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UNIVERSITY HONORS PROGRAM

SENIOR PROJECT - APPROVAL

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PROJECT TITLE: Classification, biogeography and phylogeny of Northern Hemisphere *Lentinellus* species

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I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: Karen W Hughes, Faculty Mentor

Date: May 7, 1999

Comments (Optional):

Classification, biogeography and phylogeny of Northern Hemisphere *Lentinellus* species

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Introduction

Lentinellus is a large genus of Basidiomycete fungi consisting of approximately 15 known species. In the temperate forests of Eurasia and North America, there are at least six species, *L. ursinus*, *L. vulpinus*, *L. angustifolius*, *L. omphalodes*, *L. montanus*, and *L. micheneri* but these species are poorly delineated based on morphology. Their range and distribution is also unknown.

The purpose of these studies was to develop diagnostic molecular characters that delineate species, to determine the geographical distribution of each species, to determine if gene exchange is occurring between geographically separate populations within species and to develop a phylogeny based on molecular characters. Species delineated by molecular methods were compared with the morphological species and biological species to determine the cohesiveness of these concepts. There seems to be a second biological species sheltered under *Lentinellus omphalodes* and this unnamed taxon was also examined.

During these studies, a type I intron was identified within the ribosomal 18S gene. A type I intron is a mobile, self-replicating piece of DNA. The intron is transcribed but not translated. The length of this intron is about 350 base pairs long. The presence or absence of this intron was determined for all species and collections and the phylogenetic relationships of the intron and *Lentinellus* species (based on the ribosomal ITS sequence) were compared.

History of the Genus

Many of the species currently in *Lentinellus* were originally placed in *Agaricus* (Fries 1821), then later, segregated these species into the genus *Lentinus* (Fries 1863). Karsten established *Lentinellus* over 100 years ago with the species *L. cochleatus, L. friabilis, L. omphalodes, L. umbellatus* with type specimens from France (Karsten 1879). Into this genus, he placed taxa previously included in *Lentinus,* which were originally placed in the genus *Agaricus*. At the same time as Karsten proposed *Lentinellus*, he also proposed *Hemicybe* that included some other taxa from *Lentinus* (Karsten 1879).

Lentinellus ursinus was discovered in 1821 by Fries and was first called *Agaricus ursinus* (Fries 1821). In 1825, it was moved to *Lentinus* and renamed *Lentinus ursinus* by Fries (Fries 1825). Karsten moved the species to *Hemicybe* and renamed it *Hemicybe ursina* in 1879 (Karsten 1879). According to Miller, in 1915, Murrill moved the species to *Panellus* and renamed it *Panellus ursinus* (Miller 1997). According to Miller and Stewart (Miller 1971), the species was transferred to *Lentinellus* in 1926 and renamed *Lentinellus ursinus* by Kuhner.

Lentinellus ursinus has also been called *Lentinellus castoreus* in 1946 (Romagnesi 1946). Miller and Stewart decided in 1971 that *Lentinellus castoreus* was the European version of the North American *Lentinellus ursinus* (Miller 1971). Fries had added the species to *Lentinus* in 1838 *as Lentinus castoreus* (Fries 1836-1838). Karsten moved *Lentinus castoreus* in 1879 to *Hemicybe* and renamed *Hemicybe castorea* (Karsten 1879). According to Miller, (Miller 1997), the species was placed in *Lentinellus* under the new name of *Lentinellus castoreus* in 1936 by Konrad and Maublanc.

According to Miller and Stewart, (Miller 1971), *Lentinellus ursinus* has also been called *Lentinus anastomosans* in 1938 by Rick. *Lentinellus ursinus* has also been labeled *Lentinus hepatotrichus* by Berkeley in 1860 (Berkeley 1860). In 1880, Kalchbrenner placed *Lentinellus ursinus* in *Lentinus hyracinus* (Kalchbrenner 1879-1880). Romagnesi christened the species as *Lentinellus pusio* in 1965 (Romagnesi 1965).

Lentinellus angustifolius was originally placed in *Lentinus* by Romell in 1901 as *Lentinus angustifolius* (Romell 1901). In 1952 Singer transferred the species to *Lentinellus* as *Lentinellus angustifolius* .

In 1803 *Lentinellus vulpinus* was classified as *Agaricus vulpinus* by Sow (Sow 1803). In 1821 the species was reconfirmed by Sow as being *Agaricus* (Sow 1821). According to Miller, in 1836, it was moved to *Lentinus* under the name *Lentinus vulpinus* (Miller 1997). It was transferred to *Hemicybe* by Karsten in 1879 (Karsten 1879). According to Miller, (Miller 1997), Murrill moved the species to *Panellus* in 1915. In 1934, the species was moved to *Lentinellus* by Kuhner and Maire (Kuhner 1934). Stalpers does not recognize the move to *Panellus* as being legitimate (Stalpers 1996).

Other names for *Lentinellus vulpinus* according to Miller were *Lentinus auricula* given in 1861 by Fries (Fries 1863), *Lentinus hygrophanus* given by Harz in 1889 and *Lentinus tomentellus* given by Karsten in 1887 (Miller 1997). The species was also classified as *Hemicybe tomentella* by Karsten in *Hemicybe* in 1889 (Miller 1997).

Lentinellus omphalodes was established by Karsten in 1879 (Karsten 1879). Before the establishment of this genus it was called *Lentinus omphalodes* by Fries since 1863 (Fries 1863).

Lentinellus cochleatus has also been called *Agaricus cornucopioides* (Bolton 1788), *Agaricus cochleatus* (Fries 1821), *Lentinus cochleatus* (Fries 1836-1838), according to Stalpers *Lentinus umbellatus* by Peck in 1876 (Stalpers 1996), *Clavicorona dryophila* (Maas 1976), *Lentinellus umbellatus* , and *Lentinellus cornucopioides* by Murrill (Miller 1997). It was finally transferred to *L. cochleatus* in 1971 by Miller and Stewart (Miller 1971). Miller and Stewart do not recognize the transfer to *C. dryophila* as legitimate (Miller 1971).

Lentinellus micheneri has also been known as *Agaricus dentatus* (Fries 1821) and *Lentinus omphalodes* (Fries 1863) before it was established as a separate species (Miller 1971).

According to Stalpers, *Lentinellus flabelliformis* has also been called *Claudopus subargillaceus* by Kauffm, *Lentinus scoticus* by Berkeley and Br., *Lentinus bisus* by Quel., and *Lentinus americanus* by Peck (Stalpers 1996).

Lentinellus montanus is a new species that was discovered and named by O.K. Miller in 1965 (Miller 1965).

Methods and Materials

Collections

Collections used in this study are given in Figure 1

DNA Extraction/Preparation

Cultures were maintained on Potato Dextrose, PD, agar slants at 4 C until ready for use. A portion of the culture was removed and half of the removed portion was put into liquid Potato Dextrose Media and half was transferred to a new Potato Dextrose agar slant tube. Both of these were incubated at 27°C for three weeks. At the end of three weeks the slant tube was returned to cold room storage if it had not become contaminated. The liquid culture was drained and the tissue was pressed to remove as much media as possible. The tissue was weighed, and 0.3-0.4g of tissue was removed from the culture tissue for DNA extraction. Carlson-Lysis buffer (Carlson et al 1991) and β-mercaptaethanol were heated to 74°C. The fungal tissue was ground with sterile sand and the hot Carlson-Lysis buffer and incubated at 74°C for one hour with inversion every 10 minutes. Cell debris and sand were sedimented by centrifugation for 10 minutes at 10,000 rpm. The supernatant was removed and chloroform added to precipitate proteins and polysaccharides while leaving the DNA in suspension. After shaking, the sample was centrifuged to separate the DNA from the proteins and polysaccharides. The top level was removed, being careful not to remove polysaccharides with it. Isopropanol was added to precipitate the DNA and the sample was incubated at room temperature for 30 minutes. The DNA was pelleted at the bottom of the tube by 10 minutes of centrifugation. DNA was washed off the sides with ice-cold ethanol to remove the isopropanol. The sample was centrifuged for 10 minutes to pellet the rest of the DNA. The pellet was dried and resuspended in TE buffer. The DNA was ready for further analysis.

PCR Amplification

DNA extracted from cultured tissues was used as a substrate for Polymerase Chain Reaction (PCR) amplification of the Internal Transcribed Spacer Region (ITS) between the 18 S ribosomal subunit gene and the large ribosomal subunit gene. This area is divergent enough to compare species within the same genus. The standard PCR reaction contained the following ingredients at the specified amounts:

ddH20	27.75µl
10X $MgCl_2$ Free Buffer	5µl
$MgCl_2$ 25mM	6µl
dNTP mix 10µM each	8µl

ITS4 primer	1μl
ITS5 primer	1μl
Taq polymerase	0.25μl
DNA	1μl

The thermocycler used was an Ericomp Single Block™ System in the Easy Cycler™ Series. Cycle times: Heat at 94°C for 3 minutes. Thirty-five cycles of one minute at 94°C, one minute at 52°C, and one minute at 72°C. Three minutes at 72°C. Store at 4°C when finished. PCR products were electrophoresed on a 1.5% agarose gel in TBE buffer to determine if amplification occurred.

When the DNA had been frozen for long periods of time, it would not amplify under standard conditions. To overcome this problem, Eppendorf made a Taq Enhancer that enables Taq polymerase to remain attached to the DNA strands for longer periods of time. With the addition of 20% Taq Enhancer, the PCR reaction proceeded and amplification occurred. The reaction mix for the PCR reaction with the Taq Enhancer was as follows:

ddH20	20.75μl
10X Buffer with 15 mM $MgCl_2$	5μl
$MgCl_2$ 25 mM	3μl
dNTP mix 10μM each	8μl
Taq Enhancer heated to 65°C	10μl
ITS4 primer	1μl
ITS5 primer	1μl
Taq polymerase	0.25μl
DNA	1μl

PCR products were electrophoresed on a 1.5% agarose gel in TBE buffer to determine if amplification occurred.

The primers used throughout the standard PCR reactions are ITS-4 and ITS-5 primer,. ITS-4 primer is a reverse primer that runs from the large ribosomal subunit gene into the internally transcribed spacer region. ITS-5 primer is a forward primer that starts in the 18 S ribosomal subunit gene and runs to the large ribosomal subunit gene. (Diagram of ITS area is given in Figure 2. The sequence of the ITS-4 primer is TCCTCCGCTTATGATATGC (White et al 1990). The sequence of the ITS-5 primer is GGAAGTAAAAGTCGTAACAAGG (Whiteet al 1990). These primers are also used for the sequencing of this region.

PCR Amplification of part of the 18S ribosomal DNA.

In the survey of the collections of *Lentinellus*, a portion of the 18S ribosomal RNA gene was PCR amplified to determine if the Group I Intron was present. Amplification of part of the 18S ribosomal RNA gene was accomplished using the primers SR1c and NS6. SR1c is a forward primer of the sequence, AGCAGCCGCGGTAA, (Hibbett 1992), while NS6 is the reverse primer that has a sequence of GCATCACAGACCTGTTATTGCCTC, (White et al 1990).

The thermocycler used was an Ericomp Single Block™ System in the Easy Cycler™ Series. Cycle times: Heat at 94°C for 4 minutes. Thirty cycles of thirty seconds at 94°C, thirty seconds at 60°C, and two minutes at 72°C. Three minutes at 72°C. Store at 4C when finished. PCR reaction mix:

ddH20	30.75µl
10X MgCl2 Free Buffer	5µl
MgCl2	8µl
dNTP mix 10µM each	3µl
SR1c primer	1µl
NS6 primer	1µl
Taq polymerase	0.25µl
DNA	1µl

Reaction mixes were electrophoresed on a 1.5% agarose gel in TBE buffer to determine if amplification occurred. *Hae* III – digested *Phi* X was used as a molecular weight marker.

RFLP Analysis

ITS sequences of exemplars of 10 species of *Lentinellus* were examined to identify sequence differences that were diagnostic of each species. Restriction enzymes that recognized these differences were identified using the 'map' program in GCG and 'Rebase' (http://www.neb.com/rebase/rebase.html) to determine if the enzymes were commercially available. The PCR Products were digested according to manufacturer's directions as follows. Restriction Digest mix:

ddH20	4µl
10X Buffer	1µl
DNA	4µl
Enzyme	1µl

Samples were incubated at optimal digestion temperatures for each enzyme. The sample mixes were electrophoresed on 1.5% agarose gel in TBE buffer to determine the length of the restriction fragments.

Purification of the PCR Product and Sequencing

Four 50 µl PCR products were combined and electrophoresed on a 1.5% Nuseive GTG low melting temperature agarose gel with TAE buffer, and ethidium bromide. This separated the strands based on size. The dominant band at ~700 base pairs was excised with a sterile scalpel and placed in a microcentrifuge tube and heated to 70°C until all the agarose is melted. Using Quiagen's Wizard Purification protocol, the PCR product is separated from the agarose and suspended in 70°C water so that it can be sequenced. The PCR product is amplified again with dideoxynucleotides using the ITS-4 and ITS-5 primers. The machine used in the Biology Sequencing Service Facility at the University of Tennessee is an ABI automated sequencer. The

ABI automated sequencer produces electropherograms of the amount of color tags that appear at each position.

Aligning Sequences

The sequences using the ITS-5 primer and the ITS-4 primer were automatically compared to each other using the 'gap' sub-program of the GCG program. The sequences were manually corrected based on the electropherograms from the automated sequencer. The ITS1-5.8S-ITS2 DNA sequences of multiple isolates were compared using 'pileup' and 'lineup' sub-programs in GCG (Genetics Cooperative Group). 'Lineup' incorporated multiple sequences while 'Pileup' did an initial alignment. The initial alignment was manually corrected using SeqLab in the GCG program.

Estimating a Phylogeny

The pileup file, **.msf, was adapted to work within the PHYLP program. Phylogenies were estimated using Neighbor- Joining and the strength of the branches was examined by Bootstrapping. The Neighbor-Joining program uses a Jukes-Cantor distance matrix to determine evolutionary relationships. Bootstrapping does 100 random replacements to evaluate the strength of the branches. For example, if one base replacement changes the whole topology of the tree, the branch supporting that area of the tree is very weak.

Results

Phylogeny of Lentinellus species based on sequences of the ribosomal ITS region

Aligned ITS sequences are given in Fig.3 for exemplars of each of the Lentinellus species in this study. Phylogenetic analyses (Figures 4-6) show that *L. vulpinus* and *L. cochleatus* form a single clade, differing from each other by 72 base pairs. The two collections of *L. ursinus* formed a second distinct clade. Two collections of *L. angustifolius* from the U.S. Southeast, formed a third clade and had identical sequences. *L. omphalodes* (Mating group IX), *L. montanus* and *L. micheneri* form a closely related group but another specimen identified morphologically as *L. omphalodes* (collection 9981) formed a separate clade. This collection also did not mate with *L. omphalodes* (R. H. Petersen, Pers. Com.) and is probably a new species.

