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The fungal ascomycete genus *Otidea* as represented in the Pacific Northwest of North America includes eight well known species and two poorly-known species. Cladistic analysis utilizing characters from ribosomal DNA and morphology allowed the development of a robust phylogenetic species concept for *Otidea*. The combination of characters from the ribosomal DNA internal transcribed spacer region and those characters coded from morphological descriptions proved both useful and robust towards the elucidation of a phylogenetic species concept. The resulting phylogenetic species concept was then applied to known members of the genus, and species descriptions revised to account for additional data collected in the course of this study. The following species of *Otidea* are described in detail in this work: *O. alutacea, O. concinna, O. leporina, O. onotica, O. rainierensis, O. smithii, O. tuomikoskii* and *O. umbrina*. Revised keys to the genus for both fresh and dried material are presented in Chapter Three. *O. alutacea, O. leporina, O. rainierensis, O. smithii* and *O. umbrina* are reported as rare in the Pacific Northwest due to the relatively small number of collections made of each in the course of this study.

Systematics of the genus *Otidea* in the Pacific Northwest

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Dr. Joseph Spatafora was involved in the design, molecular analysis, and writing of each manuscript. Additionally, all molecular work was performed in the lab of Dr. Spatafora. Dr. Nancy Weber was involved in the design, taxonomic analysis and description, and writing of chapter three (Revisional monograph of the genus *Otidea* in the Pacific Northwest).

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INTRODUCTION

The genus *Otidea* in the Pacific Northwest has been among the many taxa of concern to land managers and mycologists since the publication of the Forest Management and Assessment Team (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994) report on the habitat of the Northern Spotted Owl. This document, written to deal with rare and endangered species of animals, plants, and fungi associated with mature and old growth forests of the Pacific Northwestern U.S.A., states that *Otidea smithii*, *O. leporina*, and *O. onotica* are "species of concern" due to their apparent rarity and that a general survey must be taken for all three of these species before federally- and state-owned property slated for forest management activities can be approved. In addition, if *O. smithii* is found, 160 acres surrounding each population must be removed from any ground disturbing activity (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994). Implementation of this directive requires accurate identification of specimens of *Otidea*.

The only published North American work solely on the genus, Kanouse (1949), includes descriptions generated primarily from dried collections and original notes on those collections housed in the University of Michigan fungal herbarium. Kanouse's descriptions relied heavily on other worker's assessment of color and shape in the fresh state. She described one new variety and two new species, having previously described *O. smithii* (Kanouse, 1939). *O. alutacea* var. *microspora*, was separated from *O. alutacea* var. *alutacea* by paler apothecial color and slightly smaller spores (characters

that could be attributed to immaturity in collections). Kanouse (1949) separates *O. rainierensis* and *O. kauffmanii* from other members of the genus, but differentiates the pair by "size, shape and color of the apothecia and also differences in spore size" and differences in distribution; *O. kauffmanii* is reported from Michigan, *O. rainierensis* from Washington. However, if one considers the high degree of morphological plasticity and ecological variability attributed to the fungi (Alexopolus, Mims and Blackwell, 1996) and the poorly documented geographic distribution of most species, it is plausible that *O. kauffmanii* and *O. rainierensis* are synonyms. Difficulty associated with identification of these and other species of *Otidea* warranted reassessment of species concepts within the genus.

The working assumption for this study was that combining characters generated from molecular sequence data with morphological characters in phylogenetic studies would allow the generation and testing of robust phylogenetic hypotheses regarding evolution and relationships within the genus. Moreover, by constructing cladistic phylogenies one can infer patterns of character evolution in the genus and establish a phylogenetic species concepts for members of the genus (Donoghue, 1985; Kluge, 1985). The revision of the species concept within *Otidea*, presented in Chapter Two of this work, allows a reassessment of character state distribution within individual species and provide information for the taxonomic revision contained in Chapter Three of this thesis.

This introductory chapter covers a brief summary of the taxonomy of discomycetes in general and the genus *Otidea* specifically, and reviews important aspects of fungal molecular systematics. In addition, I will discuss the idea of a modern synthesis of molecular and morphological methods and how the results of this project

apply to both the genus *Otidea* and other groups of fungi. Details regarding the molecular systematics of the group will be presented in Chapter Two, and those regarding morphology and taxonomy in Chapter Three.

Morphology:

The discomycetes, or "cup-fungi", have been studied since Roman times, when Pliny (23-79 A.D.) described an organism "Belonging to the mushroom kind, also there is a species known to the Greeks by the name 'Pezica' which grows without root or stalk." (Kimbrough, 1970). Among early contributors to information about the cup fungi were Linnaeus (1753) Persoon (1796; 1801; 1822), and Fries (1822). While establishing groups based primarily on morphology of the apothecium, Fries contributed heavily to the study of the discomycetes by establishing an ordinal, subordinal, and familial classification system (Kimbrough, 1970). The Friesian system is still used and advances in the field made clear the importance of microscopic characters. Most notable were the contributions of Nylander (1869), who stressed the importance of the iodine reaction with the tip of the asci, and Crouan and Crouan (1857), who described the operculate nature of the asci. Boudier (1907) proposed a classification based on the presence or absence of ascal operculum, as well as several other macro- and microscopic characters. He was the first to separate the cup-fungi into the operculate and inoperculate discomycetes (Kimbrough, 1970; van Brummelen, 1994). According to van Brummelen (1994), Saccardo abandoned the Friesian system for his own that placed more emphasis on ascospore characters. Numerous rearrangements of taxa at higher levels continued until

Nannfeldt (1932) proposed three main groups of higher Ascomycetes: the Plectascales, Ascoloculares, and the Ascohymeniales. According to Kimbrough (1970), Nannfeldt's work provided a foundation for much of the work done at the generic level from the 1940's to the 1970's (e.g. Le Gal (1947), Rifai (1968), Eckblad (1968), Korf (1973)). Eckblad (1968) divided the Pezizales into the families Thelebolaceae, Ascobolaceae, Pyronemataceae, Pezizaceae, Helvellaceae, Morchellaceae, Rhizinaceae, Sarcoscyphaceae, and Otideaceae based primarily on morphological and anatomical characters of the excipulum. Trappe (1979) emended several families in the Pezizales to include hypogeous fungi in his re-examination of the Tuberales, and distributed most members of this "anachronistic order" in various families of the Pezizales, based primarily upon microscopic characters. A much more thorough treatment of the early history presented above is available in Kimbrough's work, *Current Trends in the Classification of Discomycetes* (1970) and van Brummelen's chapter in *Ascomycete Systematics: Problems and Perspectives in the Nineties* (1994), and a brief, but detailed synopsis of later work is available in Landvik, et al. (1997).

In regard to the operculate discomycetes (Pezizales), of which *Otidea* is a member, definition of some of the basic concepts will make the following chapters more readable. The following information follows Weber, Trappe, and Dennison (1997) and Korf (1973) unless otherwise noted. The ascocarp of the operculate discomycetes is said to be "in its commonest form, a typical apothecium, taking the shape of a saucer, a cup, or a nearly closed sphere" (Korf, 1973). A great deal of variation can be observed within the order, but most epigeous members have an exposed hymenial layer composed of asci surrounded by sterile elements (paraphyses). In most hypogeous members ascribed to the

Pezizales by Trappe (1979) the apothecium is not an open, cup-shaped structure and the ascospores are not, with the exception of *Geopora* (Trappe, 1979), forcibly discharged from the asci at maturity. Rather the asci are cylindric to globose, often enclosed in an epithelium or embedded in tramal tissue, and contained within subglobose to globose fruiting structures of various forms but mostly not apothecia. Several early classification systems were based on the macro-morphology of the ascoma, but this character can vary considerably at lower taxonomic levels, a fact that led Eckblad to conclude that within the Pezizales this character is of "low taxonomic value" (1968). The tissues of the epigeous apothecia are the hymenium, subhymenium, medullary excipulum, and ectal excipulum (from inside to outside). Shape, size, and structure of each of these layers varies a great deal among the discomycetes but their morphology is generally similar among species within a genus. Some authors place importance on the ectal excipulum, especially the presence or absence of hairs (Kimbrough, 1970). The excipular layers can be described as being of seven basic types, *textura globulosa* (Fig. 1.1a), *t. angularis* (Fig. 1.2b), *t. prismatica, t. intricata* (Fig. 1.3c), *t. epidermoidea, t. oblita,* and *t. porrecta*, first described by Stärback and diagramed by many authors including Eckblad (1968, his Figure 2) and Korf (1973, his Figure 3).

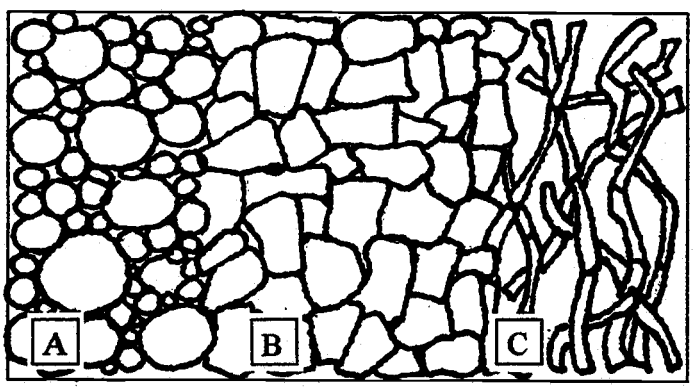

Figure 1.1: Types of excipular layers

The description of ascal dehiscence given by Crouan and Crouan (1857) remains important to the taxonomy of the discomycetes. They described the asci in the Pezizales as typically having an apical or subapical pore that opens like a trap door when the spores are discharged. The asci of the Pezizales are all unitunicate, and, while some authors have indicated that this "single-coated" wall is actually two-layered (Kimbrough, 1970), these layers never separate at spore discharge (Korf, 1973). Additionally, several authors have noted that the apex of the ascus varies enough within the Euascomycetes (ascocarp-forming Ascomycetes) to be phylogenetically informative at higher taxonomic levels (Samuelson, 1978). The ascospores of operculate discomycetes are also a source of extremely important characters, especially to delimit genera or species within a genus. Le Gal emphasized spore characters, believing that "spore characters should take precedence of all other characters" (1947). Eckblad (1968), however, stated that caution is necessary and that no segregation should be made based on spore form only. The spores are unicellular; smooth or variously ornamented; hyaline, brown, or rarely purple; with bipolar or radial symmetry. Many groups possess ascospores with obvious contents, e.g., oil droplets (guttules) (Kimbrough, 1970). Most ascospores of the Pezizales are uninucleate (excepting the Sarcoscyphaceae, Helvellaceae, Rhizinaceae, Discinaceae, Tuberacaeae, and Morchellaceae) (Kimbrough, 1970; O'Donnell, et al., 1997). This character is typically consistent within several groups, and thus thought to be phylogenetically informative (Korf, 1973).

