,00	1,50	7,50	2,00	10,00
dNTPs, Mix zu je 2.5 mM	0,40	2,00	0,60	3,00	0,80	4,00
Primer 10 µM, jeweils	0,50	2,50	0,75	3,75	1,00	5,00
Polymerase	0,20	1,00	0,32	1,60	0,40	2,00
Template	2,00					
Gesamtvolumen	10,00	50,00	15,00	75,00	20,00	100,00

Appendix III.2 peqlab Taq-Polymerase and the chemicals used.

all inklusiv peqlab taq	
Ansatz µl:	20
Anzahl Primer:	2
Primerkonzentration:	0,3
Anzahl Proben:	5
Negativkontrolle:	1
Positivkontrolle:	1

Reaktionsmaßstab [µl]	20	
	1 RK	Master
H2O [µl]	12,2	85,4
BSA [µl]	2	14
Y-Puffer [µl]	2	14
dNTPs [µl]	0,4	2,8
Primer je [µl]	0,6	4,2
taq-Polymerase [µl]	0,2	1,4
Mix [µl]	18	126
DNA [µl]	2	2

Appendix IV – Sequences retrieved from the Genbank

Appendix IV.1 Sequences from the Genbank used for the NJ Tree based on the barcode primer LCO 1490 and HCO2198 (Folmer et al., 1994) without cloning

Sequence	Sequence ID:
NORDMANNIANAE1	HQ668156
NORDMANNIANAE2	FJ502448
NORDMANNIANAE3	FJ502447
NORDMANNIANAE4	FJ502456
NORDMANNIANAE5	JX267030
NORDMANNIANAE6	EF073080
PRELLI1	GU571030
PRELLI2	GU571033
PICEAE1	FJ502471
PICEAE2	EF073086
PICEAE3	JX267033
PICEAE4	FJ502473
PICEAE5	FJ502469
GILLETTEELLA1	GU571040
GILLETTEELLA2	GU571048
APHRASTASIA	GU571012

Appendix IV.2 Sequences from the Genbank used for the NJ Tree based on the barcode primer LCO 1490 and HCO2198 (Folmer et al., 1994) after cloning

Sequence	Sequence ID:
NORDMANNIANAE1	JX267030
NORDMANNIANAE2	FJ502448
NORDMANNIANAE3	FJ502447
NORDMANNIANAE4	EF073080
PICEAE1	JX267041
PICEAE2	FJ502471
PICEAE3	EF073086
GILLETTEELLA1	GU571040
GILLETTEELLA2	GU571048
APHRASTASIA	GU571012

Appendix IV.3 Sequences from the Genbank used for the NJ Tree based on the primer pair CP1 and CP2 (Harry et al., 1998) amplifying on cytochrome b 3´ end region.

Sequence	Sequence ID:
APHIS1	HQ528293.1
APHIS2	GU324595.1
APHIS3	HQ528290.1
APHIS4	HM062865.1
PICEAE1	EF073195.1
PICEAE2	EF073194.1
PICEAE3	JX267018.1
PICEAE4	JX267017.1
NORDMANNIANAE1	EF073190.1
AULACORTHUM1	FJ752016.1
HYALOPTERUS1	GU457815.1
UROLEUCON1	HQ528302.1
GILLETTEELLA1	GU571040
GILLETTEELLA2	GU571048
APHRASTASIA	GU571012

Appendix V - Index of abbreviations

A	Adenosine
BLAST	Basic Local Alignment Search Tool
BSA	Bis(trimethylsilyl)acetamid (*BSA*)
Bp	Base pairs
C	Cytosine
° C	Degree Celsius
DNA	Deoxyribonucleic acid
dNTP	2´-deoxyribonucleoside-5´-triphosphate
E.coli	Escherichia coli
G	Guanosine
LB	Lysogeny broth
M	Molar
m	Milli
MEGA	Molecular evolutionary genetics analysis
mg	Miligramm
Mg	Magnesium
MgCl2	Magnesium chloride
Min	Minute(s)
MP	Maximum Parsimony
NJ	Neighbor-Joining
PCR	Polymerase chain reaction
RNase	Ribonuclease
rpm	Rounds per minute
sec	second(s)
T	Thymidine
T_m	Temperature
Taq	*Thermus aquaticus*
tRNA	Transfer Ribonucleic
U	Unit
UV	Ultraviolet (light)
µ	Micro
µl	Microliter
µm	Micrometer
X-Gal	5-bromo-4-chloro-3-indolyl- β-D-Galactopyranoside