Neighbor-Joining and Parsimony analyses are two different ways to group isolates. Neighbor-Joining analysis groups according to overall similarity, not according to evolutionary relationships. This method is acceptable because generally isolates that are the most similar to each other are usually the most closely related. Parsimony analysis groups according to evolutionary relationships. According to the phylogenetic trees generated with Parsimony and Neighbor-Joining analyses, the overall topology of the trees were very similar. The only difference came from the placement of collection 9981. In the Neighbor-Joining tree 9981 was in the same clade with collection 8452 from Mexico. In the Parsimony tree, collections 9981 and 8452 are not in the same clade (Fig 5 and 6). It is not known at the present time if these are the same biological species according to mating studies.

Restriction Length Fragment Polymorphisms (RFLP)

Specific restriction enzymes were identified that separated the different species of Lentinellus. *Eco* RI separated *L. ursinus* from all other *Lentinellus* species in this study, Figures 3 and 7, however, within *L. ursinus*, there were five collections that did not show the typical *L. ursinus Eco* RI RFLP pattern, Figure 1. Comparison of *L. ursinus* collections 2210 and 9986 showed that the loss of a restriction site was due to a single base pair substitution in which 9986 lost an adenosine base in the recognition site of the enzyme.

*Taq*I separates *L. angustifolius* and *L. vulpinus* from all other Lentinellus species, however, these two species are not phylogenetically related and this similarity apparently represents convergent evolution, Figure 3. Other restriction enzymes were used to try to distinguish between species but these were not species-specific (*Hpa* II, *Hinf* I, *Cla* I and *Rsa* I).

Group I Intron

Results of PCR amplifications to determine the presence or absence of a group I intron are given in Figure 1. The Group I Intron in the 18S ribosomal DNA (Diagram of Group I Intron Figure 8) seems to have a geographic and species distribution. The Group I Intron occurs most frequently in the Southern United States around the Appalachian Mountains as can be seen in Figures 9-16. It occurs uniformly in *L. omphalodes* (Mating group IX), *L. micheneri* and *L. omphalodes* (Mating group VIII). It appears to be variable in *L. angustifolius*. Thus far the Group I Intron has not been found in *L. ursinus*. The phylogeny of the Group I Intron is similar to the phylogeny of the ITS region of the isolates containing the Group I Intron as can be seen in Figure 17.

Analysis of Placements

Lentinellus is closely related to the genera, *Clavicorona* and *Panellus*. At different times in history, there has been much debate about whether some *Lentinellus* species belong to these genera. The placement of *L. vulpinus* into the genus *Panellus* was not justified as shown by the alignment between 7996 and a *Panellus* isolate. The comparison between a *Panellus* isolate and *L. vulpinus* isolate can be seen in Figure 18. There is little similarity between the isolates. This data supports the stand taken by Stalpers that this species belongs in *Lentinellus* . The placement of *L. cochleatus* in the *Clavicorona* genus was not justified as shown by the alignment of 9985 and a *Clavicorona* isolate. The comparison between the *Clavicorona* and *L. cochleatus* can be seen in Figure 19. There is little similarity between these isolates. This data supports placement of this species in *Lentinellus* by Miller and Stewart .

Conclusions

Phylogenetic trees generated by neighbor-joining and parsimony analysis correspond well to the mating study data. Mating groups correspond to clades identified by phylogenetic analysis (Fig. 1 and Fig. 5). Branch lengths suggest that *L. vulpinus* and *L ursinus* are well separated from the remainder of the *Lentinellus* species and from each other. *L. omphalodes*

(Mating group IX), *L. montanus* and *L. micheneri* are closely related but still form separate clades and thus the separation of the latter two from *L. omphalodes* is justified. An unexpected finding was the separation of *L. omphalodes* (Mating group VIII) into a distinct clade associated with *L. sp.* from Mexico. This suggests that morphology was conserved or was convergent but that these are indeed separate species.

The biogeography of many of the species was previously unknown. The *L. ursinus* clade groups the two isolates from NC and SC. The mating study data indicated that the biological species was definitely cosmopolitan in its range. Samples from Russia, Sweden, Mexico and the United States confirmed this wide geographic distribution. The *L. angustifolius* clade groups two isolates that are very similar in sequence and are able to mate with each other. The isolates come from different areas within the southeast. The mating study data indicated that the biological species was cosmopolitan in its range. Collections from Russia, Austria, Costa Rica, Australia, and the United States confirmed this wide geographic distribution. The *L. cochleatus-L. vulpinus* clade occupies a boreal forest climate from MN and Austria. The *L. montanus-L. omphalodes* clade occupies a northern United States distribution. *L. montanus* according to Miller has a geographical range from Montana to Washington to Oregon (Miller, 1965). In the *Lentinellus omphalodes* complex (*L. omphalodes* mating group IX, *L. micheneri*, *L. montanus* and *L. omphalodes* Mating group VII), clades corresponding to the following geographic areas; boreal forests of North America and Europe, the TN/NC area, and global Northern Hemisphere. *Lentinellus omphalodes* inhabits a northern boreal forest climate with a short growing season and cool summers. The collections from Finland, Russia, Sweden and Alaska confirmed the northern boreal forest distribution of *L. omphalodes*. *Lentinellus micheneri* inhabits a southern Appalachian climate with a longer growing season and mild winters. Mating study data indicated that the TN/NC *L. omphalodes* were one biological species. The remaining clade is a cosmopolitan group containing isolates from Mexico, Austria, and Washington and may be comprised of more than one species.

The intercontinental distribution of *L. omphalodes* mating group IX has several possible explanations: 1) There is intercontinental gene flow by spores or by human-mediated transport of wood; 2) There is no significant intercontinental gene flow and the current intercontinental distribution represents an ancient connection between the continents. The most recent connection was via a North Atlantic land bridge in the Tertiary Period (Graham 1993); 3) The constipated duck theory states that birds and other animals carry the spores and tissue to other continents in their feces and on their bodies. The biogeographical disjunct between the species, *L omphalodes, L. micheneri,* and *L. montanus*, is similar to the disjunct seen in *Flammulina* (Petersen and Hughes, Pers. Com.) and *Pleurotus* (Vilgalys and Sun 1994).

Collection 9981 is an unknown species at present. Morphological examinations need to be conducted to determine if it is a known species.

L. vulpinus and *L. cochleatus* are in the same clade. Based on sequence data alone, they are probably not the same species. The percent difference between the L. vulpinus and L. cochleatus isolates is 10.9%. Normally, disjunct populations of the same species have a percent difference of about 1%. To determine if this assumption is correct, mating studies need to be conducted to see if these two species inter-mate.

L. ursinus from NC and SC were sequenced. The sequence differences suggest some divergence in the Appalachian area. This does agree with other studies suggesting a high level of diversity in this region (Currie and Paquin 1987).

Diagnostic Molecular Characters to Delineate Species

There are few distinguishing morphological characters that can be used to characterize each of the different species of *Lentinellus* and these characters vary significantly with the age of the mushroom (Miller 1997). Restriction Fragment Length Polymorphisms can be used as molecular tools to help distinguish species. For example, *L. ursinus* can be distinguished uniquely by the presence of two *Eco* RI sites (if there are two sites present, the species is *L. ursinus*), yet some *L. ursinus* isolates do not have the second *Eco* RI site and will be missed by this diagnostic character. *Taq* I separates *L. angustifolius*, and *L. vulpinus* from all the other species. The sites that *Taq* I recognizes are not the same in these two species however, and there are difficulties with similar sized fragments that are produced upon digestion. By sequence comparison, two enzymes have tentatively been identified to distinguish species when used in combination, *Mbo* II and *Sph* I. Future studies will test these to see if they are reliable.

Group I Intron

The intron was probably vertically transferred. The phylogenetic relationship of species with the intron indicates that the intron was either lost or gained in an ancestor of present day species (Figure 20). If the intron had been horizontally transferred, there would have been no phylogenetic signal and the placement of the intron would be random, however, that is not the case. Species with the intron are phylogenetically related. Within *Lentinellus*, the loss or gain of the intron was apparently due to a single event that then evolved. The comparison of the intron tree vs. the ITS tree shows that there is some similarity between the two trees (Figure 17), however there are some major differences. The two trees are not entirely congruent. There are several explanations for these differences in the intron tree and the ITS tree. The two genes used for comparison may have evolved independently of each other and at different rates. The other explanation is that there is another mechanism at work here that is unknown at the present. As far as the geographical distribution of the intron, the intron could have been spread the same way that the organism was spread.

Further evidence for an ancient intron insertional event can be found by examining members of the family Auriscalpiaceae, including *Lentinellus, Clavicorona* and *Auriscalpium*, all of which have this intron. Comparison of a phylogenetic tree based on ribosomal 18S sequences with a phylogeny based on intron sequences shows concordance between these trees and indicates that this is an old element in this family (Ed Lickey, Pers. Com.).

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Figures

FIGURE 1 - LIST OF COLLECTIONS USED IN THIS STUDY				Mating Group	Intron?	Exemplars	Hpa II	Hinf I	Cla I	Eco R I	Rsa I	Taq I
	Name Collected Under						Re-patterned					
7966	LENTINUS VULPINUS	USA	MN	III - VULPINUS	no	ITS sequenced	3	1	1	1		1
	LENTINELLUS URSINUS COMPLEX											
7497	L. VULPINUS	FINLAND	ETELA-HAME	I - URSINUS	no		2	2/1 het	2	1		1
8456	L. ursinus ?	MEXICO	EST. MEXICO	I - URSINUS	no		2	1	1	2		1
6259	L. ursinus ?	MEXICO	TLAXCALA		no		2	1	1	2		1
8711	L. ursinus ?	MEXICO	TLAXCALA	I - URSINUS	no		2	1	1	2		1
7104		NEW ZEALAND	SOUTH ISLAND	I - URSINUS								
3307	L. URSINUS ANM	Russia	PRIMORSK	I - URSINUS	no							
6556	L. URSINUS ANM	RUSSIA	PRIMORSKI REG.	I - URSINUS	no		2	2	2	1		1
7280	L. VULPINUS	SWEDEN	UPPLAND	I - URSINUS	no		?	2	1	1		1
9010	L. cochleatus	USA	CA	I - URSINUS	no		2	1	1	1		1
2078	L. VULPINUS	USA	GA	I - URSINUS	no		2	1	1	2		1
5641	L. COCHLEATUS	USA	ID	I - URSINUS	no		5	2	1	1		1
2414	L. URSINUS ANM	USA	IL	I - URSINUS	no		2	1	1	2		1
ANM 497	L. URSINUS ANM	USA	IL	I - URSINUS	no		1			2		
ANM 508	L. URSINUS ANM	USA	IN	I - URSINUS	no		1			2		
ANM 510	L. URSINUS ANM	USA	IN	I - URSINUS	no							
ANM 480	L. URSINUS ANM	USA	IO	I - URSINUS	no							
ANM 482	L. URSINUS ANM	USA	IO	I - URSINUS	no		1			2		
ASM 8109	L. URSINUS ANM	USA	MI	I - URSINUS	no		1			2		
ANM 491	L. URSINUS ANM	USA	MO	I - URSINUS	no							
2082	L. VULPINUS	USA	NC	I - URSINUS	no		2	1	1	2		1
2209	L. VULPINUS	USA	NC	I - URSINUS	no		2	1	1	2		1
2210	L. URSINUS ANM	USA	NC	I - URSINUS	no	ITS sequenced	2	1	1	2		1
6631	L. SP.	USA	NC		no		2	1	1	2		1
8862	L. sp.	USA	NC	I - URSINUS	no		2	1	1	2		2
9963	L. URSINUS	USA	SC	I - URSINUS	get culture							
9986	L. URSINUS	USA	SC	I - URSINUS	no	ITS sequenced	1			1		
3404	L. VULPINUS	USA	TN	I - URSINUS	no							
5324	L. SP.	USA	TN		no		2	1	1	1		1
ANM 321	no data in thesis			I - URSINUS	get culture	poor crosser						
ANM 438	no data in thesis			I - URSINUS	no							
ANM 479	no data in thesis			I - URSINUS	no							
ANM 493	no data in thesis			I - URSINUS	no							
3538	L. SP.	AUSTRALIA	N.S.W.	II - ANGUSTIFOLIUS	no							
ANM 511	L. ANGUSTIFOLIUS ANM	AUSTRIA	BURGENLAND	II - ANGUSTIFOLIUS	no							
7808	L. ANGUSTIFOLIUS ANM	COSTA RICA	PROV. SAN JOSE	II - ANGUSTIFOLIUS	no		3	1	1	1		2
7880	L. ANGUSTIFOLIUS ANM	COSTA RICA	PROV. SAN JOSE	II - ANGUSTIFOLIUS	no		3	1	1	1		2
7876	L. ANGUSTIFOLIUS ANM	COSTA RICA	PROV. SAN JOSE	II - ANGUSTIFOLIUS	no		3	1	1	1		2
8946	L. URSINUS	RUSSIA	CAUCASIA	II - ANGUSTIFOLIUS	no		3	1	1	1		2
4321	L. BISUS	SWITZERLAND	MAGGIA	II - ANGUSTIFOLIUS	yes		3	1	1	1		2
9547	L. FLABELLIFORMIS	USA	CA	IV THEN II	no							
DDL 9369		USA	CALIFORNIA	II - ANGUSTIFOLIUS	get culture							
8270	L. SP.	USA	FL	II - ANGUSTIFOLIUS	no		1	1	1	1		2
9149	ISG1	USA	FL	IV THEN II	yes		2			1		