Paraphyses are sterile hyphae interspersed among the asci in most operculate discomycetes. They vary in form throughout the group but usually are "filiform to clavate, sometimes lance-shaped, pyriform, curved, hooked or branched, and most always

septate" (Kimbrough, 1970). Within the Otideaceae paraphyses can be extremely useful for differentiating genera and individual species (e.g., the hooked paraphyses of *Otidea*).

Additionally, pigments in the discomycetes are useful for identification at higher taxonomic levels. Arpin (1968) studied carotenoid pigments in nearly 100 species of discomycetes. His work led to the creation of the Aleuriaceae within the Pezizales (from taxa formerly placed in the Ciliarieae and Humarieae *sensu* Le Gal) and reported an absence of carotenoid pigments in the Morchellaceae, Helvellaceae, Pezizaceae, and Otideaceae.

Taxonomy and nomenclature of *Otidea*:

Members of the genus *Otidea* are described as having roughly ear-shaped apothecia that are yellow, brown, or orange in color. While ascomal shape varies from species to species, all members of the genus *sensu stricto* have a split in the apothecium on one side giving the ascoma an irregular appearance (Figure 1.2). Microscopically members of the genus typically have smooth, biguttulate ascospores and hooked paraphyses, although exceptions do occur. Additional information regarding the taxonomy and nomenclature of the genus is presented in Chapter Three.

Figure 1.2: *Otidea tuomikoskii* showing the irregular appearance of the ascoma observed in the genus.

Molecular systematics:

Despite the integral roles fungi play in most terrestrial ecosystems, little is known about their evolutionary history as compared to other groups of eukaryotes (e.g., plants and animals). Problems of assessing characters and character state homology have plagued historical studies of the fungi and have resulted in contradictory classification schemes (Bruns, White and Taylor, 1991). Recently developed methods of molecular systematics offer new approaches to study fungal evolution. Specifically, development of objective criteria for analyzing data, increases in computational power to conduct analyses, and the ability to rapidly generate large data sets of nucleotide characters which are independent of morphology have allowed molecular characters to become integral components of study in fungal systematics (Hibbett and Donoghue, 1998; Bruns, White and Taylor, 1991; Hibbett, 1992; Hillis, Huelsenbeck and Cunningham, 1994). Many different methods are available to compare fungal organisms, including DNA-DNA hybridization, restriction enzyme analysis, electrophoretic karyotyping, fungal diagnostics, and nucleotide sequencing (Hibbett, 1992; Bruns, White and Taylor, 1991). Of these, nucleotide sequencing has been embraced by many authors due to the relative ease of generating large numbers of phylogenetically informative characters. Also, the polymerase chain reaction (PCR, Saiki, et al., 1988; Mullis and Faloona, 1987) relies on a relatively small amount of DNA template (typically around 1-10 μg), thereby facilitating the study of rare or difficult-to-isolate organisms (Bruns, White and Taylor, 1991). Because many of the genes encoded by genomic DNA are ubiquitous, homologous data can be compared across taxa of varying morphology (Bruns, White and Taylor, 1991).

While phylogenetic studies of nucleotide characters have become commonplace within fungal systematics, Hibbett is careful to point out that "the characters themselves are only as good as the method of analysis" (1992).

Several authors have discussed the need to study a gene or gene region that is appropriate to answer the question of interest (Bruns, White and Taylor, 1991; Hibbett, 1992; Kohn, 1992; Lutzoni and Vilgalys, 1995). Bruns, et al. (1991) cite the following factors to consider when selecting a nucleotide region for phylogenetic analysis: (1) The region should be evolving at an appropriate rate to answer the question of interest. (2) The region should either be present as, or evolve as, a single copy within the genome. (3) The region should have the same function in all the organisms being studied. (4) The effect of codon bias (if applicable) and base composition should be examined, to prevent a distorted estimate of sequence divergence. Both Bruns, et al. (1991) and Hibbett (1992), as well as many others, have suggested the genes which encode for the ribosomal RNA (rDNA) satisfy the above criteria extremely well. rDNA consist of 5.8S, 18S, and 25S subunits that are transcribed along with internal and external transcribed spacer regions (ITS and ETS). The DNA that comprises these units is present in multiple copies as tandem repeats within the genome, but the multiple copies evolve in unison and therefore act as a single copy gene (Hibbett, 1992). In addition, and most importantly, each region of the rDNA unit appears to evolve at a different average rate, thereby providing characters most appropriate to answer questions at different taxonomic levels (Hibbett, 1992; Bruns, White and Taylor, 1991). Hibbett and other authors suggest that, in general, the 18S and 25S regions are most appropriate to answer high to moderate level taxonomic questions (how are families, orders, and perhaps genera related), and that the

ITS regions flanking the 5.8S region are most appropriate to answer lower-level taxonomic questions (how are these species, or populations of a single species, related?) (Hibbett, 1992; Liston, et al., 1996; Downie, et al., 1998). For a much more thorough treatment regarding rDNA see Hibbett (1992).

Numerous workers have used rDNA nucleotide data to answer taxonomic questions about fungi. Molecular characters provide an independent source of data from which phylogenetic hypotheses can be generated to test morphological hypotheses (Blackwell and Spatafora, 1994; Spatafora and Blackwell, 1994; Bruns, White and Taylor, 1991; Lutzoni and Vilgalys, 1995). New hypotheses have been developed regarding relationships amongst the fungi (Bruns, White and Taylor, 1991), classes of ascomycetes (Berbee and Taylor, 1992), the discomycetes (Gargas and Taylor, 1995; O'Donnell, et al., 1997), the origin of the lichen symbiosis (Gargas, et al., 1995), as well as a variety of lower-level taxonomic questions (see Hibbett and Vilgalys, 1993; Vilgalys and Sun, 1994; Lutzoni and Vilgalys, 1995; Hibbett, et al., 1995; Kretzer, et al., 1996; Harrington, 1998). The current trend in fungal systematics is to combine independent data sets, particularly from molecular and morphological sources, to infer the most robust phylogeny possible (Hibbett and Vilgalys, 1993; Lutzoni and Vilgalys, 1995; Tehler, 1994). Many authors are looking critically at the combinability of data and weighting schemes for characters within data sets to better resolve taxonomic relationships (Lutzoni and Vilgalys, 1995; Chippindale and Wiens, 1994; Bull, et al., 1993; Farris, 1969; Cunningham, 1997; Barrett, Donoghue and Sober, 1991). Additionally, molecular tools are now being used to assess rarity within fungal groups (Hibbett, et al., 1995; Kerrigan, et al., 1998).

To return the focus to *Otidea* and related groups, it makes sense to briefly examine how molecular sequence data has been used to examine hypotheses within the cup-fungi. Gargas and Taylor (1995) examined relationships within the "discomycetes" (*sensu* Korf (1973), those fungi having a cup-like apothecium, e.g., the class Discomycetes). Using characters from the rDNA small subunit (SSU), these authors tested the monophyly of the "discomycetes" and high-level taxonomic groups within this artificial taxonomic assemblage. Relevant to my work, they showed that the Pezizales are a monophyletic order (within their sampling) and basal within the discomycetes. The authors speculate, based on their most-parsimonious tree, that the Pezizales as a group may possess ancestral (primitive) characters within the ascomycetes. Spatafora (1995) confirmed the monophyly of the Pezizales, again using nucleotide characters from the rDNA SSU region, although his work did not unequivocally place the Pezizales as basal within the Discomycetes *sensu* Korf (1973). Rather, his work showed that the Pezizales were in a clade that was sister to two groups of cleistothecial and perithecial ascomycetes (Pyrenomycetes and Pleosporales) but that the root from the Saccharomycetes to several Euascomycete groups was equivocal, thereby questioning the ability of SSU rDNA alone to resolve the radiation within the Euascomycetes (Spatafora, 1995).

O'Donnell, et al. (1997) used nucleotide characters from the rDNA tandem repeat to study relationships between epigeous and hypogeous sporocarp-forming genera within a subset of the Pezizales (*sensu* Trappe, 1979). Although it was generally accepted that these hypogeous sporocarp-forming genera belong in the order Pezizales, relationships with epigeous genera were based on few characters, and familial assignments were provisional. These authors combined data from the SSU rDNA and a portion of the LSU

rDNA to test and refine phylogenetic hypotheses of epigeous and hypogeous evolution within the Pezizales. Their data showed a 9.5% nucleotide divergence in the SSU data set, and a 26.9% nucleotide divergence in the LSU data set. These values suggest that within the Pezizales that the SSU and LSU regions can be analyzed to answer questions at different taxonomic levels. Their combination yielded a single most-parsimonious tree with strong bootstrap support for most groupings already recognized based on morphology. The analysis provided a great deal of information regarding the initial question of the relationship between the epigeous and hypogeous Pezizales, enabling several changes in assignment of genera to families.

Addressing a similar problem as O'Donnell, et al. (1997), Landvik, Egger, and Schumacher (1997) evaluated subordinal relationships within the Pezizales. They examined 37 taxa representing as many genera placed within the Pezizales *sensu lato*. Theirs is the most recent work on the Pezizales and the only one that examines hypotheses about relationships within the Otideaceae by use of nucleotide characters. They address the taxonomic difficulties encountered within the Otideaceae, the family being a figurative "dumping ground" for taxa that are not readily classified elsewhere. Within their sampling of the Otideaceae *sensu* Hawksworth, et al. (1995), high bootstrap support was shown for the grouping of *Otidea* with *Trichophaea*, *Aleuria, Scutellinia*, *Byssonectria* and *Pyronema*. Additionally, *Otidea* was shown to be basal in this assemblage in one of their four most parsimonous trees (their Figure 1), although this relationship collapsed into unresolved or poorly resolved polytomies in their consensus trees (their Figures 2 & 3). This work provides support for the wide range of characters and character states exhibited by the family as currently recognized. Their work also

suggests that since *Pyronema* is embedded within this assemblage, the proper name for the family is actually Pyronemataceae (since the Pyronemataceae was erected first, in 1842), but they stop short of recommending we use this name preferentially, noting that the group is both large and poorly sampled to date. While these authors sampled *Otidea* in their work no new information about the genus was provided.

Synthesis of morphological and molecular methodologies:

To address the taxonomic problems within the genus *Otidea*, a modern synthesis of morphological and molecular methods was employed in my work. Combining the traditional methods of ascomycete taxonomy with newer methods of molecular systematics will permit a phylogenetically-based system of classification to be proposed and tested for the genus. This new classification will in turn facilitate the refinement of descriptions and tools for identification, making it easier to identify members of the genus. More accurate identifications will allow for better assessments of rarity within the range of the Northern Spotted Owl, and this new combination of systematic methods will then contribute to practical ends, those of conservation biology and land-use concerns.

Phylogenetic analysis:

The synthesis of morphological and molecular methods involves the combining of these two seemingly independent sets of characters into a combined parsimony analysis. Before this combination can be undertaken several potential problems must be considered. The problems of positional homology and gap coding in use of nucleotide

characters and those of definition of characters and character states of morphological characters must be addressed. Additionally, the combination of data sets and the weighting of characters within and between data sets is currently an active area of research in systematics.