Appendix V – Curriculum Viatae

GERHARD FRANZ BLABENSTEINER

Lohn 18 • 3633 Schönbach • Tel.0664/4512105

Personal data:

Date of Birth	April 30, 1986
Place of Birth	Zwettl, Austria
Citizenship	Austria
Cell	0043664/4512105
Email	g.blabensteiner@hotmail.com

Education:

Since April 2013	Masters programme *Forestry*, BOKU Vienna
March 2013	Graduation of Bachelor's degree *Forestry*, BOKU Vienna
October 2008	Bachelor programme *Forestry*, BOKU Vienna
2005 – 2008	Vocational Matriculation Examination Horn/NÖ
2000 – 2004	Business School Zwettl/NÖ
1996 - 2000	Secondary Modern School Schönbach/NÖ
1992 – 1996	Primary School Schönbach/NÖ

Bachelor and Master Thesis:

Bachelor Thesis:

- *„Untersuchung der Nadelmasse in einem Fichten Kiefern Mischbestand"*
- *„ALS Airborne Laserscanning Daten zur Erfassung und Beurteilung forstlicher Erschließungssysteme"*

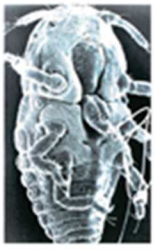
Master Thesis

- *„DNA Barcoding of Dreyfusia nordmannianae"*

Appendix VI – Poster of the Masterseminar 06.05.2014

Taxonomie und Endosymbionten von der Tannentrieblaus

Gerhard Blabensteiner, Fisnik Batusha
BetreuerInnen: Christian Stauffer, Susanne Krumböck



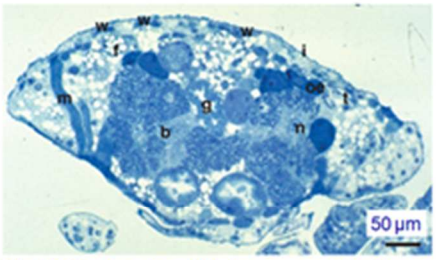
Kitzberger (1995)

Biologie

Um 1840 wurde die Tannentrieblaus, *Dreyfusia nordmannianae*, vom Kaukasus nach Mitteleuropa eingeschleppt. Im Kaukasus vollzieht sie einen zweijährigen Holozyklus mit Wirtswechsel zwischen dem Primärwirt *Picea orientalis* und dem Sekundärwirt *Abies nordmanniana*. In Europa lebt *D. nordmannianae* rein anholozyklisch-parthenogenetisch auf *Abies alba*. Das Insekt saugt vor allem an sonnenexponierten Trieben. Dies kann zum Absterben von Jungtannen führen.

Endosymbiose

Ein Beispiel für Endosymbiose ist die mutualistische Beziehung zwischen Tannentrieblaus und den Proteobakterien, *Candidatus Steffania adelgidicola und Candidatus Ecksteinia*, welche in Bakteriozyten leben. Dabei beeinflussen die Endosymbionten deren Wirt in der Ernährung, Reproduktion und Abwehr gegen Umweltstress und Parasitoide.



Die Bakteriozyten (b) der Tannentrieblaus
Toenshoff et al. ISME J 2012

Verwandtschaftsverhältnisse von *Dreyfusia*
Zurovcova et al. JAE 2010

Taxonomie - Barcoding

Morphologisch sind die Arten innerhalb der Gattung *Dreyfusia* nicht eindeutig definiert. Bei DNA Barcoding wird die Abfolge der Basenpaare einer DNA-Sequenz eines bestimmten Gens zur Bestimmung einer Art verwendet. Jedoch führte diese Methode bisher bei den Arten der Gattung *Dreyfusia* zu keiner klaren taxonomischen Abgrenzung.

Arbeiten:

- Analyse der Endosymbionten
- Barcoding mit polymorpheren Genen

Danksagung:

FWF

Institute of Entomology
Biology Centre AS CR, v. v. i.