ANM 495	L. ANGUSTIFOLIUS ANM	USA	IL	II - ANGUSTIFOLIUS	yes							
9208	L. ANGUSTIFOLIUS	USA	LA	II - ANGUSTIFOLIUS	yes							
4101	L. ANGUSTIFOLIUS	USA	NC	II - ANGUSTIFOLIUS	yes	ITS sequenced	1	1	1	1		2
8768	L. ANGUSTIFOLIUS	USA	NC	II - ANGUSTIFOLIUS	yes							
3402	L. SP.	USA	TN		yes		1	1	1	1		2
4065	L. SP.	USA	TN	II - ANGUSTIFOLIUS	yes		1	1	1	1		2
2036	L. ANGUSTIFOLIUS ANM	USA	TN	II - ANGUSTIFOLIUS	yes		3	1	1	1		2
9254	L. ?VULPINUS	USA	TN	II - ANGUSTIFOLIUS	yes							
8685	L. ISG IV	USA	LA	II - ANGUSTIFOLIUS	yes	ITS sequenced	3			1		
7803				II - ANGUSTIFOLIUS	get culture							
ANM 492	no data in thesis			II - ANGUSTIFOLIUS	yes							
9405		Costa Rica		II - ANGUSTIFOLIUS	get culture							
9319		USA	CA	II - ANGUSTIFOLIUS	get culture							
ANM XXX	L. flabelliformis ss Miller			II - ANGUSTIFOLIUS	get culture							
okm 27329-7	L. MONTANUS	USA	MONTANA	V - MONTANUS	yes	ITS sequenced						
5702	L. FLABELLIFORMIS	USA	WA		no		3	1	1	1		1
7491	L. OMPHALODES	FINLAND	ETELA-HAME	IX - OMPHALODES BOR	yes		3	1	1	1		1
7468	L. OMPHALOIDES	FINLAND	ETELA-HAME	IX - OMPHALODES BOR	yes		no dna	1	1	1		1
9978	L. OMPHALODES	RUSSIA	LAKE BAIKAL	IX - OMPHALODES EUR	yes	ITS sequenced	determine from sequence					
4243	L. OMPHALODES	SWEDEN	VASTERGOTLAND		yes	ITS sequenced	3	1	1	1		1
6701	L. OMPHALODES	USA	AK		yes	ITS sequenced	3	1	1	1		1
8199	L. OMPHALODES	USA	AK	IX - OMPHALODES BOR	yes		3	1	1	1		1
4433	L. OMPHALODES	USA	NC	VII - OMPHALODES SE	yes		3	1	1	2		1
6293	L. OMPHALODES	USA	NC	VII - OMPHALODES SE	yes		3	1	1	1		1
9177	L. OMPHALODES	USA	NC	VII - OMPHALODES SE	yes							
9159	L. OMPHALODES	USA	TN	VII - OMPHALODES SE	yes	ITS sequenced	determine from sequence					
9712	L. micherni	USA	TN	VII - OMPHALODES SE	yes							
6303		USA	TN	VII-OMPHALODES SE	get culture							
9981	L. omphalodes	AUSTRIA		VIII - OMPHALODES EUR	yes	ITS sequenced	determine from sequence					
9980	L. omphalodes	AUSTRIA		VIII - OMPHALODES EUR	yes	bad sequence						
7292		SWEDEN	UPPLAND	VIII - OMPHALODES EUR								
9985	L. cochleatus	Austria		cochleatus	yes	ITS sequenced						
8452	L. mexicanus ?	MEXICO	EST. MEXICO		heterozygous	ITS sequenced	3	1	1	1		1
8617	L. SP.	ARGENTINA	PROV. CHUBUT		no							
3533	L. SP.	AUSTRALIA	N.S.W.		no							
3911	L. SP.	AUSTRALIA	TASMANIA		no							
3955	L. SP.	AUSTRALIA	TASMANIA		no							
3997	L. (GRIFOLA)	AUSTRALIA	TASMANIA		no							
4027	L. SP.	AUSTRALIA	TASMANIA		no							
3400/16	L. SP.	CANADA	BC									
7886	L. SP.	COSTA RICA	PROV. SAN JOSE		no							

7904	L. SP.	COSTA RICA	PROV. SAN JOSE		no							
7827	L. SP.	COSTA RICA	PROV. SAN JOSE		no							
7831	L. SP.	COSTA RICA	PROV. SAN JOSE		no							
9489	L. sp	Costa Rica			no							
6681	L. CASTOREUS	FINLAND	ETELA-HAME		no							
2344	L. SP.	JAPAN	TOCHIGI PREF.		no							
KL 4245	L. SP.	MEXICO	DPTO TLAXCALA		yes							
ME-1146	L. OMPHALODES	MEXICO	DPTO. TLAXCALA		no							
8759	L. SP.	MEXICO	NAYARIT		heterozygous							
6233	L. SP.	MEXICO	TLAXCALA		no							
6272	L. SP	MEXICO	VERACRUZ		no							
7035	L. SP	NEW ZEALAND	FIORDLAND		no							
7426	L. SP	NEW ZEALAND	NORTH ISLAND		no							
7118	L. SP	NEW ZEALAND	SOUTH ISLAND		no							
7128	L. SP	NEW ZEALAND	SOUTH ISLAND		no							
2589	L. SP.	NEW ZEALAND			no							
8967	L. sp.	Russia	Caucasia		no							
3211	L. SP.	Russia	PRIMORSK		no							
9976	L. ursinus	Russia		X - "URSINUS"								
7267	L. CASTOREUS	SWEDEN	UPPLAND		no							
7320	L. FLABELLIFORMIS	USA	HUMBOLDT CO.		no							
5641A	L. ?COCHLEATUS	USA	ID				``					
ASM 5463	L. URSINUS	USA	IL									
ASM 6771	L. URSINUS	USA	IL		no							
TJV 95-96	L. angustifolius	USA	MS									
6295		USA	NC	VII-OMPHALODES SE	GET							
KWH	L. URSINUS	USA	NC									
4058	L. SP.	USA	TN		yes							
4051	L. SP.	USA	TN		yes							
5146	L. SP.	USA	TN		no							
4047	L. SP.	USA	TN									
5325	L. SP.	USA	TN		no							
5881	L. FLABELLIFORMIS	USA	WA. JEFFERSON CO.		no							
9984	L. cochleatus	Austria			no							
3869					get culture							
6303					get culture							
10233					get culture							
ANM 1	no data in thesis				get culture							
ANM 506	no data in thesis				get culture							

Diagram of ITS region

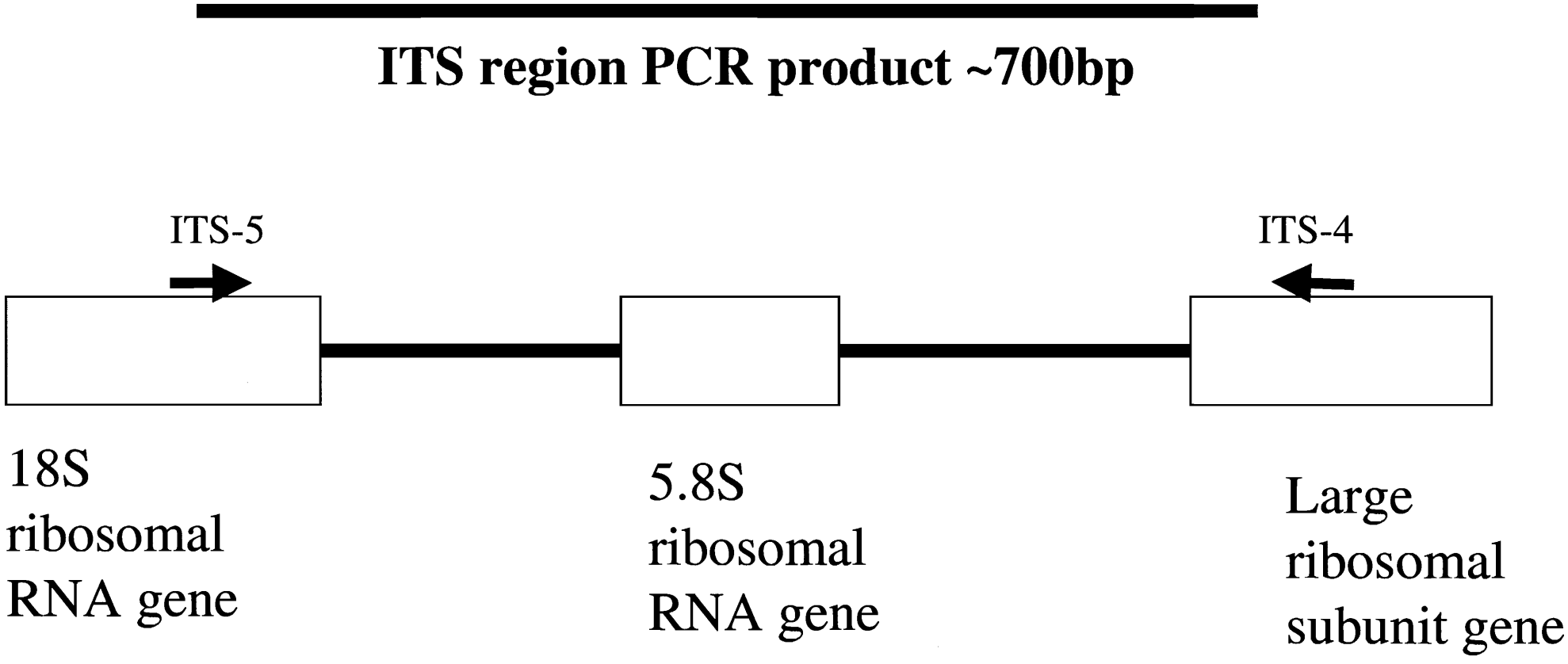

Figure 2

Figure 3

MSF File with *Eco* RI sites Highlighted in Blue with red lettering of the recognition site.
MSF file with *Taq* I sites Highlighted in Yellow with Purple lettering of the recognition site.

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 9985 L. cochleatus                CG TAGGTGAACC TGCGGAAGGA
 7966 L. vulpinus                  CG TAGGTGAACC TGCGGAAGGA
 9986 L. ursinus                   CG TAAAACAAAG GCCGATAGGT
 2210 L. ursinus                   CG TAAAACAAAG GCCGATAGGT
 8685 L. angustifolius             CG TAGGTGAACC TGCGGAAGGA
 4101 L. angustifolius             CG TAGGTGAACC TGCGG.AGGA
 9978 L. omphalodes                CG TAGGTGAA.C TGCGGAAGGA
 4243 L. omphalodes                CG TAGGTGAA.C TGCGGAAGGA
 6701 L. omphalodes                CG TAGGTGAA.C TGCGGAAGGA
  OKM L. montanus                  CG TAGGTGAACC TGCGGAAGGA
 5702 L. omphalodes                CG TAGGTGAA.C TGCGGAAGGA
 9159 L. micheneri                 CG TAGGTGAACC TGCGGAAGGA
 8452 L. sp. nov. 1                CG TAGGTGAA.C TGCGGAAGGA
 9981 L. sp. nov. 2                GC TGCAGGCAGT GCAGGAAAGA
 4027 L. sp. nov. 3                AG GTCGAGAGCA TGAGGAAGGA

      51                                                  100
 9985 TCATTATCGT AAACAA.AGG CCGTGGTTTT GCTGTTGCTG GCCCCCCTT.
 7966 TCATTATCGA AAACAAGAGG CCGCGGTACG GCTGTCGCTG GCCCCCCCTC
 9986 TGTCGCTGGT CCCCTAGGGA CATGTGCACG CCTTTGGTCG ATATCCTTC
 2210 TGTCGCTGGT CCCCTAGGGA CATGTGCACG CCTTTGGTCG ATAATCCTTC
 8685 TCATTATC.. .GTAAACAAA AAGGCC..TT GGGTTGTCGC TGGTCCTCCG
 4101 TCATTATC.. .GTAAACAAA AAGGCC..TT GGGTTGTCGC TGGTCCTCCG
 9978 .CATTAC... TGTAA..ACA AAGGCTGAAC .GGTTGTCGC TGGTCCTCCG
 4243 .CATTAC... TGTAA..ACA AAGGCTGAAC .GGTTGTCGC TGGTCCTCCG
 6701 .CATTAC... TGTAA..ACA AAGGCTGAAC .GGTTGTCGC TGGTCCTCCG
  OKM TCATTAC... TGTAA..GCA AAGGCCGAAC .GGTTGTCGC TGGTCCTCCG
 5702 TCATTAC... TGTAA..ACA AAGGCCGAAC .GGTTGTCGC TGGTCCTCCG
 9159 TCATTAC... TGTAA..ACA AAGGCCGAAC .GGTTGTCGC TGGTCCTCCG
 8452 .CATTAT... TGTAA..ACA AAGGCCGAGC GGGTTGTTGC TGGTCCTCCG
 9981 CTATTAC... TGGTATAACA GAAGGCCGAG CGGTTGTAGC AGGTCCTCCG
 4027 TCATTATCTG TAAAAAGCAT GAGGCCGAGC GGCTGTGCGC TGGTCCTCCG

      101                                                 150
 9985 .GCGGGAGGC ATGTGCACGC CCATGGTCGC ATCCTTCACA CCCCTGTGCA
 7966 GGGGGGGGGC ATGTGCACGC CCGCGGTCGC ATCCTTCACA CCCCTGTGCA
 9986 ACACCCCTGT GCACCTCTGC GTGTG...GT TCTCTTTTTT TCCCCCTCCT
 2210 ACACCCCTGT GCACCTCTGC GTGTG...GT TCTCTTTTTC CTCCCTTCCT
 8685 GGACATGTGC ACGCCCTCGG TCGTT...AC ATCCTTCATA CCCCTGTGCA
 4101 GGACATGTGC ACGCCCTCGG TCGTT...AC ATCCTTCATA CCCCTGTGCA
 9978 GGACATGTG. CACA.CCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 4243 GGACATGTG. CACA.CCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 6701 GGACATGTG. CACA.CCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
  OKM GGACATGTG. CACG.CCCTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 5702 GGACATGTG. CACG.CCCTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 9159 GGACATGTG. CACA.CCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 8452 GGACATGTG. CACACCCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
 9981 GGACATGTGT CACACCTGTC GGTCA...AC ATCCTTCACA CCCCTGTGCA
 4027 GGACATGTG. .CACGCCTTC GGTCG...AC ATCCTTCACA CCCCTGTGCA
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Taq I site == TCGA

Eco RI site == GAATTC

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      151                                                        200
9985  CCTCTGCGTG GGTTTGTTGG CTTGTGTCTT C......... GAGCCCGCGT
7966  CCTCTGCGTG GGTTCGTCGG CTTGCGCCTT C......... GAGCCCGCGT
9986  ATCGACCCGT TCATTCGGGT TGTAAGGTTG GAGAAGGGGG GGACCCGCGT
2210  ATCGACCCGT TCATTCGGGT TGTAAGGTTG GAGAAGGGGG GGACCCGCGT
8685  CCTCTGCGTG TGGTTCTCTC CCTCCTCTTC GGCGGGGGGT TTGGGCCTGC
4101  CCTCTGCGTG TGGTTCTCTC CCTCCTCTTC GGCGGGGGGT TTGGGCCCGC
9978  CCTCTGCGTG TGGCT...CT CCT..CGCTT CGGCTTGTGG GGGCCCGCGT
4243  CCTCTGCGTG TGGCT...CT CCT..CGCTT CGGCTTGTGG GGGCCCGCGT
6701  CCTCTGCGTG TGGCT...CT CCT..CGCTT CGGCTTGTGG GGGCCCGCGT
 OKM  CCTCTGCGTG TGGTT...CC CCT..CGCCT CGGCTTGTGG GGGCCCGCGT
5702  CCTCTGCGTG TGGTT...CC CCT..CGCCT CGGCTTGTGG GGGCCCGCGT
9159  CCTCTGCGTG TGGTTCCCCT CCT..CGCTT CGGCTTGTGG GGGGCGCCCG
8452  CCTCTGCGTG TGGCT..CCC CCT..TGCCT CGGCTTGTGG GGGCCCGCG.
9981  CCTCTGCGTG TGGCT..CCC CCT..CGCTT CGGCTTGTGG GGGCCCGCG.
4027  CCTCTGCGTG TGGTCTTCCC CTTGCTTCTT AAAAACGGCG GGGTTGGCCC