The first step after nucleotide data are collected is to generate a sequence alignment for the taxa of interest. The sequence alignment is the basis of the phylogeny, but this step is poorly understood and often overlooked (Swofford, et al., 1996; Wheeler, Gatesy and DeSalle, 1995). For sequences to be useful in phylogenetic analysis *positional homology* must be attained to the highest degree possible. Positional homology is the idea that in a sequence, at any given site, all nucleotides at that position can trace their ancestry to a single character that occurred in a common ancestor. *Homoplasy*, as opposed to homology, is the idea that nucleotides at a site appear to be homologous but have arisen due to parallelism, reversal, or convergence instead of common ancestry (Swofford, et al., 1996). To assure positional homology it is often necessary to place gaps in the alignment. These gaps are hypothesized to have originated from insertion or deletion events in the organism's evolution. Gaps can be treated either by ignoring positions that contain them (in all taxa in the analysis), by coding them as a new state (fifth nucleotide), or by creating multiple sequence alignments and "eliding" (Wheeler, Gatesy and DeSalle, 1995) the sequences to down-weight gaps (as well as ambiguous sites in the alignment). Swofford, et al. (1996) state that since the mechanism that causes insertion and deletion events in nucleotide sequences is not well understood mechanistically, they suggest removing gaps from phylogenetic analysis, either by pairwise deletion (in two taxa being compared) or complete deletion (the site in question

in all taxa). Other authors propose treating insertion/deletion events as a new state, or fifth nucleotide (Downie, et al., 1998; Bruns, et al., 1992). Keeping gaps in the analysis recognizes that, even if we do not fully understand them, the events leading to the insertion or deletion of nucleotides are part of the organism's evolution. Wheeler, et al. (1995) point out that removal of gaps can result in "robust but grossly unresolved hypotheses of relationships" (citing their own work). For this reason they advocate keeping the gaps and creating multiple sequence alignments by assigning different gap penalties, and then combining the alignments into one "grand alignment" (elision, their term).

Conservation concerns:

When the final phylogenetic analysis is complete, and a more robust hypothesis regarding evolutionary relationships within *Otidea* in the Pacific Northwest has been generated, these data can be used to better assess rarity within the genus. By clearly stating a hypothesis about the number of species in the genus and their characteristics, additional steps will have been taken to enable the survey and management of rare and threatened species of *Otidea* and the habitat in which they occur. This work can serve then as a model for taxonomists to use when working with nomenclaturally difficult and morphologically plastic genera.

My research will help to clarify the following taxonomic questions in Chapter Three of this manuscript. (1) What species is *O. leporina* as listed in the FEMAT Record of Decision (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994)? The FEMAT report lists *O. leporina* as a strategy three fungus (extensive survey must be taken before forest

management activity can occur), but includes no varietal name. This work will clarify the relationship between what must be *O. leporina* (Batsch) Fuckel var. *leporina* (= var. *typica* Kanouse) and *O. leporina* (Batsch) Fuckel var. *minor* Rehm, as well as help determine which taxon, if either, is actually rare in the range of the Northern Spotted Owl. Preliminary results indicate that the two morphotypes are quite divergent in terms of ITS sequence similarity, suggesting the presence of cryptic species. (2) What names should be applied to the two provisional new species identified in the course of this study? In the course of this work, one species that was unknown to Nancy Smith Weber was collected on several occasions. This fungus is quite distinct morphologically and forms a natural (monophyletic) group separate from other species of *Otidea* in the phylogenetic analysis. A second species, that superficially resembles *O. rainierensis* but has significantly larger spores has also been collected, and this species also forms a monophyletic group in my analysis. Have these species been described from other parts of the world? If so, by what names? If not, what new names should be applied to them? (3) For the rest of the *Otidea* spp. in the Pacific Northwest, what are the best names to apply to each, based on original morphological descriptions? By using the phylogenetic hypotheses in combination with original descriptions (obtained thanks to the staff at the Farlow Herbarium, Harvard University) we hope to answer this question, as well as comment on how names have been misapplied in the past.

References

Alexopolus, C. J., C. W. Mims, M. Blackwell. 1996. *Introductory Mycology*. John Wiley & Sons, Inc., New York.

Arpin, N. 1968. *Les Carotenoides de Discomycetes: Essai Chimiotaxonomique*, pp. 170. University of Lyons, Villeurbanne, France.

Barrett, M., M. J. Donoghue, E. Sober. 1991. Against consensus. *Systematic Zoology* 40 (4): 486-493.

Berbee, M. L., J. W. Taylor. 1992. Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequences. *Molecular Biology and Evolution* 9 (2): 278-284.

Blackwell, M., J. W. Spatafora. 1994. *Molecular data sets and broad taxon sampling in detecting morphological convergence*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 243-248. Plenum Press, New York.

Brummelen, J. v. 1994. *Problems in the systematics of the Pezizales*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 303-314. Plenum Press, New York.

Bruns, T. D., R. Vilgalys, S. M. Barns, D. Gonzales, D. S. Hibbett, D. J. Lane, L. Simon, S. Stickel, T. M. Szaro, W. G. Weisburg and M. L. Sogin. 1992. Evolutionary relationships within the fungi: Analysis of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution* 1 (3): 231-241.

Bruns, T. D., T. J. White, J. W. Taylor. 1991. Fungal Molecular Systematics. *Annual Review of Ecology and Systematics* 22 : 525-564.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42 (3): 384-397.

Chippindale, P. T., J. J. Wiens. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. *Systematic Biology* 43 (2): 278-287.

Crouan, P. I., H. M. Crouan. 1857. Note sur queleues *Ascobolus* nouveaux et sur une espece nouvelle de *Vibrissea*. *Annales des Sciences Naturelles. A. Botanique* IV (7): 173-178.

Cunningham, C. W. 1997. Is congruence between data partitions a reliable predictor of phylogentic accuracy? Empirically testing an iterative proceedure for choosing among phylogenetic methods. *Systematic Biology* 46 (3): 464-478.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 88 : 172-181.

Downie, S. R., S. Ramanath, D. S. Katz-Downie, E. LLanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *RPOC1* intron sequences. *American Journal of Botany* 85 (4): 563-591.

Eckblad, F.-E. 1968. The Genera of Operculate Discomycetes. *Nytt Magasin for Botanikk* 15 (1-2): 1-191.

Farris, J. S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18 : 374-385.

Fries, E. M. 1822. *Systema Mycologicum*. II. Greifswald.

Gargas, A., P. T. DePriest, M. Grube, A. Tehler. 1995. Multiple origins of lichen symbioses in fungi suggested from SSU rDNA phylogeny. *Science* 268 : 1492-1495.

Gargas, A., J. W. Taylor. 1995. Phylogeny of discomycetes and early radiations of the apothecial Ascomycotina inferred fron SSU rDNA sequence data. *Experimental Mycology* 9 : 7-15.

Harrington, F. A. 1998. Relationships among *Sarcoscypha* species: evidence from molecular and morphological characters. *Mycologia* 90 (2): 235-243.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, P. N. Pegler. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*. CAB, London.

Hibbett, D. S. 1992. Ribosomal RNA and fungal systematics. *Transactions of the Mycological Society of Japan* 33 : 533-556.

Hibbett, D. S., M. J. Donoghue. 1998. Integrating phylogenetic analysis and classification in fungi. *Mycologia* 90 (3): 347-356.

Hibbett, D. S., Y. Fukumasa-Nakai, A. Tsuneda, M. J. Donoghue. 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. *Mycologia* 87 (5): 618-638.

Hibbett, D. S., R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Systematic Botany* 18 (3): 409-433.

Hillis, D. M., J. P. Huelsenbeck, C. W. Cunningham. 1994. Application and accuracy of molecular phylogenies. *Science* 264 : 671-677.

Kanouse, B. B. 1939. Notes on new or unusual discomycetes. *Papers of the Michigan Academy of Science, Arts & Letters* 24 : 28.

Kanouse, B. B. 1949. Studies in the genus *Otidea*. *Mycologia* 41 : 660-677.

Kerrigan, R. W., D. B. Carvalho, P. A. Horgen, J. B. Anderson. 1998. The indigenous coastal Californian population of the mushroom *Agaricus bisporus*, a cultivated species, may be at risk of extinction. *Molecular Ecology* 7 : 35-45.

Kimbrough, J. W. 1970. Current trends in the classification of discomycetes. *The Botanical Review* 36 (2): 92-161.

Kluge, A. G. 1989. A concern for evidence and phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Systematic Zoology* 38: 7-25.

Kohn, L. M. 1992. Developing new characters for fungal systematics: An experimental approach for determining the rank of resolution. *Mycologia* 84 (2): 139-153.

Korf, R. P. 1973. *Discomycetes and Tuberales*. In: Anisworth, Sparrow, Sussman (eds) *The Fungi IVA: An advanced treatise*, pp. 249-319. Academic Press, New York, New York.

Kretzer, A., Y. Li, T. Szaro, T. D. Bruns. 1996. Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: Phylogenetic and taxonomic implications. *Mycologia* 88 (5): 776-785.

Landvik, S., K. N. Egger, T. Schumacher. 1997. *Towards a subordinal classification of the Pezizales (Ascomycota)*, PhD Thesis in part, Umea University, Sweden. pp. 1-29.

Le Gal, M. 1947. Recherches sur les ornamentations sporales des Discomycetes opercules. *Annales des Sciences Naturelles. A. Botanique* XI (8): 73-297.

Linneaus, C. 1753. *Species Plantarum*. II. Laurentii Salvii, Stockholm.

Liston, A., W. A. Robinson, J. M. Oliphant, E. R. Alvarez-Buylla. 1996. Length variation in the nuclear ribosomal DNA internal transcribed spacer region of non-flowering seed plants. *Systematic Botany* 21 (2): 109-120.

Lutzoni, F., R. Vilgalys. 1995. Integration of morphological and molecular data sets in estimating fungal phylogenies. *Canadian Journal of Botany* 73 (Supplement 1): S649-S659.

Lutzoni, F., R. Vilgalys. 1995. *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichens. *Cryptogamic Botany* 5 : 71-81.

Mullis, K. B. and F. A. Fallona. 1987. Specific synthesis of DNA in vitro via polymerase-catalyzed chain reaction. *Methods of Enzymology* 155: 335-350.

Nylander, W. 1869. Observationes circa Pezizas Fenniae. *Notiser ur Saellskapets Pro Fauna et Flora Fennica Foerhandlinger* 10 : 1-97.

O'Donnell, K., E. Cigelnik, N. S. Weber, J. M. Trappe. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *Mycologia* 89 (1): 48-65.

Persoon, C. H. 1796. *Observationes mycologicae*. Petrum Phillippum [sic] Wolf, Leizig.

Persoon, C. H. 1801. *Synopsis Methodica Fungorum*. Henricum Dieterich, Gottingen.

Persoon, C. H. 1822. *Mycologia Europaea*. Joanni Jacobi Palmii, Erlangae.

Rifai, M. A. 1968. The Australasian Pezizales in the Herbarium of the Royal Botanic Garden Kew. *Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, afd. Natuurkunde* 57 (3): 1-295.

Saiki, R., D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. Horn, K. Mullis and H. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 : 487-494.

Samuelson, D. A. 1978. Asci of the Pezizales. II. The apical apparatus of representatives in the *Otidea-Aleuria* complex. *Canadian Journal of Botany* 56 : 1876-1904.

Schaffer, H. B., P. Meylan, M. L. McKnight. 1997. Tests of turtle phylogeny: Molecular, morpholgical, and paleontological approaches. *Systematic Biology* 46 (2): 235-268.

Spatafora, J. W. 1995. Ascomal evolution of filamentous ascomycetes: Evidence from molecular data. *Canadian Journal of Botany* 73 (Supplement 1): S811-S815.

Spatafora, J. W., M. Blackwell. 1994. *Cladistic analysis of partial ssrDNA sequences among unitunicate perithecial ascomycetes and its implacations on the evolution of centrum development*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 233-242. Plenum Press, New York.

Swofford, D. L., G. J. Olsen, P. J. Waddell, D. M. Hillis. 1996. *Phylogenetic Inference*. Sinauer Associates, Inc, Sunderland, Massachusetts.

Tehler, A. 1994. Morphological data, molecular data, and total evidence in phylogenetic analysis. *Canadian Journal of Botany* 73 (Supplement 1): S667-S676.

Trappe, J. M. 1979. The orders, families, and genera of hypogeous ascomycotina (truffles and their relatives). *Mycotaxon* 9 (1): 297-340.

U.S.D.A. F.S., U.S.D.O.I. B.L.M. 1994. *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents within the Range of the Northern Spotted Owl*, U.S. Government Printing office. 74p. Plus Attachment A: Standards and Guidelines.

Vilgalys, R., B. L. Sun. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. *Proceedings of the National Academy of Science* 91 : 4599-4603.

Weber, N. S., J. M. Trappe, W. C. Denison. 1997. Studies on Western American Pezizales. Collecting and describing ascomata -- Macroscopic features. *Mycotaxon* 61 : 153-176.

Wheeler, W. C., J. Gatesy, R. DeSalle. 1995. Elision: A method for accomodating multiple molecular sequence alignments with alignment-ambiguous sites. *Molecular Phylogenetics and Evolution* 4 (1): 1-9.

Chapter 2

CLADISTIC ANALYSIS OF THE GENUS *OTIDEA* IN THE PACIFIC NORTHWEST

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Abstract

The genus *Otidea* in the Pacific Northwest is reported to include as many as 10 species. Collections were made over a two year period in Northern California, Idaho, Oregon and Washington to examine morphological diversity in the genus. Sequence data was generated for the ribosomal DNA internal transcribed spacer (ITS) and large subunit (LSU) regions from 37 and 28 collections, respectively. Data were also generated by coding micro- and macroscopic characters. Two different methods were examined for coding continuous data into discreet, phylogenetically useful characters: generalized gap-coding and Duncan's Multiple Range Test. The partition homogeneity test as implemented in PAUP* was used to examine combinability of individual data sets. It was discovered that the ITS and LSU regions were heterogenous due to the presence of three different LSU types that apparently were derived from at least two ancient gene-duplication events. The ITS and morphological data were determined to be homogenous and combined in our final analysis. Eight phylogenetic species were determined to occur in the Pacific Northwest and relationships between these species are described.

Introduction

The genus *Otidea* in the Pacific Northwest includes eight species that are difficult to identify. Compounding this problem is their rarity. Three species of *Otidea* are currently recognized as being "species of concern" under the U.S. Forest Service Forest Ecosystem Management Assessment Team's 1994 Record of Decision regarding the habitat of the Northern Spotted Owl (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994). *O. smithii* Kanouse is listed as a strategy one fungus, requiring that 160 acres surrounding all known populations be excluded from all ground disturbing activities. *O. onotica* (Persoon) Fuckel and *O. leporina* (Batsch) Fuckel are strategy three fungi, requiring active survey for each fungus before any ground disturbing activity can occur. All three species are superficially easy to identify, but this work proves more complicated when one considers the remainder of the genus as it occurs in the Pacific Northwest. The most recent key to all North American material was published in 1949 by Kanouse in which she treated 10 species from the Pacific Northwest.

Our work represents half of a regional monographic revision of members of *Otidea* as they occur in the Pacific Northwest. The working assumption was that combining characters generated from molecular sequence data with morphological characters in phylogenetic studies would allow the generation of robust phylogenetic hypotheses regarding evolution and relationships within the genus. Moreover, by coding and combining characters from both molecular and morphological data sets, one can infer patterns of character evolution in the genus and establish a phylogenetic species concept (Donoghue, 1985). Phylogenetic analysis of data collected from ribosomal DNA (rDNA)

nucleotides and coded from morphological descriptions allows inference of evolutionary relationships within the genus. Support for nomenclatural revision is presented based on the phylogenetic species concept; the results will be presented elsewhere (Peterson, et al., Chapter Three). Little has been written regarding the combination of molecular and morphological data in phylogenetic analysis of the fungi (but see Lutzoni and Vilgalys, 1995; Hibbett and Vilgalys, 1993; and Harrington, 1998). We examine four different strategies to code spore measurements (continuous data), the combination of these data with molecular data and the taxonomic implications of our combined analysis.

Materials and Methods

Materials

Specimens used in this study were collected and examined per Weber, et al. (1997). Fresh material was used whenever possible to minimize errors in measurement caused by distortion due to drying (Baral, 1992). Collections were made throughout the Pacific Northwest in the Fall of 1996 and 1997 and Spring 1998. Fresh collections were supplemented with materials from Nancy Smith Weber's personal herbarium (NSW), the mycological collection of the Oregon State University herbarium (OSC; Holmgren, Holmgren and Barnett, 1990), and The University of Michigan Fungus Collection (MICH). Paratypes of *O. rainierensis* Kanouse, *O. kauffmanii* Kanouse, and *O. alutacea* var. *microspora* Kanouse and the holotype of *O. smithii* Kanouse were made available courtesy of Dr. Robert Fogel, curator of the mycological collection at MICH. GenBank accession numbers for all sequences are given in Table 2.1.

Table 2.1: Collections used in this study. (P) indicates paratype material and (H) indicates holotype. All type material provided by MICH.

Name	Herbarium Accession #	GenBank ITS Accession #	GenBank LSU Accession #
O. concinna	OSC 56749	AF072082	AF086592
	OSC 56760	AF072081	AF086591
	OSC 56809	AF072080	AF086590
	NSW 7574	AF072083	AF086594
O. leporina	OSC 56784	AF072077	AF086597
	OSC 56824	AF072079	AF086589
	OSC 56825	AF072078	AF086588
O. tuomikoskii	OSC 56826	AF072086	AF086596
	OSC 56756	AF072084	AF086594
	OSC 56761	AF072085	AF086595
O. rainierensis	OSC 56829	AF072087	AF086597
	NSW 6354	AF072088	AF086598
	OSC 56745	AF072089	AF086599
O. rainierensis (P)	EGS 2179 (MICH)	AF072093	
O. alutacea var. *microspora (P)*	AHS 30502 (MICH)	AF072094	
O. kauffmanii (P)	AHS 21147 (MICH)	AF072095	
O. grandis	ML 941947 (MICH)	AF072096	
O. smithii (H)	AHS 8843 (MICH)	AF072065	
O. smithii	OSC 56753	AF072062	AF086574
	OSC 56799	AF072063	AF086575
	OSC 56811	AF072060	AF086572
	OSC 56823	AF072061	AF086573
	OSC 56830	AF072092	
	NSW 7536	AF072064	AF086576
O. onotica	OSC 56734	AF072066	AF086577
	OSC 56759	AF072068	AF086579
	OSC 56801	AF072067	AF086578
O. alutacea	OSC 56747	AF072070	AF086580
	OSC 56754	AF072069	
	OSC 56770	AF072073	AF086585
	OSC 56777	AF072071	AF086582
	OSC 56798	AF072072	AF086583
O. umbrina	OSC 56758	AF072074	AF086581
	OSC 56782	AF072076	AF086586
	OSC 56813	AF072075	AF086584
Aleuria aurantia	OSC 56831	AF072090	AF086600
Scutellinia sp.	NSW 7387	AF072091	AF086601

Molecular data

Thirty-seven individual collections representing 10 species of *Otidea* were examined in this study. Ribosomal DNA (rDNA) internal transcribed spacer (ITS) sequence data was generated for 37 collections. rDNA large subunit (LSU) sequence data were generated for a subset of the collections (28 sequences total). In repeated attempts we were unable to amplify LSU sequences for specimens over 50 years old from MICH. ITS sequence data from these collections were collected by amplifying the ITS1 and ITS2 regions separately.

DNA was extracted from both fresh and dried collections by the following modification of the CTAB technique of Bult, et al. (1992). Thirty to fifty μg of material were carefully cleaned (surface sterilized) in bleach, 70% EtOH, and distilled H_2O. This material was then suspended in 500 μl CTAB (containing 0.5% B-mercaptoethanol) in 1.5 ml Eppendorf tubes (USA Scientific, Ocala, FL) and placed in a 65° C water-bath for 30 minutes. After 30 m the tubes were removed from the bath and the material was macerated with a Ryobi Cordless drill and reusable Kontes Pellet Pestles (Fisher Scientific, Pittsburgh, PA) that had been previously autoclaved. After maceration the material was returned to the water-bath for 30 m. The final step in the process was to further lyse the cells by submerging the Eppendorf tubes in ice-cold EtOH for 5 minutes, then return the tubes to the 65° bath for 5 minutes. The freeze/thaw process was repeated twice.

The resulting material was then extracted by a chloroform:IAA (24:1) extraction as described in Bruns, et al. (1990). When the interface was clear, 500 μl isopropyl alcohol was added to the supernatant and the mixture was spun in a microcentrifuge for

15 m at 12,000 rpm. The supernatant was then poured off and the resulting pellet cleaned by adding 500 µl 70% EtOH and spinning for 15 m. Cleaned DNA pellets were then resuspended in 20 µl dd H_2O, heated in a 55° water-bath for 5 m, and vortexed for 30 s.