      201                                                        250
9985  CTTATATCAT ATACAC.... CTGTATGTCT TCAGAATGTC AAC.ATGCGA
7966  CCCCCTTCCT ACACACACCT TTGTATGTCT TCAGAATGTC AAC.ATGCGA
9986  C..TCATTAT .AAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
2210  C..TTATTAT AAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
8685  GTCTCCTTAT AAACACCCCT TGTATGTTCT TATGAATGTC TACTATGCGA
4101  GTCTCCTTAT AAACACCCCT TGTATGTTCT TATGAATGTC TACTATGCGA
9978  C..TCTTATA AAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
4243  T..TCTTATA AAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
6701  C..TCTTAT. AAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
 OKM  C..TCTTATA AACA..CCCT TGTATG.TCT TACGAATGTC TACTATGCGA
5702  C..TCTTATA AACAC.CCCT TGTATG.TCT TACGAATGTC TACTATGCGA
9159  CGTCTTCTTA TAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
8452  ...TCTCTTA TAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
9981  ...TCTCTTA TAAACACCCT TGTATG.TCT TACGAATGTC TACTATGCGA
4027  GCGTCTCTTA TAAACACCCC TCAATG.TCT TACGAATGTC TACTATGCGA

      251                                                        300
9985  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
7966  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
9986  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
2210  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
8685  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
4101  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
9978  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
4243  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
6701  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
 OKM  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
5702  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
9159  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
8452  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
9981  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
4027  TAAAAAGCAT CTAATACAAC TTTCAACAAC GGATCTCTTG GCTCTCGCAT
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      301                                                        350
9985  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
7966  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
9986  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
2210  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
8685  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
4101  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
9978  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
4243  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
6701  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
 OKM  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
5702  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
9159  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
8452  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
9981  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA
4027  CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA

      351                                                        400
9985  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
7966  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
9986  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
2210  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
8685  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
4101  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
9978  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
4243  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
6701  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
 OKM  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
5702  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
9159  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
8452  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
9981  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG
4027  GTGAATCATC GAATCTTTGA ACGCACCTTG CACCCCTTGG TATTCCGAGG

      401                                                        450
9985  GGTACGCCTG TCTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
7966  GGTACGCCTG TCTGAGTGTC G.TGAAATTC TCAACCCGGC CCCCTTTTGC
9986  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCGC CCCCTTTTGC
2210  GGTACGCCTG TTTGAGTGTC GTTGAAATTC TCAACCCCGC CCCCTTTTGC
8685  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
4101  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
9978  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
4243  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCGC CCCCTTTTGC
6701  GGTACGCCTG TTTGAGTGTC GTTGAAATTC TCAACCCCGC CCCCTTTTGC
 OKM  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
5702  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
9159  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCGC CCCCTTTTGC
8452  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCAC CCCCTTTTGC
9981  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCGC CCCCTTTTGC
4027  GGTACGCCTG TTTGAGTGTC G.TGAAATTC TCAACCCCGC CCCCTTTTGC
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      451                                                        500
9985  GAGGGGCAT. ..TGGGGATT GGACTTGGAG GCTTTGCTGG AACCC.....
7966  GAGGGGTGT. ..CGGGGATT GGACTTGGAG GCTTTGCCGG AACCCGGTGT
9986  GAGGGGTTTG TCGGTGGCTT GGACTTGGAG GCTTTT.GCC GGGGG.....
2210  GAAGGGTTTG TCGGTGGCTT GGACTTGGAG GCTTTTTGCC GGGGGA....
8685  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCCTTTGCCG TTAAAA....
4101  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCCTTTGCCG TTAAAA....
9978  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GC.TTTGCC. GGGAAA..GG
4243  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCTTTTGCC. GGGAAA..GG
6701  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCTTTTGCC. GGGAAA..GG
 OKM  GAGGGG..TG TCGGTGGCTT GGACTTGGAG GC.TTTGCCG GGGAAA..GG
5702  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCTTTTGCCG GGGGAG..GG
9159  GAGGGG.TTG TCGGTGGCTT GGACTTGGAG GCTTTTGCC. GGGAAA..GG
8452  GAGGGG..TG TTGGTGGCTT GGACTTGGAG GTTTTGCCGG GAAAGG..GT
9981  GAGGGG..CG TCGGTGGCTT GGACTTGGAG GCTTTGCCGG GGAAAAAGGG
4027  GAGGGG.CTG TCGGTGGCTT GGACTTGGAG GCTTTTGCCG GGGAGCGTGT

      501                                                        550
9985  CCCCCCCCCC CCTCGGTG.. ......GGTG GGATCGGCTC CTCTCAAAGG
7966  GCCCTCCCCT TCTCGGGGTG GCGCGTGTCG GGATCGGCTC CTCTCAAAGG
9986  ......ATTC GTTCC..... .......... ...TCGGCTC CTCTCGAAGG
2210  ......ATTC ATTCC..... .......... ...TCGGCTC CTCTCGGAGG
8685  ........CC CTTTG..... .......... ...TCGGCTC CTCTCGAATG
4101  ........CC CTTTG..... .......... ...TCGGCTC CTCTCGAATG
9978  GTTTCGACCC ACTTC..... .......... ...TCGGCTC CTCTCGAAGG
4243  GTTTCGACCC ACTTC..... .......... ...TCGGCTC CTCTCGAAGG
6701  GTTTCGACCC ACTTC..... .......... ...TCGGCTC CTCTCGAAGG
 OKM  GTTTCGACCC CACTC..... .......... ...TCGGCTC CTCTCGAAGG
5702  GTTTCGACCC CACTC..... .......... ...TCGGCTC CTCTCGAAGG
9159  GTTTCGACCC GC.TC..... .......... ...TCGGCTC CTCTCGAAGG
8452  TTCAAACCCC TGCTC..... .......... ...TCGGCTC CTCTCGAAGG
9981  TTTCGACCCC CGCTC..... .......... ...TCGGCTC CTCTCGAAGG
4027  AT.......A CGCTC..... .......... ...CCGGCTC CTCTCGAAGG

      551                                                        600
9985  CATTAGCGGG A.CCCTTTGC GGCCTCGGTG TGATAAATCA TCTACGCCAT
7966  CATTAGCAGG ACCCCTCTGC GGCCTCGGTG TGAT.AATTG TCTACGCCCT
9986  CATTAGCAGG ACCCTT...G CGGCCTCGGT GTGATAATTG TCTAGCCCGT
2210  TATTAGCAGG ACCCTT...G CGGCCTCGGT GTGATAATTG TCTACGCCGT
8685  CATTAGCAGG ACCCTT...G CGGCCTCGGT GTGATAATTG TCTACGCCGT
4101  CATTAGCAGG ACCCTT...G CGGCCTCGGT GTGATAATTG TCTACGCCGT
9978  CATTAGTAAG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
4243  TATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
6701  TATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
 OKM  CATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
5702  CATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
9159  CATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
8452  CATTAGTAAG ACCCTTTGC. .GGCCTCCGT GTGATAATTG TCTACGCCGT
9981  CATTAGTAGG ACCCTTTGC. .GGCCTCGGT GTGATAATTG TCTACGCCGT
4027  CATTAGTAGG ACCCTTTGCC GGGCCTCGGT GTGATAATTG TCTACGCCGT
```

```
      601                                                  650
9985  GGGTTTAGTT CTT..GTGGG GGACTTGCTT CCAACCGTCT CGTGAGGGAC
7966  GGGCTTAGCT CTC..GTGGG GGACCCGCTT CCAACCGTCC CGCGAGGGAC
9986  GGGCTTAGC. ..TCCTCTGG GACCCTGCTT ACAANCGTCT CGCAAGGGAC
2210  GGGCTTAGC. ..TCCTCTGG GA.CCTGCTT ACAACCGTCT CGCAAGGGAC
8685  GGGCTTAGCT GTC....TGG GA.CCCGCTT CCAACCGTCT CGCAAGAGAC
4101  GGGCTTAGCT GTC....TGG GA.CCCGCTT CCAACCGTCT CGCAAGAGAC
9978  GGGTTTAGCA TGTCAT.GGG .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
4243  GGGTTTAGCA TGTCAT.GGA .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
6701  GGGTTTAGCA TGTCAT.GGG .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
 OKM  GGGTTTAGCA TGCCAT.GGG .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
5702  GGGTTTAGCA TGCCAT.GGG .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
9159  GGGTTTAGCA TGTCATGGGG .A.CCCGCTT CCAACCGTCT CGCAAGGGAC
8452  GGGTTTAGCA TGCCATGGGG GACCCTGCTT CCAACCGTCT CGCAAGGGAC
9981  GGGTTTAGCA TGTCATGGG. ..ACCCGCTT CCAACCGTCT CGCAAAGGGA
4027  GGGCTTCAGC ATGCTATGGG ..ACCCGCTT CCAACCGTCT CGCAAGGGAC

      651                                                  700
9985  ACTTTT...A TCGAAACTTG ACCTCAGATC AGGTGGACTA CCC
7966  ACCTTC...A TCGAAACTTG ACCTCAGATC AGGCGGACTG ACT
9986  ACTTT...CA TCGAAACTTG ACCTCAGATC AGGCGGGATA CCC
2210  ACTTT...CA TCGAAACTTG ACCTCAGATC AGGCGGACTG TAC
8685  AAATTTCAAT CGGAAACTTG ACCTCAGATC AGGCGGGACT AAC
4101  AAATTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT .AC
9978  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
4243  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
6701  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGATA CCG
 OKM  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
5702  A.CTTTCAAT C.GA.ACTTG ACCTCAGATC AGGCGGGACT ACC
9159  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
8452  ACCTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
9981  CACTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
4027  A.CTTTCAAT C.GAAACTTG ACCTCAGATC AGGCGGGACT ACC
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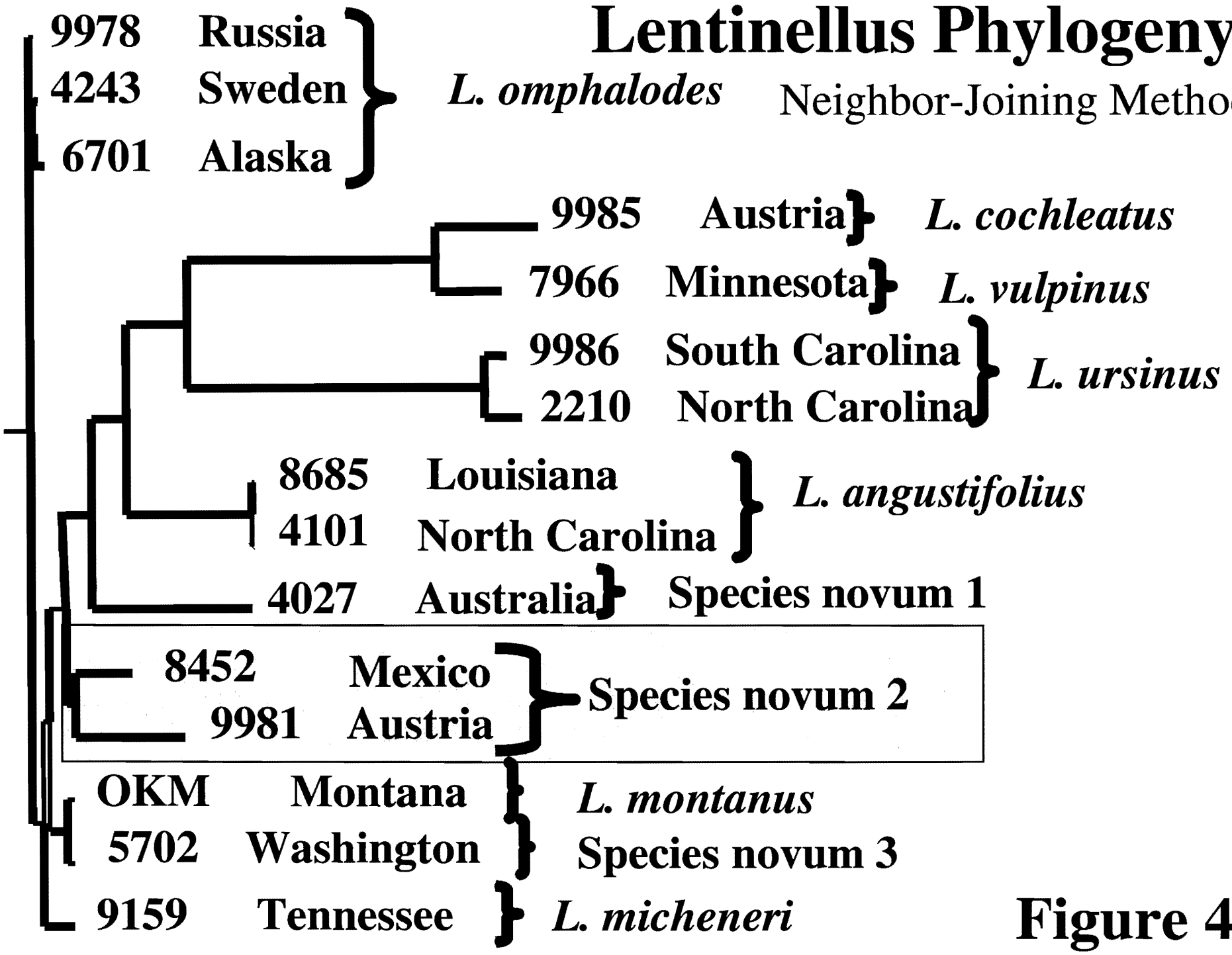

Lentinellus Phylogeny
Neighbor-Joining Method
9978 Russia
4243 Sweden
6701 Alaska
L. omphalodes
9985 Austria
L. cochleatus
7966 Minnesota
L. vulpinus
9986 South Carolina
2210 North Carolina
L. ursinus
8685 Louisiana
4101 North Carolina
L. angustifolius
4027 Australia
Species novum 1
8452 Mexico
9981 Austria
Species novum 2
OKM Montana
L. montanus
5702 Washington
Species novum 3
9159 Tennessee
L. micheneri