Specific regions of DNA were amplified via the PCR reaction (Saiki, et al., 1988; Mullis and Faloona, 1987) with the following primer pairs: ITS4 and ITS5, LR0R and LR3 (White, et al., 1990; Vilgalys and Hester, 1990). In a few instances the ITS4/5 primer pair did not amplify suitable product, so another set of primer-pairs (ITS5/2 and ITS4/3) were used to amplify the ITS1 and ITS2 regions separately. In all cases breaking the target region into smaller pieces was successful, and the sequences were easily joined due to the conservation of the rDNA 5.8S region separating ITS1 and ITS2. The few sequences for which we were unable to generate LSU sequence data were 50-plus years old (from the MICH collection). Regardless of primers used, the following program was run on a PTC-100 Programmable Thermocycle Controller (M.J. Research, Inc.) to amplify the target sequence: 35 cycles of denaturation for 45 s at 94° C, annealing for 45 s at 50° C, extension for 1 m at 72° C, followed by incubation for 5 m at 72° C. PCR product mass and concentration was estimated by ethidium bromide staining with Lambda DNA (Gibco BRL, Gaithersburg, MD) that had been cut using restriction enzymes EcoRI and HindIII (New England Biolabs, Beverly, MA). PCR product was purified using NH_4OAc and isopropyl alcohol. For sequencing reactions, PCR product was diluted in dd H_2O to concentrations of 25 - 50 ng/5 µl, depending upon fragment size, and submitted to Oregon State University's Central Services Lab, where sequences were determined on an ABI Prism 373 or ABI Prism 377 automated DNA sequencer

(Perkin-Elmer Corporation, Norwalk, CT). Bi-directional sequences were generated for all taxa using appropriate primers.

Sequence alignment

Alignment of ITS sequences were performed by direct examination with Genetic Data Environment 2.2 (GDE v. 2.2, Millipore Imaging Systems, Ann Arbor, MI) run on a UNIX workstation with the highly conserved 5.8S region as a guide. Alignment of LSU sequences was initially performed with the CLUSTAL V (Higgins, Bleasby and Fuchs, 1991) option in GDE and then adjusted where necessary. In each region, gaps were inserted as minimally as possible, and in the ITS region the gap coding technique of Bruns, et al. (1992) was used to preserve information contained in these gaps. While sequence similarity was high between morphologically similar taxa, it was quite low between distantly related taxa and alignment was ambiguous and homology difficult to assess, especially in the ITS1 region. Because of this difficulty in assessment, 76 ambiguous sites were excluded from the ITS region, leaving 211 phylogenetically informative characters; this analysis is hereafter termed *culled* ITS (cITS). Additionally, seven taxa representing the *O. concinna* - *O. leporina* group had a 200 b.p. insert in the ITS1 region that was not present in any other members of the genus sampled. This insertion is believed to be a single event and aligned and coded as such. It appears to be highly conserved, differing little within and between the two species (pairwise distances within species having this insertion range from 0.00889 to 0.06730 mean adjusted character differences, and from 0.03561 to 0.08540 mean adjusted character differences between species). These two species did however have larger numbers of total character

differences between collections than species not having the insertion event (see Table 2.2). The 200 base pair insertion event was coded using the technique of Bruns, et al. (1992, see above).

Table 2.2: Raw number of nucleotide changes within species examined in this study. Percentages indicate the maximum amount of within-species sequence divergence. Note extreme values in *O. smithii* and *O. leporina* in LSU region, indicated in **bold** type.

Species	LSU	ITS
O. smithii	5 - 83 (**15.6%**)	0 - 2 (0.4%)
O. onotica	5 - 13 (2.3%)	4 - 16 (3.3%)
O. leporina	34 - 58 (**10.3%**)	6 - 27 (4%)
O. alutacea	5 - 18 (3.1%)	0 - 3 (0.5%)
O. concinna	0 - 2 (0.3%)	11 - 43 (6.3%)
O. tuomikoskii	5 - 11 (1.9%)	0 - 1 (0.2%)
O. rainierensis	0 - 2 (0.3%)	2 - 8 (1.6%)
O. umbrina	5 - 10 (1.7%)	0 - 1 (0.2%)

Morphological data

Collections were examined as described in Peterson, et al. (Chapter Three). Morphological characters were coded according to the analysis in Peterson, et al. (Chapter Three) with the exception of characters 16 through 22. Morphological character 16, apothecium color group, was coded per Raitviir's (1972) analysis of the genus *Otidea* as well as fresh notes. The light-colored group included collections colored as some variant of yellow or tan. The dark-colored group included those described as some variant of orange or brown. To code morphological character 17, Duncan's spore ratio (length/width) group, 25 paired length and width measurements were made in distilled water (as described in Chapter Three). These were entered into an Excel spreadsheet (Microsoft Corporation, Redmond, Washington) along with the collection number, which

was then imported into the statistical package SAS 6.12 (SAS Institute, Inc., Cary, North Carolina). In SAS, an entry was created for the ratio of length to width for each paired measurement. This variable was then analyzed with the ANOVA analysis of variance option in SAS Assist. Means for each collection were analyzed with Duncan's Multiple Range Test, a powerful statistical method for detecting differences in means (Montgomery, 1997). Duncan's test orders the groups (species) from lowest mean to greatest mean, then makes all possible comparisons based on the least significant difference (LSD) (Montgomery, 1997). Collections having spore length/width ratios that did not differ significantly different at the $\alpha = 0.05$ level were grouped. Multistate, unordered characters were then coded from these groupings, resulting in 3 statistically significant groups. The same general procedure was followed for only the length measurements (character 18) yielding 5 statistically significant groups, and width measurements (character 19) yielding 2 statistically significant groups. Characters 20 through 22 were coded following the generalized gap-coding method proposed by Archie (1985) and expanded by Goldman (1988). Character 20 is the gap-coded ratio of length to width, yielding 5 groups. Characters 21 and 22 are the gap-coded length and width groups, yielding 4 and 3 groups, respectively. Population standard deviations are reported in parentheses with each character and the value of *c* for each group was 1.0 (per Goldman's suggestion, 1988). Morphological data were analyzed without spore characters included to examine the value of including characters coded from continuous data. All character states are unordered, and missing data is coded as "?". To better examine resolution provided by morphological data, two rounds of successive approximation based on the rescaled consistency index (R.C.) were used to weigh

characters. This approach applies weight to individual characters based on the observed level of character homoplasy in an unweighted analysis, and effectively downweights homoplasious characters (Farris, 1969). It should be noted that in any analysis that only one spore coding strategy was implemented at a time to satisfy the requirement of independence of characters in cladistic analyses. All characters and character states are listed in Table 2.3. Character state distributions for all taxa included in this analysis are presented in Table 2.4.

Table 2.3: Morphological characters and character states for *Otidea* species and outgroup. Characters and character states used in morphological analysis of *Otidea*. Characters with numbers in ***bold-italic*** are phylogenetically uninformative and were excluded from the final analysis. Characters 17 - 22 represent different spore-coding strategies.

Character Number	Character	Character states
1	Apothecium shape	0 = irregular; 1 = short-eared; 2 = long-eared; 3 = spathulate; 3 = cupulate
2	Apothecium apex	0 = truncate; 1 = pointed
3	Split in apothecium	0 = absent; 1 = present
4	Stipe	0 = astipitate; 1 = substipitate; 2 = stipitate
5	Stipe ornamentation	0 = concolorous w/hymenium; 1 = covered w/mycelium (white to cream)
6	Abhymenial ornamentation	0 = absent; 1 = present (pustulate)
7	Abhymenium hygrophanous	0 = not hygrophanous; 1 = hygrophanous
8	Hymenium w/rosy tints	0 = absent; 1 = present
9	Apical cell of paraphyses globose to subglobose	0 = absent; 1 = present
10	Apical cell of paraphyses having "protuberances"	0 = absent; 1 = present
11	***Paraphyses coloration***	***0 = hyaline; 1 = colored somehow***
12	Paraphyses containing granulose ornamentation	0 = absent; 1 = present
13	***Ascospore shape***	***0 = subglobose to globose; 1 = ellipsoid***
14	Ascospore ornamentation	0 = absent; 1 = present (in some form)
15	***Ascospores containing guttules***	***0 = absent; 1 = present***
16	Apothecium color group	0 = light colored; 1 = dark colored
17	Duncan's spore ratio group (length/width) (mean standard error = 0.014)	0 = mean 2.34; 1 = mean 2.19 -- 1.74; 2 = mean 1.24
18	Duncan's spore length group (mean standard error = 0.386)	0 = mean 15.77 -- 15.84; 1 = mean 15.40; 2 = mean 14.93; 3 = mean 14.05 -- 14.14; 4 = mean 11.70 -- 13.39; 5 = mean 10.23 -- 11.31; 6 = mean 8.91
19	Duncan's spore width group (mean standard error = 0.128)	0 = mean 7.18 -- 7.44; 1 = mean 5.48 -- 6.89
20	Generalized gap-code ratio group (length/width) (s = 0.180)	0 = mean 1.25; 1 = mean 1.73 -- 1.89; 2 = mean 1.91 -- 2.06; 3 = mean 2.13 -- 2.19; 4 = mean 2.34
21	Generalized gap-code length group (s = 1.93)	0 = mean 8.912; 1 = mean 10.23 -- 12.10; 2 = mean 12.51 -- 14.14; 3 = mean 14.93 -- 15.80
22	Generalized gap-code width group (s = 0.699)	0 = mean 5.48 -- 6.07; 1 = mean 6.20 -- 6.75; 2 = mean 6.84 -- 7.44

Table 2.4: Character state assignments for morphological data.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
OSC 56826	2	1	1	1	1	1	1	0	0	1	0	0	1	0	1	0	1	5	1	1	1	0
OSC 56758	1,2	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	1	4	1	2	2	1
OSC 56756	2	1	1	1	1	1	1	0	1	1	0	1	1	0	1	0	1	5	1	2	1	0
NSW 7574	1,4	0	1	2	1	1	?	0	0	1	0	0	1	0	1	1	1	5	1	1	1	1
NSW 6354	4	0	1	0,1	0	1	0	0	1	0	0	1	1	0	1	0	1	5	1	1	1	0
OSC 56734	2	1	1	2	1	1	0	1	0	0	0	0	1	0	1	0	1	4	1	2	1	0
OSC 56745	4	0	1	1	0	0	1	0	1	0	0	1	1	0	1	0	1	5	1	1	1	0
OSC 56747	2,3,4	0,1	1	1,2	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	3	3	2
OSC 56749	3,4	0	1	2	1	1	?	0	0	1	0	1	1	0	1	1	2	6	0	0	0	2
OSC 56753	2	1	1	1	1	1	1	0	0	1	0	1	1	0	1	1	1	4	1	2	2	2
OSC 56754	1,3	0	1	1	1	1	1	0	0	1	0	1	1	0	1	0	0	0	1	4	3	1
OSC 56759	3	0	1	2	1	1	?	1	0	0	0	1	1	0	1	0	1	4	1	1	1	1
OSC 56760	1	0	1	2	1	1	?	0	0	1	0	1	1	0	1	1	1	5	1	1	1	1
OSC 56761	2	1	1	1,2	1	1	1	0	0	1	0	1	1	0	1	0	1	5	0	1	1	0
OSC 56770	1	0	1	0,1	0	1	1	0	1	0	0	1	1	0	1	1	1	1	1	3	3	2
OSC 56777	1	0	1	1,2	1	1	1	0	0	1	0	1	1	0	1	0	1	2	0	2	3	2
OSC 56782	2,3,4	0	1	0,1	0	1	1	0	0	1	0	1	1	0	1	1	1	4	1	1	1	1
OSC 56784	1	0	1	1,2	1	1	1	0	0	1	0	1	1	0	1	0	1	4	1	1	2	2
OSC 56798	1	0	1	0,1	0	1	1	0	0	1	0	0	1	0	1	1	1	0	0	3	3	2
OSC 56799	1	0	1	2	1	1	1	1	0	1	0	1	1	0	1	0	1	4	1	1	2	2
OSC 56809	1	0,1	1	2	1	1	1	1	0	1	0	1	1	0	1	1	1	5	1	1	1	0
OSC 56813	2,3,4	0	1	0,1	0	1	1	0	0	1	0	1	1	0	1	1	1	4	1	2	2	1
OSC 56820	2	0	1	0,1	0	1	?	0	0	1	0	1	1	0	1	1	1	4	1	2	2	1
OSC 56823	1	0,1	1	1,2	1	1	?	0	0	1	0	1	1	0	1	0	1	4	0	1	2	2
OSC 56824	1	0,1	1	1,2	1	1	0	0	0	1	0	1	1	0	1	0	1	4	0	1	2	2
AHS 30502	1,4	0	1	1,2	1	0	?	0	1	0	0	1	1	0	1	1	1	5	1	1	1	0
OSC 56829	1,4	0	1	1	0	0	?	0	1	0	0	1	1	0	1	0	1	5	1	1	1	0
AHS 21147	1	0	1	2	1	0	1	0	1	0	0	1	1	0	1	?	1	4	1	2	1	0
OSC 56831	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1	3	0	2	2	2
AHS 8843	1	0	1	1,2	0	1	?	0	0	0	0	1	1	0	1	1	1	3	1	2	2	2
ML 941947	1,4	0	1	0,1	0,1	1	?	0	0	0	0	0	1	1	1	1	1	0	0	3	3	2
EGS 2179	1,4	0	1	1,2	1	0	?	0	1	0	0	1	1	0	1	0	1	5	1	1	1	0

Choice of outgroup

Aleuria aurantia (Persoon : Fries) Fuckel was chosen as an outgroup for this analysis because of its placement in the Otideaceae *sensu lato* and its large, irregular fruiting body. The work of Landvik, et al. show strong support for the relationship between *Otidea* and *Aleuria* (97% bootstrap support, their Figure 1) (Landvik, Egger and Schumacher, 1997). Samuelson (1978) relates these genera on the basis of the apical apparatus of the asci. Microscopically *Aleuria* is similar to *Otidea* in having biguttulate ellipsoid ascospores (although highly ornamented in *Aleuria*) of similar size and having a subglobose to globose apical cells of the paraphyses, as is observed in *O. rainierensis* and *O. kauffmanii*. In the molecular analysis *Scutellinia* sp. was also used as an outgroup based on its placement in the Otideaceae *sensu lato* to examine the placement of the root from outgroup to ingroup.

Combination of data

The incongruence length difference (ILD) test as described by Farris, et al. (1995) as implemented in PAUP* 4d64 (as the partition homogeneity test, Swofford, 1998) was used to examine congruence between the more slowly evolving LSU region and the more quickly evolving ITS region. We also tested the relationship between nucleotide data and morphological data (Cunningham, 1997a; Cunningham, 1997b). To examine the accuracy of the ILD test, the ITS1 region was compared to the ITS2 region. Additionally, where possible, weighting using successive approximation was used to examine the effects of differential weighting on partition homogeneity. ITS sequence

data that was not gap coded was also examined after applying transversion weighting. Fifteen partition comparisons were analyzed (Table 2.5). Partitions were created for each individual data set using the 'charpartition' command in PAUP*4d64, and partition homogeneity was compared using nearest-neighbor interchange (NNI) for branch swapping and the MULPARS option was turned off. 1000 repetitions of the test were performed, and each heuristic search was performed once. All phylogenetically uninformative characters were removed prior to the analysis.

Weighting methods

Characters were weighted by three different methods to examine the relationship between the more conservative LSU gene region and more variable ITS gene region. The methods employed were maximum parsimony, transversion parsimony and successive approximation weighting. Transversion parsimony assumes that transitions are more common than transversions, and therefore gives transitions a weight of zero (Cunningham, 1997). Successive approximation (successive weighting) relies on the set of most parismonious trees from an equally-weighted analysis to set character weights. Each character is re-weighted based on the degree of homoplasy it had in the equally-weighted analysis (Farris, 1969).

Phylogenetic analysis

All analyses were carried out using PAUP* 4d64 (Swofford, 1997) run on a Sun Sparc 10 (Sun Microsystems, Palo Alto, California). All trees were generated with the

heuristic search option using branch swapping with tree bisection-reconnection (TBR) and ten repetitions of random sequence addition unless otherwise noted. Support for each clade was assessed via the bootstrap (Felsenstein, 1985) implementing the following options: TBR branch swapping, MULPARS option not in effect, five repetitions on each heuristic search and 100 bootstrap replicates (resulting in 500 bootstrap replications). Gaps in the data set were analyzed as missing using the coding scheme of Bruns, et al. (1992). Only parsimonious informative characters were analyzed.

Results

Combinability of data tests

The results of fifteen partition homogeneity test comparisons are presented in Table 2.5. This table lists the type of data compared and the P-value returned by the partition homogeneity test. These results suggest that the LSU and ITS data should not be combined, but the ITS and morphological data are sufficiently homogenous and combination is warranted if successive weighting is applied.

Table 2.5: Results of fifteen partition homogeneity tests. KEY: cLSU (= characters 51 - 100 excluded from LSU analysis; cITS = ambiguous ITS characters excluded; morph (*nn*) indicates that spore-coding character(s) *nn* included in analysis; S.A. = characters weighted in 2nd analysis by successive approximation; Ti/Tv = characters weighted using transition parsimony.

Comparison	p-value	Notes
All ITS - all LSU	p = 0.001	
All ITS – cLSU	p = 0.001	
cITS - all LSU	p = 0.029	* = all taxa representing O. smithii and O. leporina removed
cITS - cLSU	p = 0.001	
cITS - cLSU; S.A.	p = 0.001	
cITS - morph	p = 0.01	
cITS – morph; S.A.	p = 0.22	
cITS - morph (20)	p = 0.01	
cITS - morph (18 & 19)	p = 0.001	
cITS - morph (18 & 19); S.A.	p = 0.219	
cITS - morph (21 & 22)	p = 0.002	
cITS - morph (21 & 22); S.A.	p = 0.192	
All ITS 1 - all ITS 2	p = 0.030	
All ITS 1 - all ITS 2; S.A.	p = 0.021	
All ITS 1 - all ITS 2; Ti/Tv	p = 0.093	All transversions given weight of 1; transitions given weight of 0

Morphological characters

Five of 22 morphological characters proved to be phylogenetically uninformative and were excluded, leaving 17 phylogenetically informative characters. Nine characters were multistate and potentially polymorphic, and coded as such (Table 2.3, characters 1, 2, 4, 5, 18 - 22). The remaining eight characters were binary and scored as present or absent. Care was taken to accurately describe the character states scored in this analysis, and details regarding character states are included in Peterson, et al. (Chapter Three). Four separate analyses of the morphological data were undertaken to examine different spore-size coding strategies.

Excluding all spore coding strategies initially resulted in 407 MPTs, and 787 MPTs of 6.123295 steps (C.I. = 0.804, R.I. = 0.925) after 2 rounds of successive weighting. The strict consensus of these trees was poorly resolved, only a single species was represented by a monophyletic clade (*O. onotica)*. When spores are coded by character 20, the generalized gap-coded ratio of length to width, 205 MPTs resulted initially and 3 MPTs of 8.97777 steps (C.I. = 0.611, R.I. = 0.711) after 2 rounds of successive weighting. Resolution in the strict consensus of these trees was worse than that of no spore coding and did not group any species as monophyletic (results not shown). Coding spores using characters 18 and 19, Duncan's spore length and width, initially resulted in 303 MPTs, and 39 MPTs of 10.16348 steps (C.I. = 0.695, R.I. = 0.822) after 2 rounds of successive weighting (Figure 2.1a). The strict consensus of the 39 MPTs resolved the monophyly of *O. umbrina* but no other species. Coding spores using characters 21 and 22, generalized gap-coded spore length and width, initially resulted in 560 MPTs, and 117 MPTs of 9.75533 steps (C.I. = 0.670, R.I. = 0.845) after 2 rounds of successive weighting (Figure 2.1b). The strict consensus tree was well resolved in terms of proximal relationships between species but no monophyletic clades were observed.

The analyses that included spore characters coded by Duncan's Multiple Range Test were as powerful as those characters coded using the more tedious generalized gap-coding technique. We decided to include spore characters 18 and 19, generated with Duncan's MRT, in the combined analysis with cITS data because (1) resolution of MPTs was greater in terms of monophyly of a single clade (*O. umbrina*); (2) fewer MPTs were generated both initially and following the successive weighting; (3) homoplasy values

were similar for both sets of characters (C.I. = 0.500 for character 18 and 0.333 for character 19 vs. C.I. = 0.500 for both characters 21 and 22); (4) the conversion from continuous to discreet data was more easily accomplished with Duncan's MRT than by the generalized gap-coding technique; (5) the P-value reported for the combination of these data with the cITS data (using characters 18 and 19) was higher (p = 0.219 vs p = 0.192, see Table 2.5).

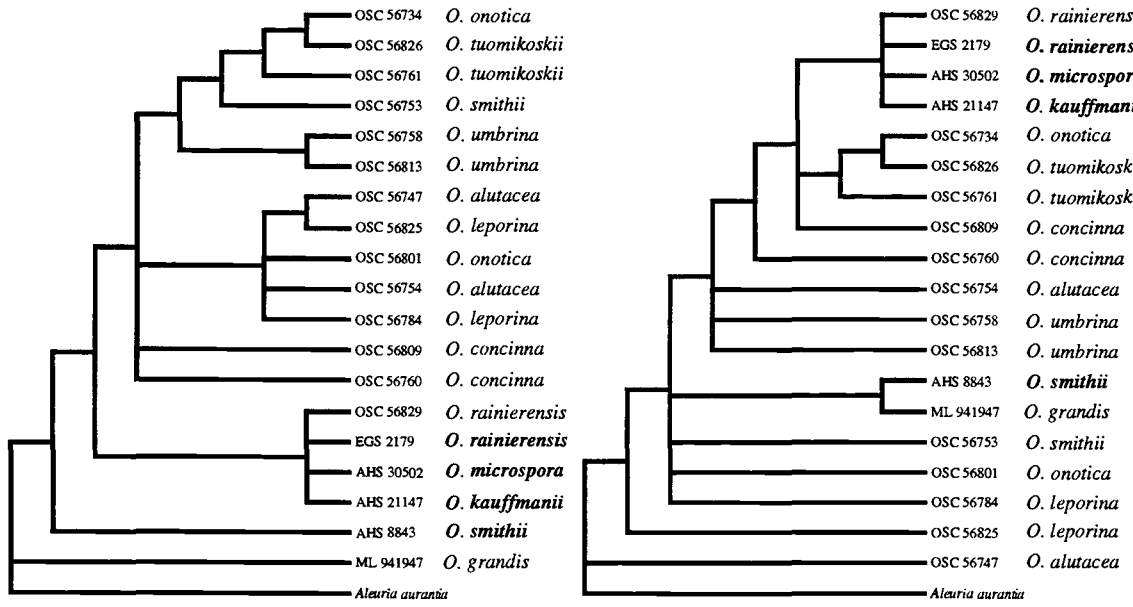

Figure 2.1: Strict consensus trees of morphological data comparing different spore-coding strategies used in this study. **1a** (left): Strict consensus of morphological data coding spores using Duncan's MRT (spore characters 18 & 19). **1b** (right): Strict consensus of morphological data, coding spores by generalized gap-coding technique (spore characters 21 & 22). Names in **bold** indicate type collections (MICH).