Figure 4

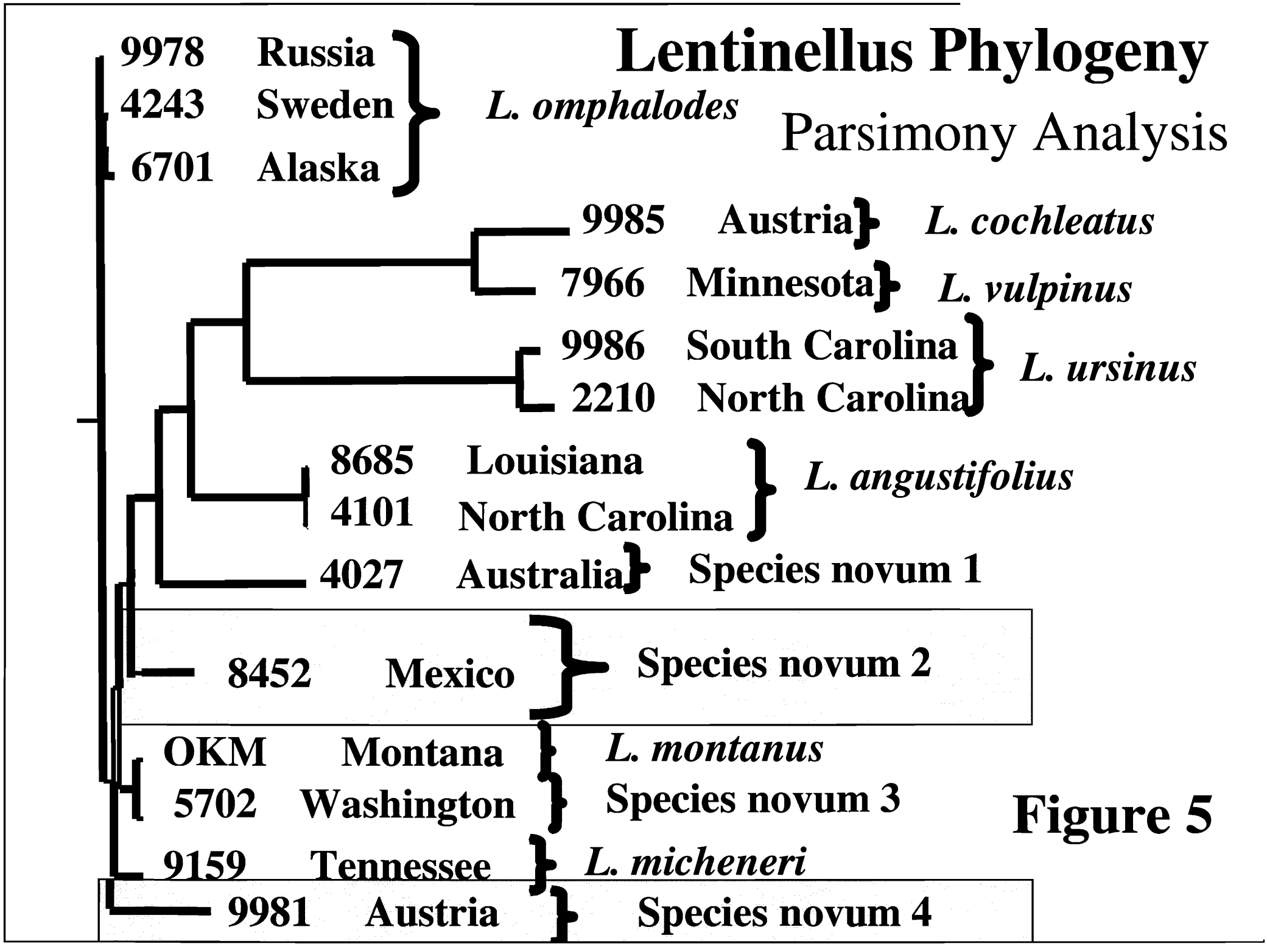
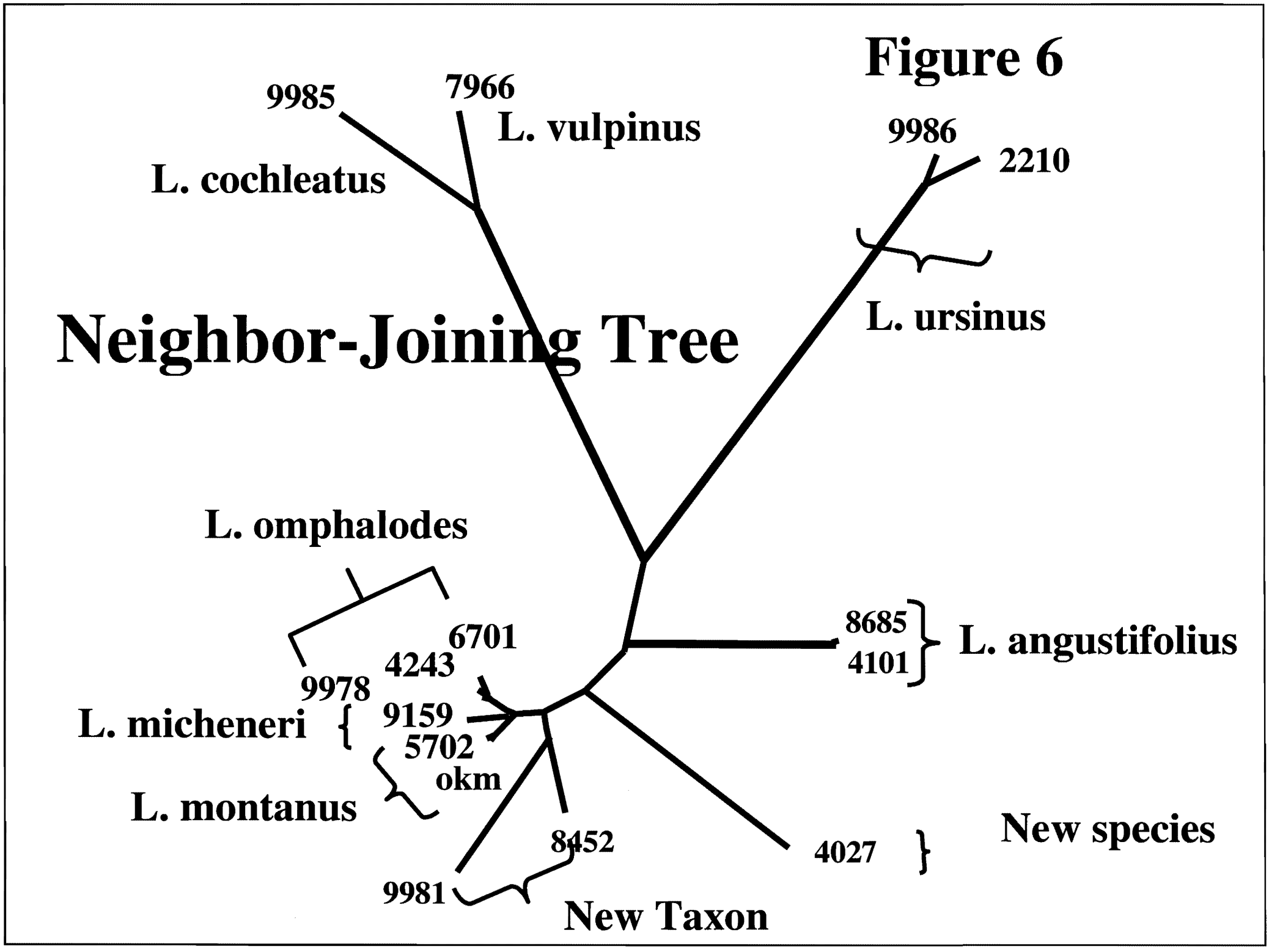

Lentinellus Phylogeny
Parsimony Analysis
9978 Russia
4243 Sweden
6701 Alaska
L. omphalodes
9985 Austria
L. cochleatus
7966 Minnesota
L. vulpinus
9986 South Carolina
2210 North Carolina
L. ursinus
8685 Louisiana
4101 North Carolina
L. angustifolius
4027 Australia
Species novum 1
8452 Mexico
Species novum 2
OKM Montana
5702 Washington
L. montanus
Species novum 3
9159 Tennessee
L. micheneri
9981 Austria
Species novum 4
Figure 5

Figure 6


9985
7966
L. vulpinus
L. cochleatus
9986
2210
L. ursinus
Neighbor-Joining Tree
L. omphalodes
6701
4243
9978
L. micheneri
9159
5702
okm
L. montanus
8685
4101
L. angustifolius
8452
9981
4027
New species
New Taxon