Molecular characters

Analysis of 127 phylogenetically informative, equally weighted nucleotide characters from the rDNA LSU region yielded 232 MPTs of 357 steps (C.I. = 0.709, R.I. = 0.869). The strict consensus of these trees is shown in Figure 2.2, with bootstrap values reported above the nodes. The LSU consensus tree has greater resolution than the consensus of either morphological analyses, but there is still little terminal resolution. Additionally, collections representing two morphologically distinct species, *O. smithii* and *O. leporina*, were dispersed throughout the entire tree. Examination of the nucleotide sequence data for these collections reveals a high degree of divergence between same-morphotype collections (up to 10% for *O. smithii* and 16% for *O. smithii*, Table 2.2). Collections representing these two species group together in three different clades and are well supported based on bootstrap values. A single round of successive approximation based on the R.C. values from the previous analysis reduced the number of MPTs to 12 (115.29 steps, C.I. = 0.814, R.I. = 0.948) but did not change the topology of the strict consensus tree. Possible reasons for the ambiguous placement of *O. leporina* and *O. smithii* are discussed below.

The ITS-only analysis included several taxa not sampled in the morphological and LSU analysis, including the holotype of *O. smithii* and paratypes of *O. rainierensis, O. kauffmanii*, and *O. alutacea* var. *microspora*, as well as *O. grandis*. Two heuristic searches were performed, one with all nucleotides present and one with all ambiguous sites excluded (culled ITS), to examine the effect of removing characters with questionable positional homology on tree support. Analysis of all nucleotide positions (262 phylogenetically informative characters) yielded 130 MPTs of 629 steps (C.I. =

0.700, R.I. = 0.911). A similar analysis excluding 207 of ambiguously aligned characters from the ITS1 region yielded 88 MPTs of 475 steps (C.I. = 0.680, R.I = 0.905). The strict consensus of these trees is shown in Figure 2.3, with bootstrap values reported above the nodes. A single round of successive approximation based on the R.C. values from the previous analysis found 23 MPTs of 291.66 steps (C.I. = 0.789, R.I. = 0.942) (results not shown).

A combined analysis of 225 phylogenetically informative sites from unambiguously aligned ITS characters and morphological characters (spore characters 18 and 19 and weighted once using successive approximation after the initial, unweighted analysis) resulted in 12 MPTs of 297.48019 steps (C.I. = 0.784, R.I. = 0.933). The strict consensus of these trees is in agreement with all other topologies presented in this paper with the exception of the LSU analysis, and is presented in Figure 2.4. A phylogram, showing branch lengths but without bootstrap values, is presented in Figure 2.5. In contrast to the LSU analysis, *O. leporina* and *O. smithii* each form monophyletic clades that are well supported by bootstrap values.

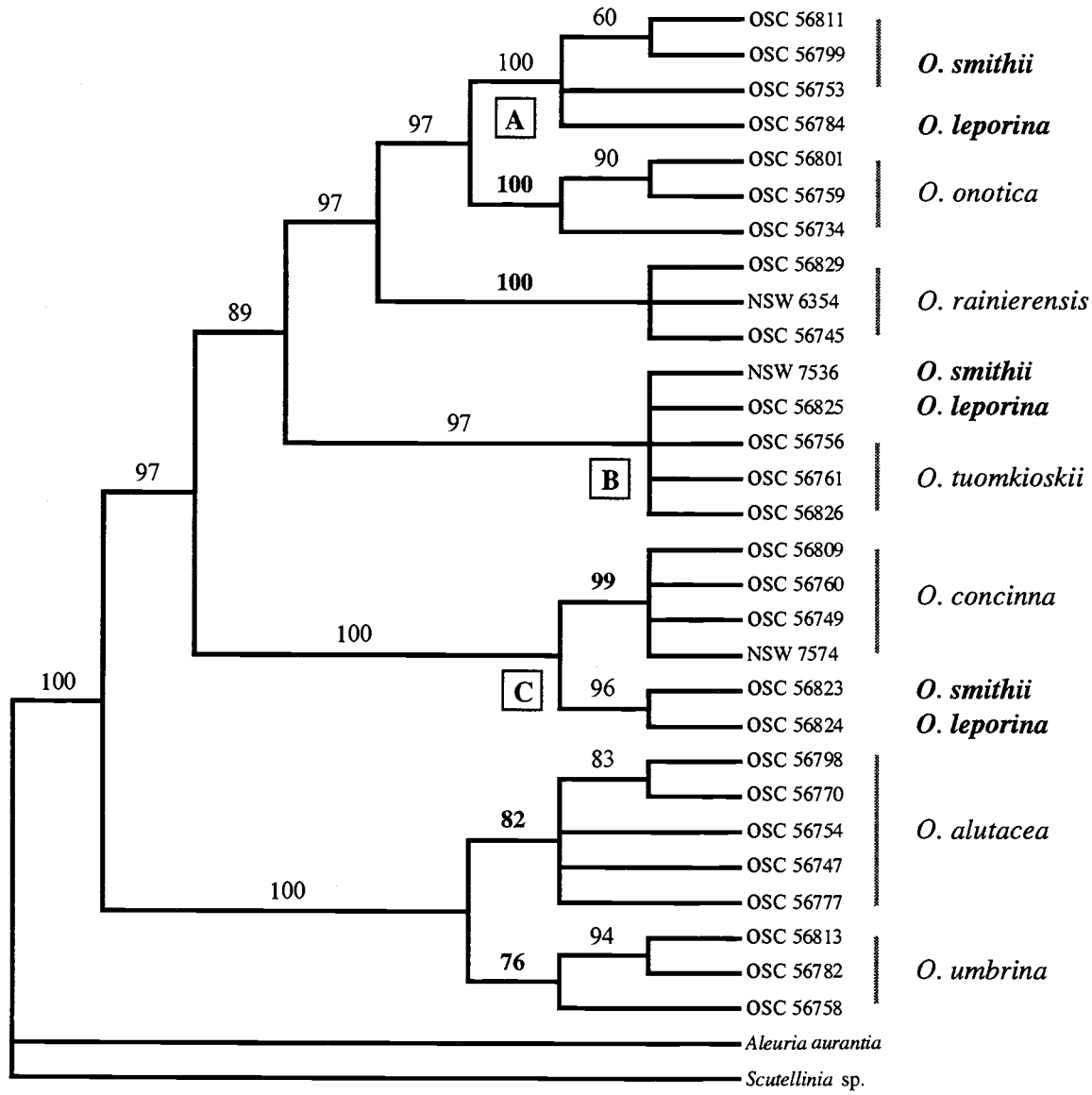

Figure 2.2: Strict consensus 232 MPTs from large subunit (LSU) data with gaps coded and no sites excluded. Trees are 357 steps (C.I. = 0.709, R.I. = 0.869). All characters are equally weighted. Bootstrap values are reported above the node, those supporting monophyletic species are in **bold**. Boxed letters (***A, B, C***) discussed in text. Paraphylletic species discussed in text are indicated with names in **bold**.

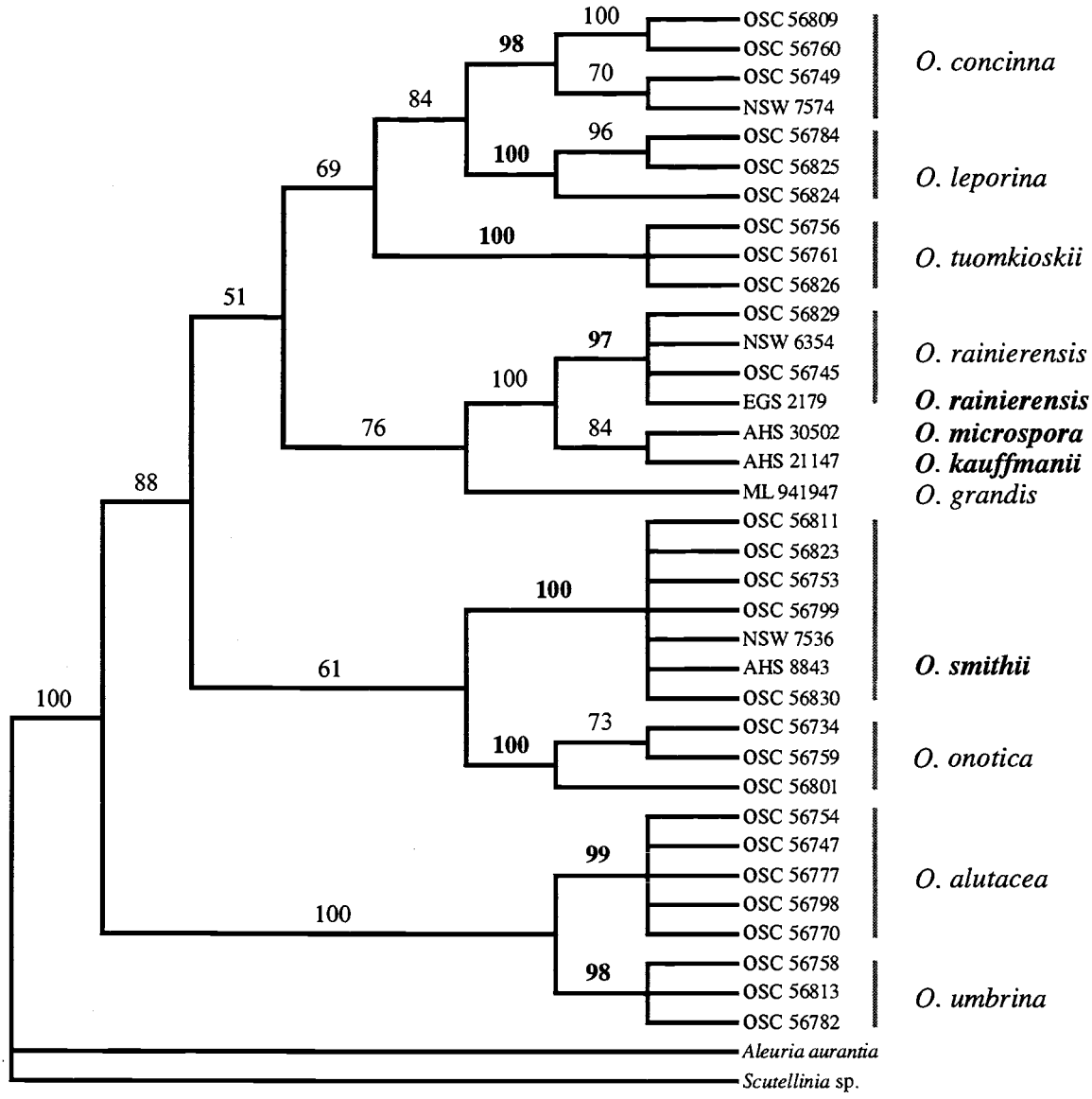

Figure 2.3: Strict consensus of 130 MPTs from internal transcribed spacer (ITS) data with gaps coded and all ambiguous characters excluded. Trees are 638 steps (C.I. = 0.697, R.I. = 0.909). All characters are equally weighted. Bootstrap values are reported above the node. Collections representing type or paratype materials indicated with names in **bold**.