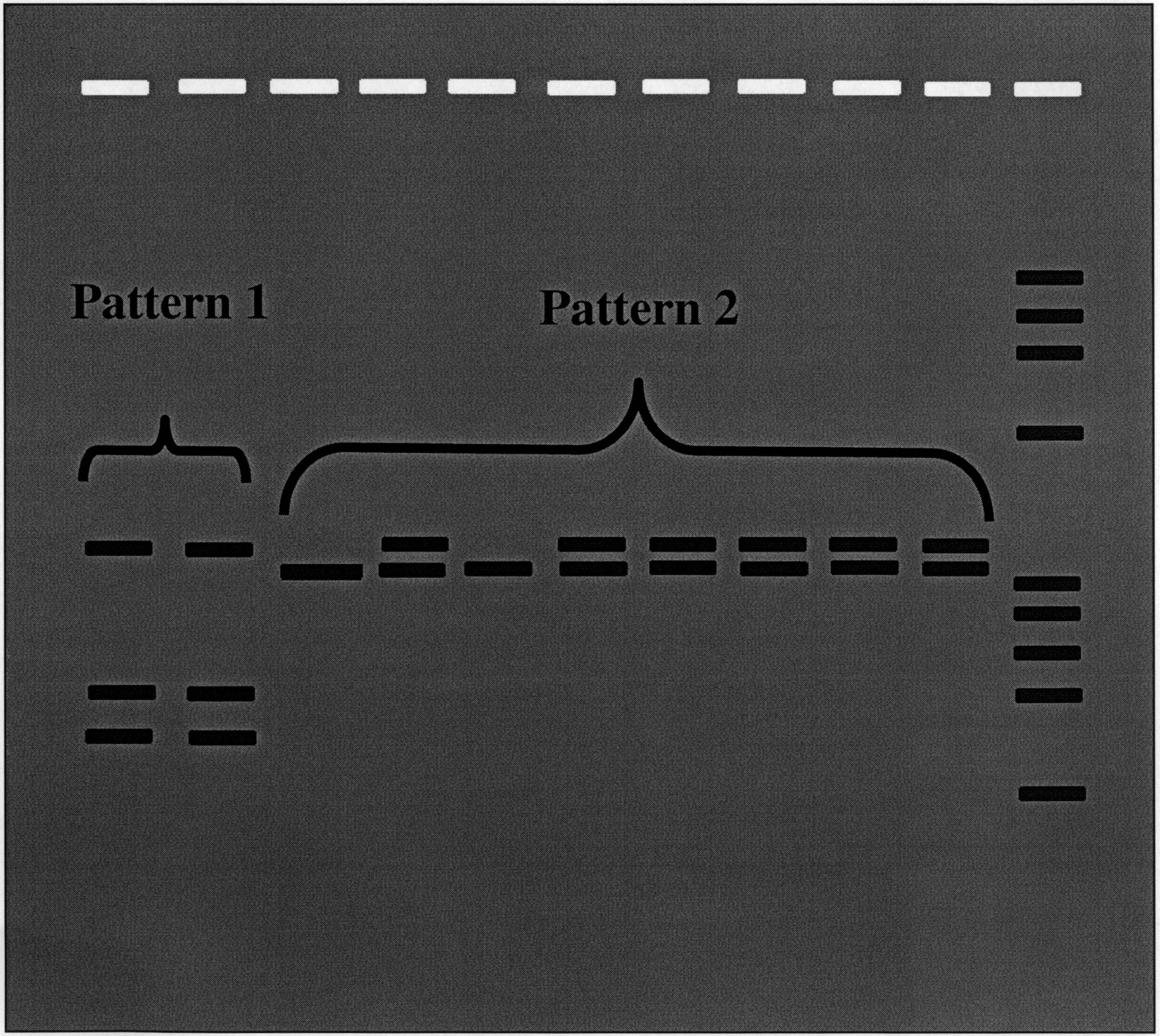


Figure 7

***Eco* RI Banding Pattern**

- Pattern 1 is *L. ursinus*
- Pattern 2 is any species

Diagram of 18S intron

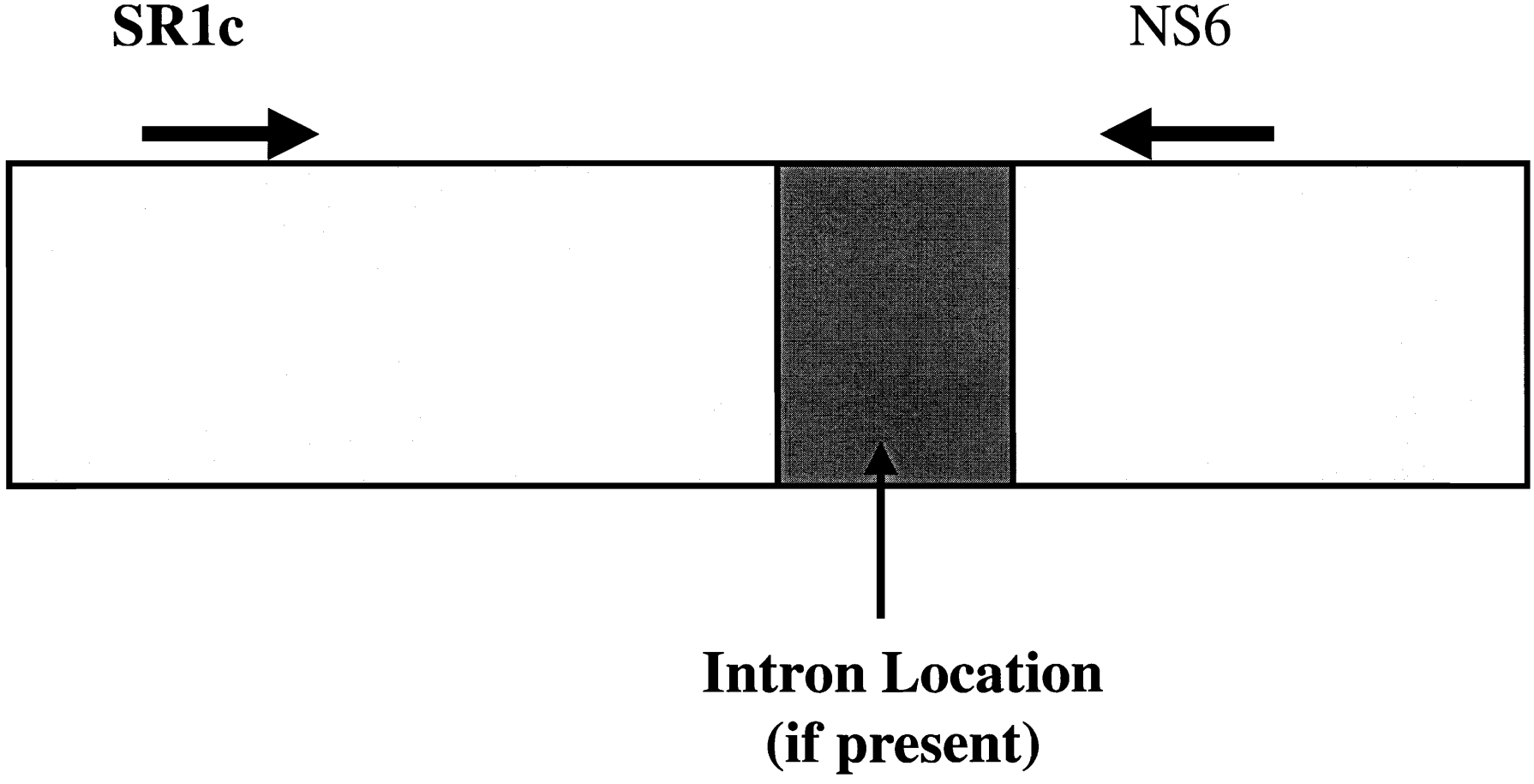
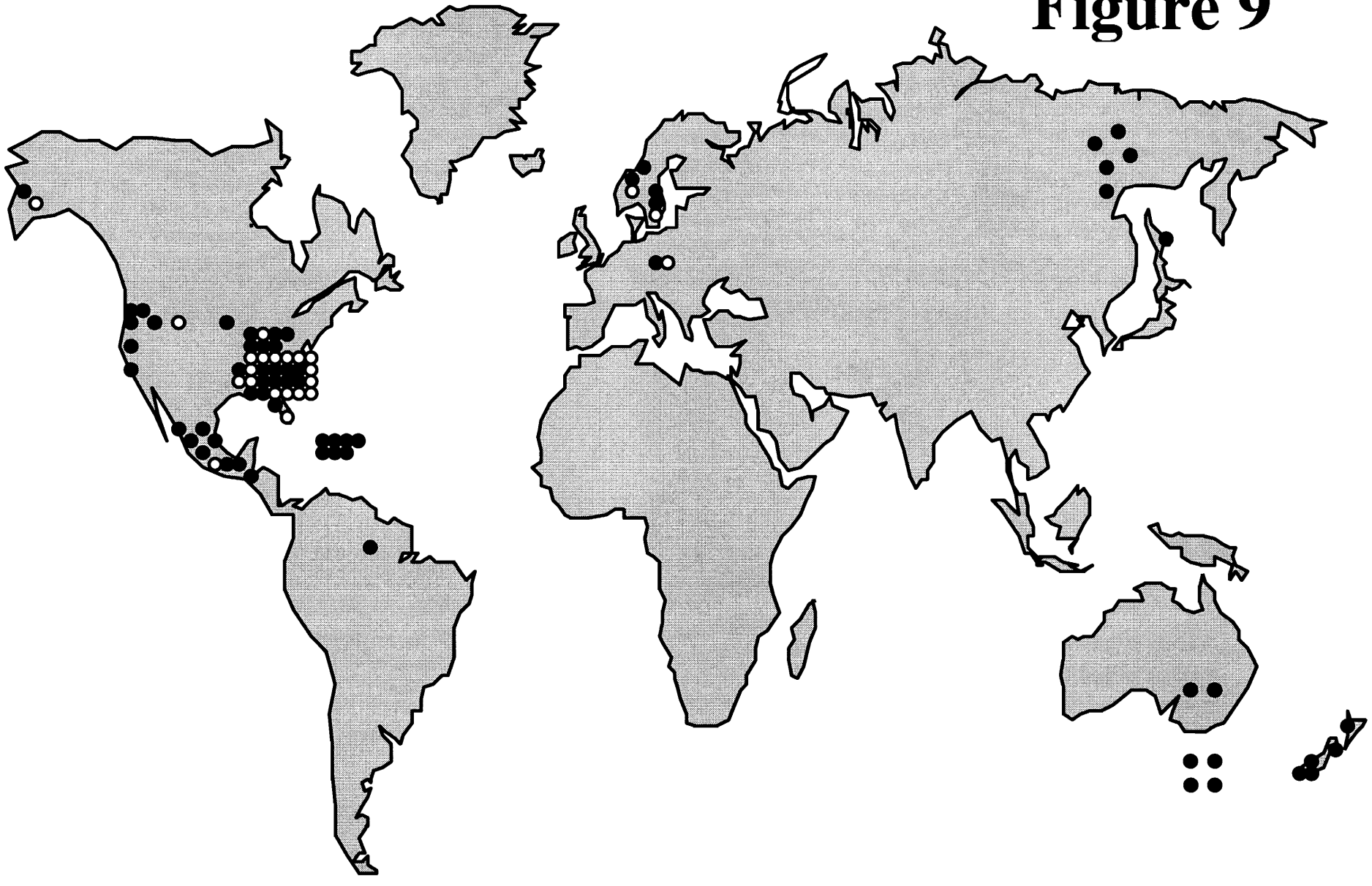


Figure 8

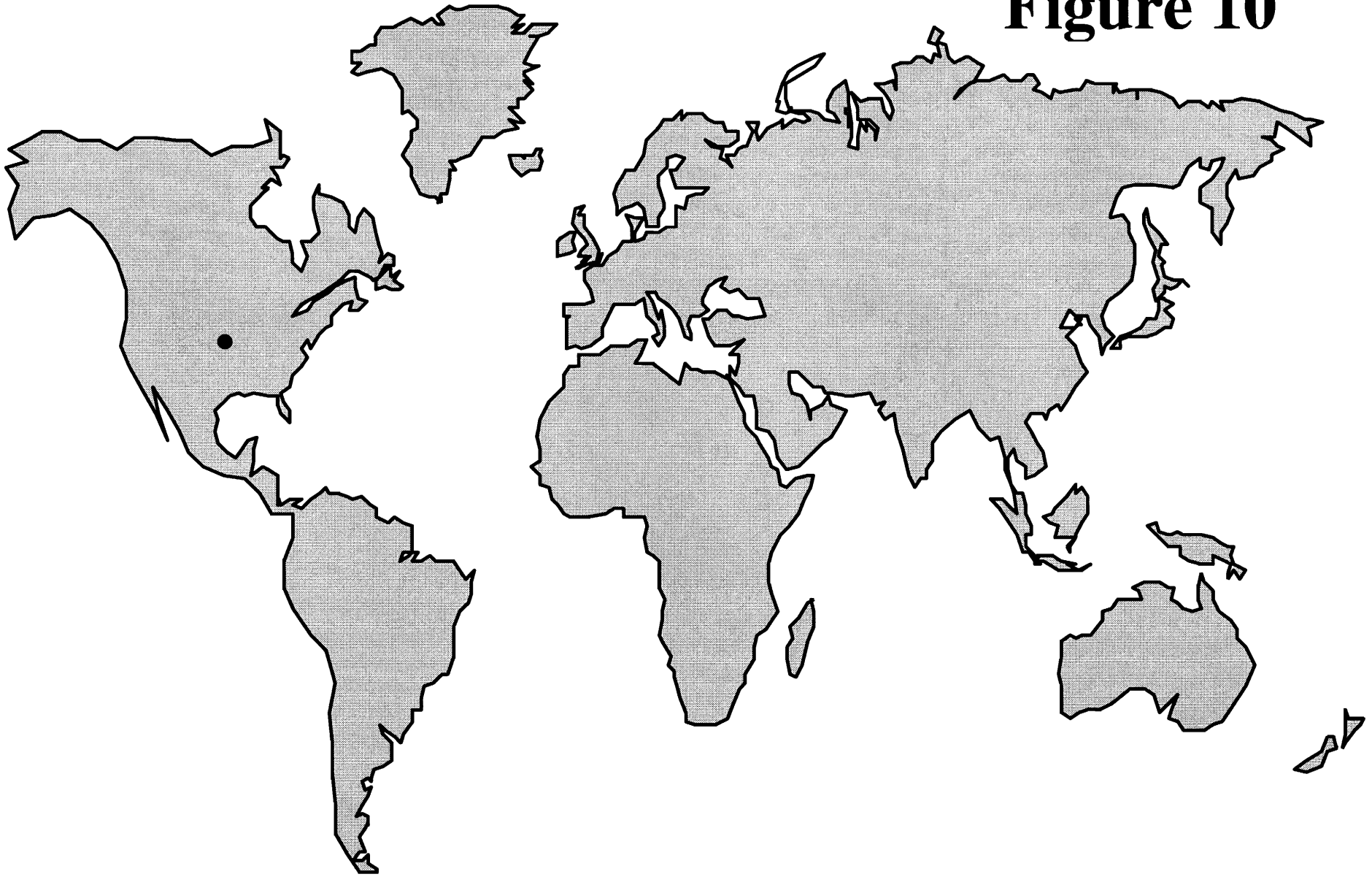
Introns within Lentinellus

Figure 9



Introns within *Lentinellus vulpinus*

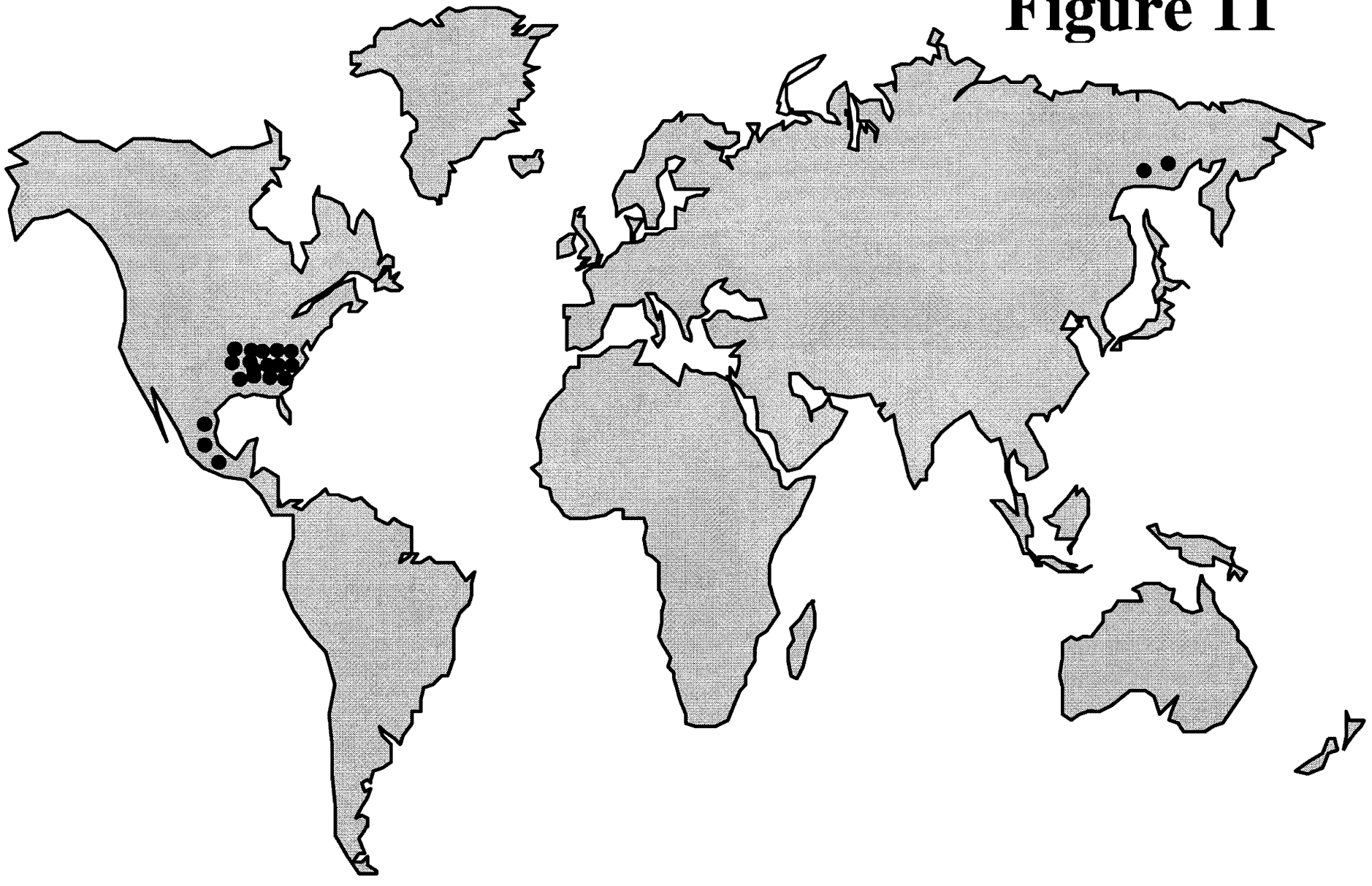
Figure 10



● No Group I Intron ● Group I Intron ● Heterozygous

Introns within *Lentinellus ursinus*

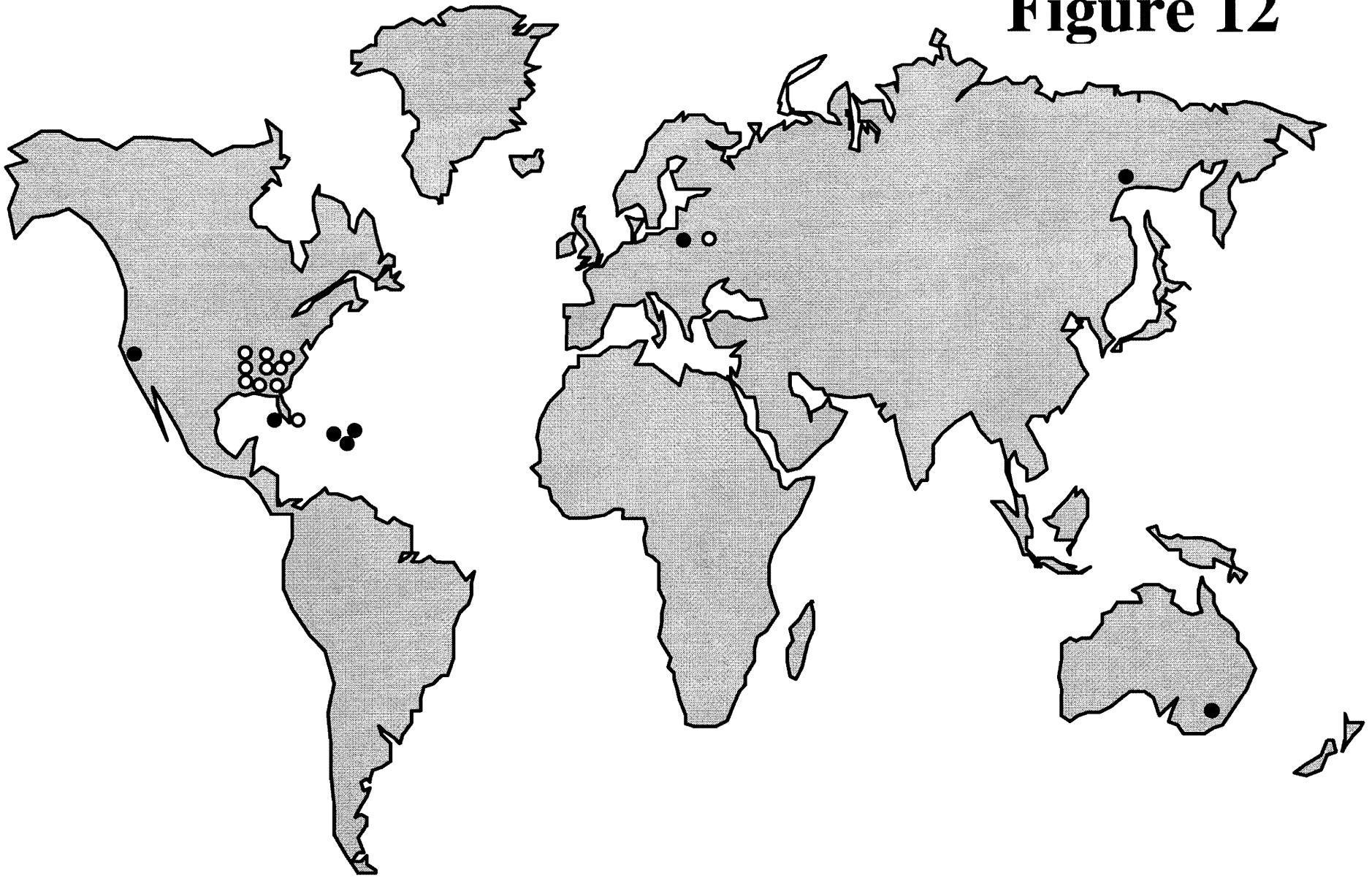
Figure 11



● No Group I Intron ● Group I Intron ● Heterozygous

Introns within *Lentinellus angustifolius*

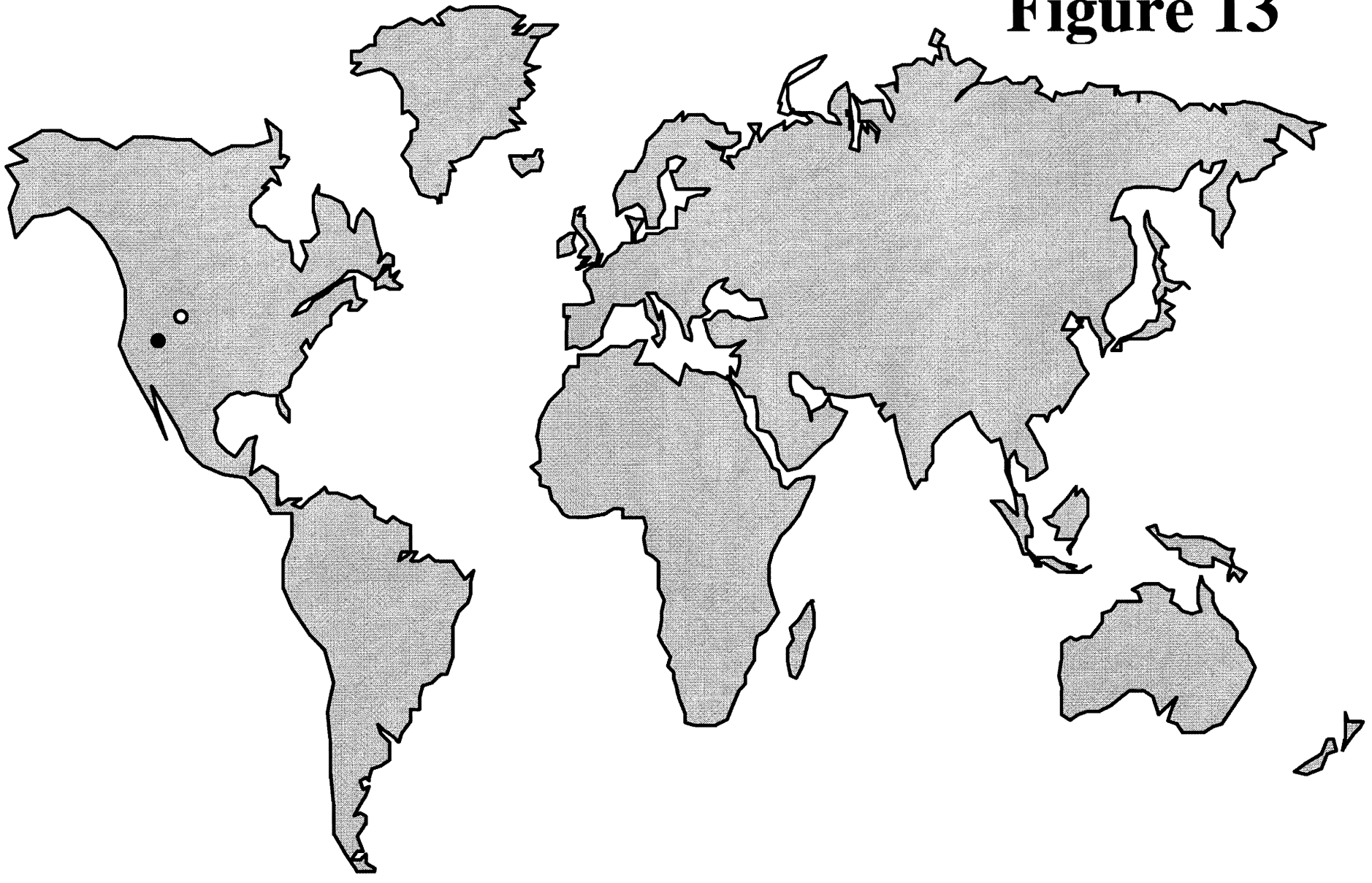
Figure 12



● No Group I Intron ○ Group I Intron ● Heterozygous

Introns within *Lentinellus montanus*

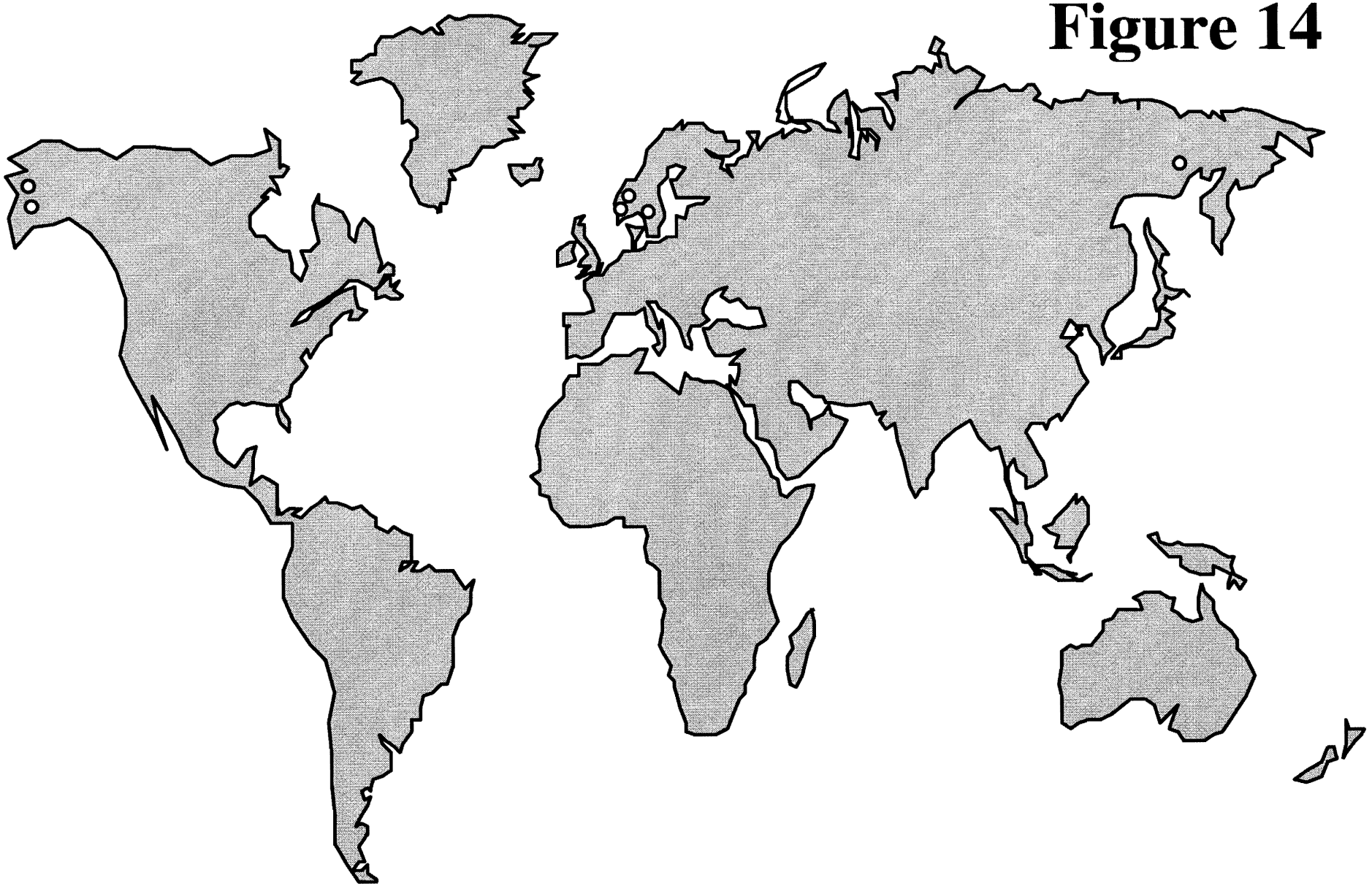
Figure 13



● No Group I Intron ○ Group I Intron ● Heterozygous

Introns within *Lentinellus omphalodes* (IX)

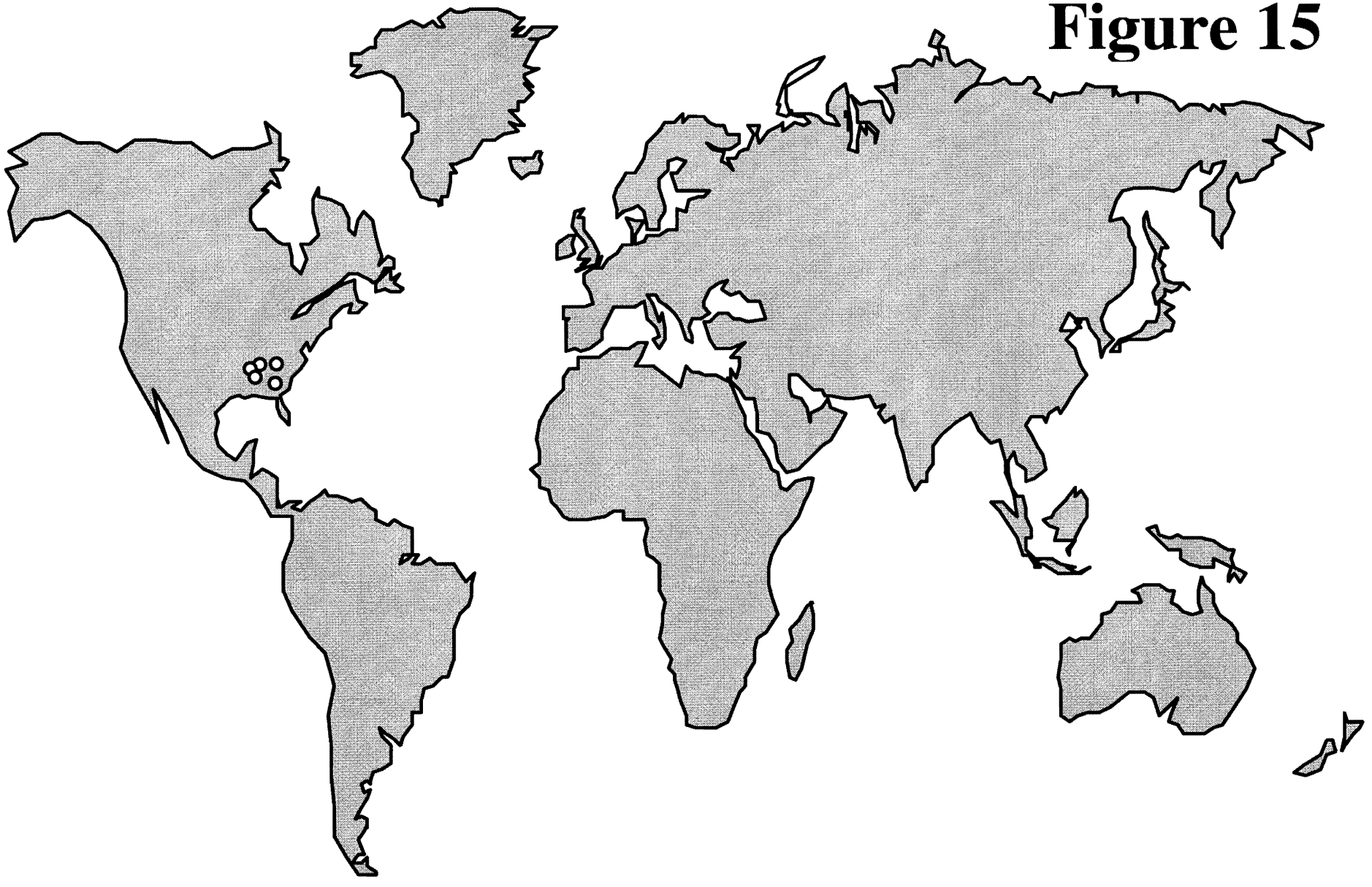
Figure 14



● No Group I Intron ○ Group I Intron ● Heterozygous

Introns within *Lentinellus omphalodes* (VII) *L. micheneri*

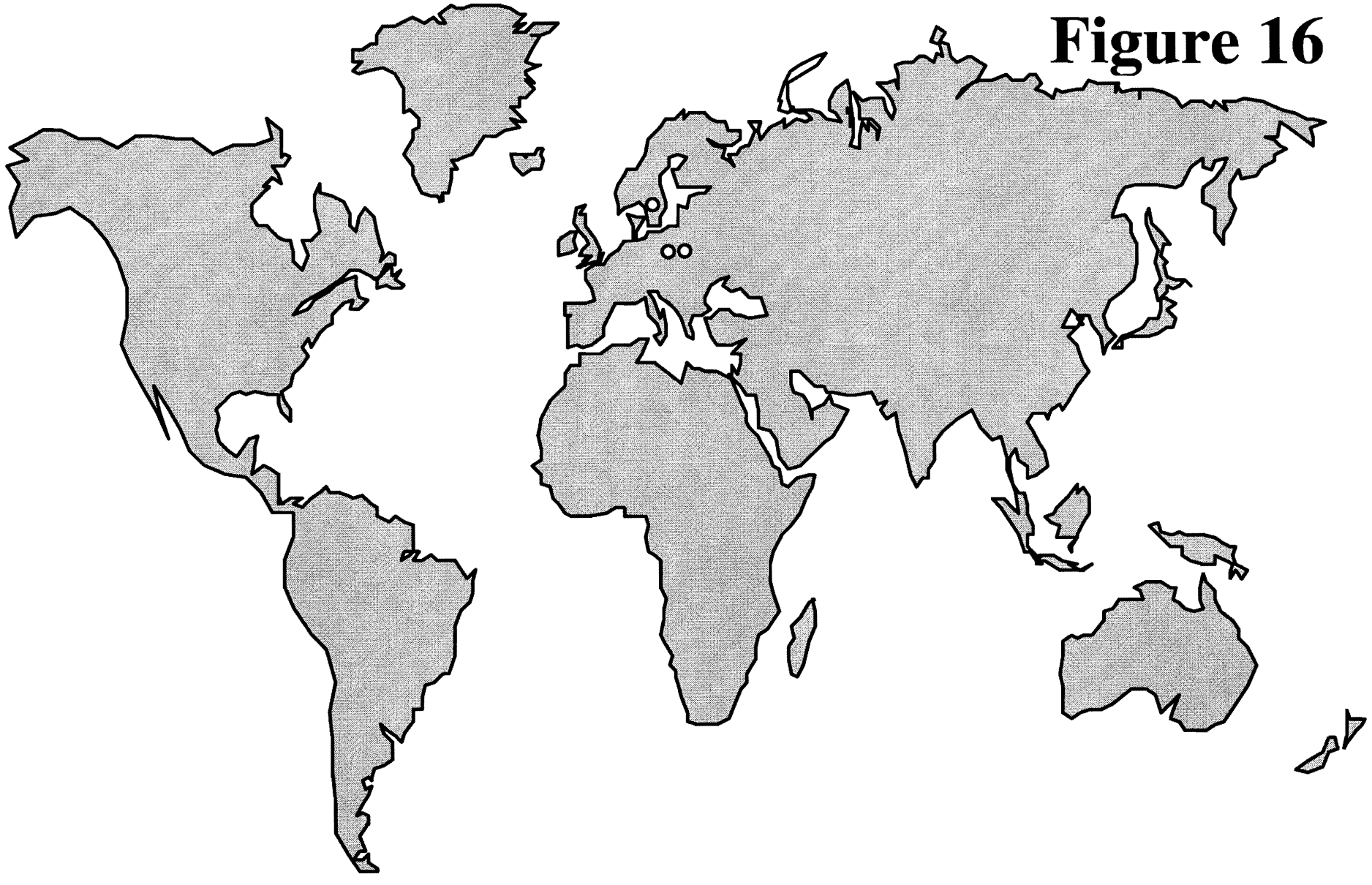
Figure 15



● No Group I Intron ○ Group I Intron ● Heterozygous

Introns within *Lentinellus omphalodes* (VIII) New Taxon

Figure 16



● No Group I Intron o Group I Intron ● Heterozygous

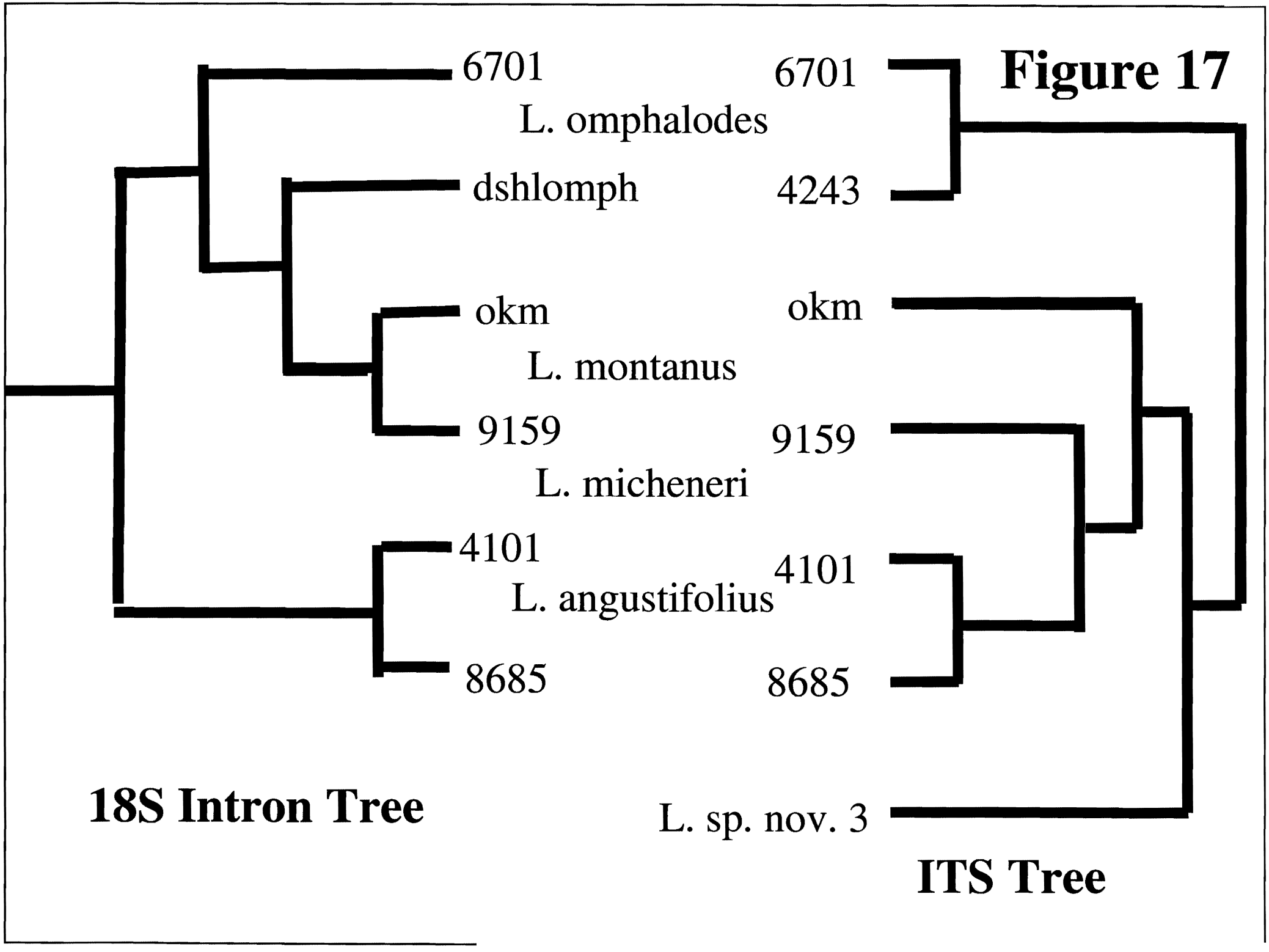

6701
L. omphalodes
dshlomph
okm
L. montanus
9159
L. micheneri
4101
L. angustifolius
8685
18S Intron Tree
6701
4243
okm
9159
4101
8685
L. sp. nov. 3
ITS Tree

Figure 17

Figure 18

Panellus and *Lentinellus*
Percent Similarity: 65.719
7966 *Lentinellus* x 2675 *Panellus*

FIGURE 19

CLAVICORONA AND *LENTINELLUS*
PERCENT SIMILARITY: 67.097
4242 *Clavicorona* X 9985 *Lentinellus*

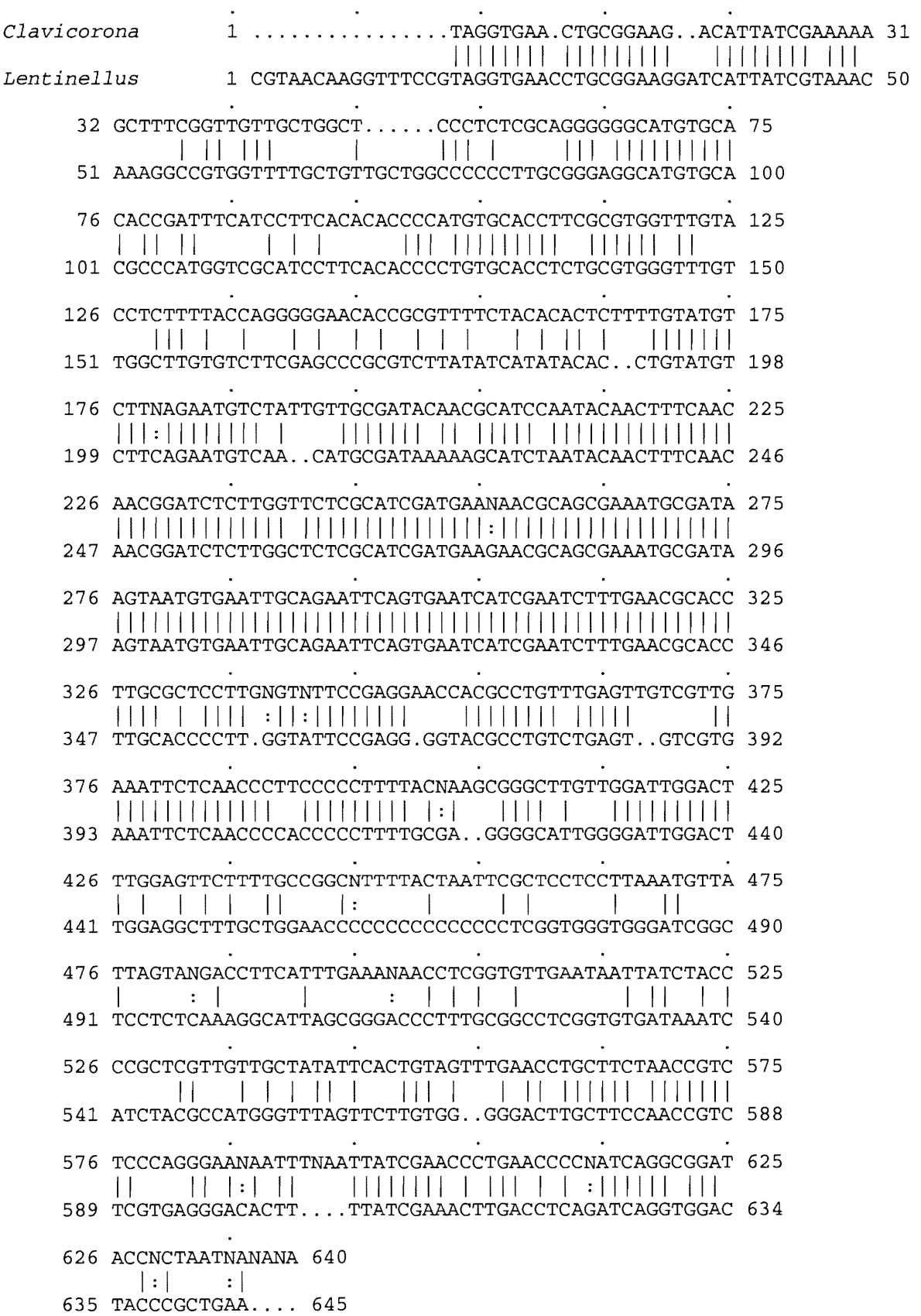


Figure 20

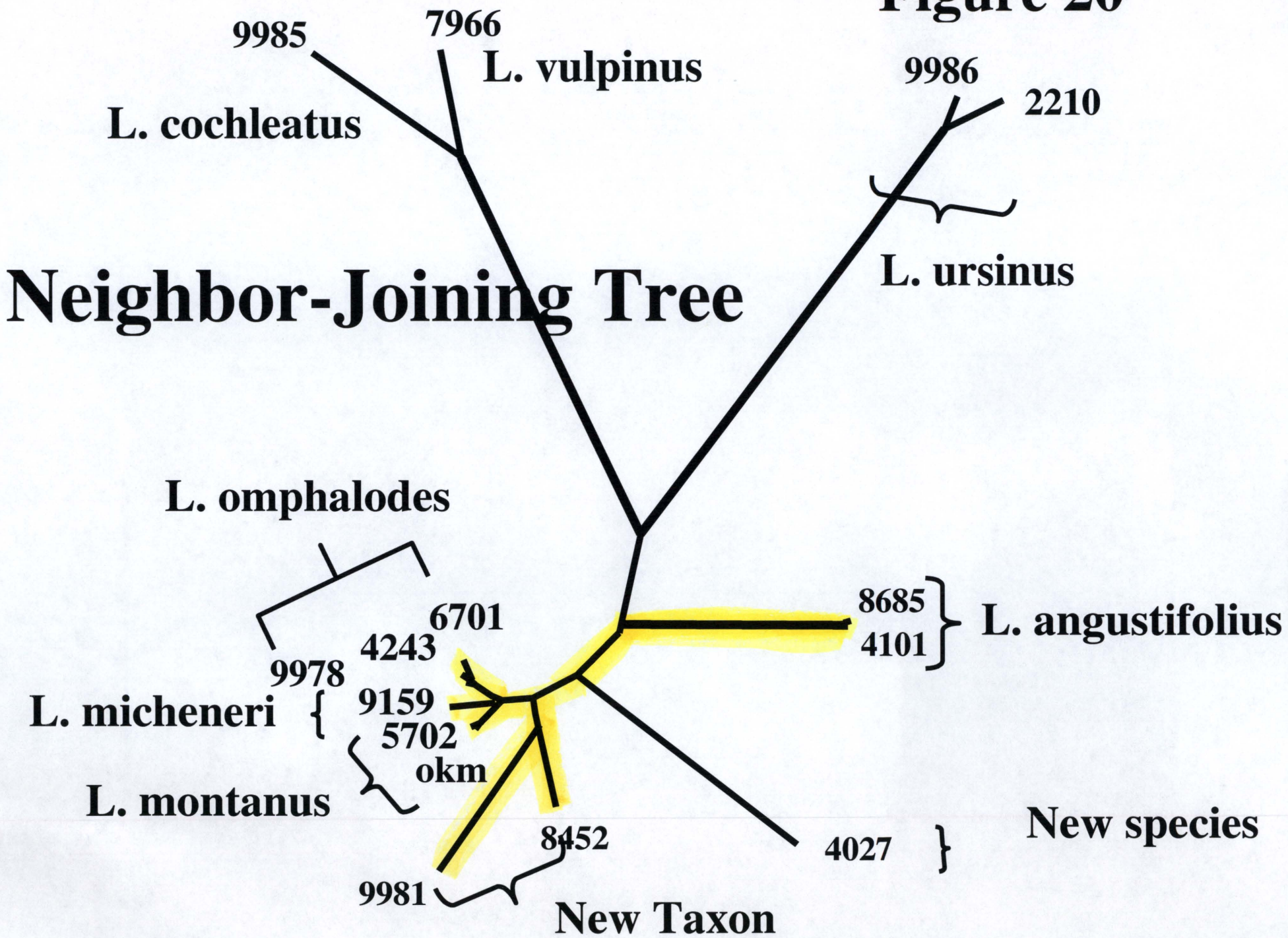

9985
7966
L. vulpinus
L. cochleatus
9986
2210
L. ursinus
Neighbor-Joining Tree
L. omphalodes
6701
4243
9978
L. micheneri
9159
5702
okm
L. montanus
8685
4101
L. angustifolius
8452
9981
4027
New species
New Taxon

Figure 21

L. vulpinus and *L. cochleatus*
PERCENT SIMILARITY: 91.339
L. vulpinus 7966 X *L. cochleatus* 9985