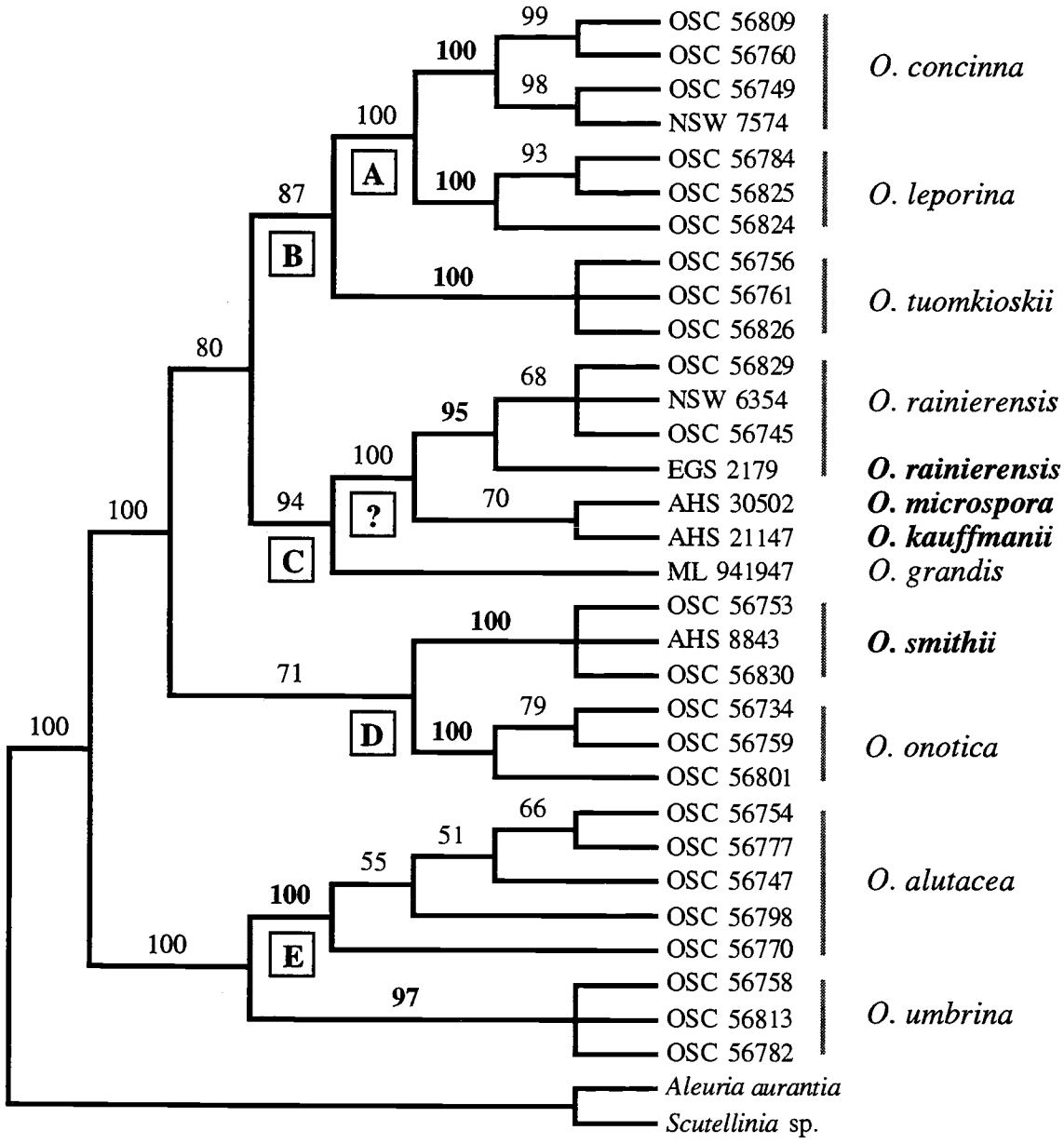

Figure 2.4: Strict consensus of 12 MPTs from combined cITS and morphological data (using spore-coding characters 18 and 19). Trees are 297.48019 steps (C.I. = 0.784, R.I. = 0.933). Bootstrap values are reported above the node, those values in **bold** indicate nodes supporting phylogenetic species. Type material indicated by names in **bold** type. Branches with boxed-letters (*A* - *E* and *?*) below node are discussed in text.

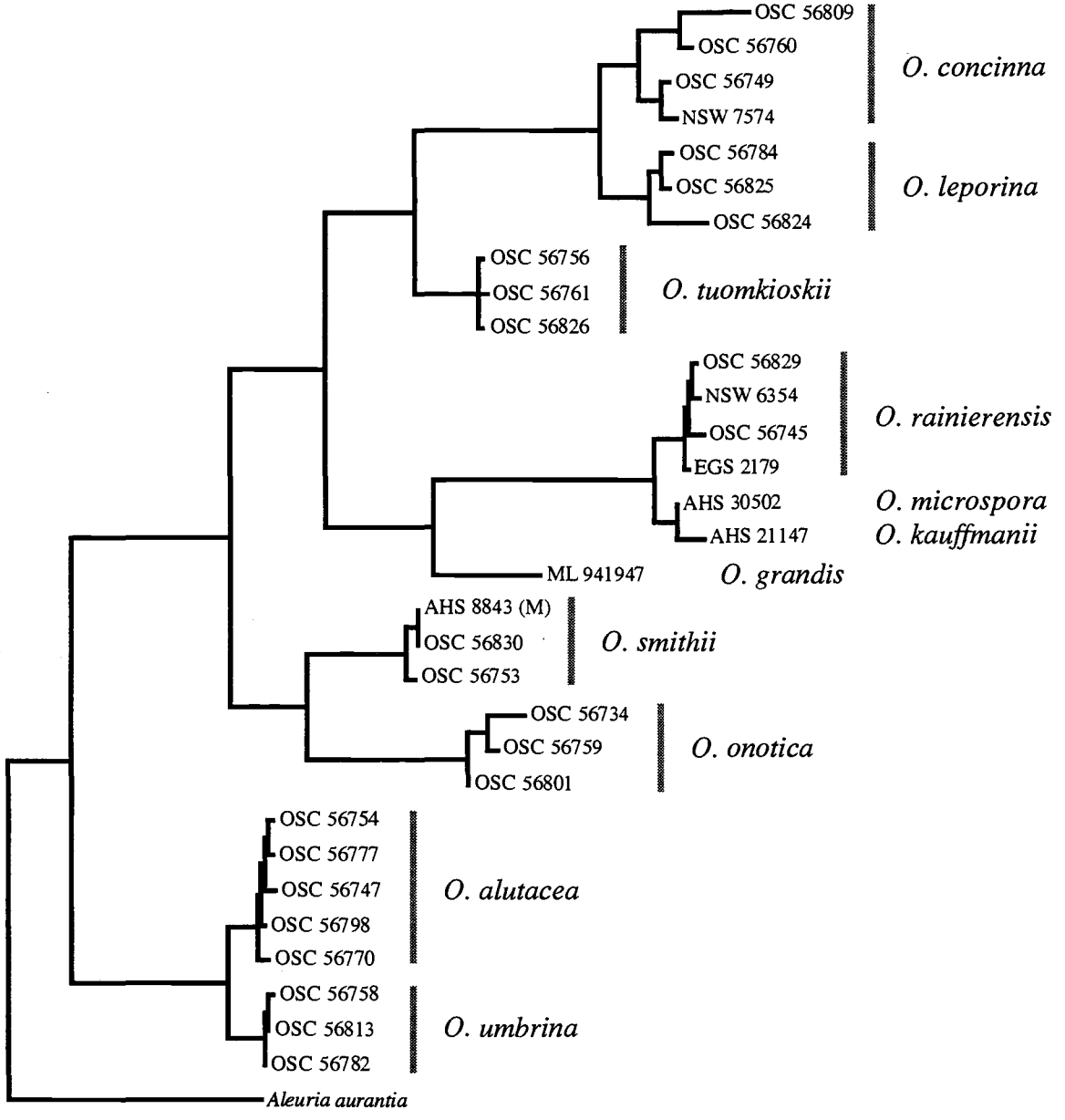

Figure 2.5: Phylogram of 1 of 12 MPTs generated from the combined analysis of cITS and morphological data (including spore-coding characters 18 and 19) weighted using successive approximation (279.56479 steps, C.I. = 0.802, R.I. = 0.941)

Discussion

Large subunit placement of O. leporina *and* O. smithii.

Careful examination of all LSU sequences of *O. leporina* and *O. smithii* revealed sequences unambiguously aligned with other taxa in this study. This examination also revealed that within-sequence differences scored on a per-nucleotide basis were as high as 10.3% for *O. leporina* and 15.6% for *O. smithii* (Table 2.2). All other LSU conspecific sequences used in this study ranged from 0.3% to 3.1%, the latter value is for the morphologically variable species *O. alutacea*. ITS sequence divergence for these taxa was as expected for conspecifics, 4% for *O. leporina* and 0.4% for *O. smithii*, well within the limits of divergence for other species in this study. Data presented by O'Donnell, et al (1998) show that different ITS2 types have become fixed in populations of *Gibberella fujikuroi* sampled from Africa, Asia, and America. The different ITS2 types are homoplastic in the gene trees presented in their work, and appear to be a result of an ancient gene duplication or interspecies hybridization event, prior to the radiation of the genus. These authors report that sequence divergence in the ITS2 region is as high as 19.4%, comparable to our findings for *O. smithii* and *O. leporina*, especially considering the expected difference in conservation between the ITS and LSU gene regions.

An attempt to map a biogeographical origin onto the placement of taxa in the *O. leporina* - *O. smithii* sequence divergence was inconclusive. Collections in the clade indicated by box *A* (Figure 2.2) are from Oregon and Washington. Collections in the clade indicated by box *B* (Figure 2.2) are from Idaho and California, and those in the clade indicated by box *C* (Figure 2.2) are from California. As in O'Donnell, et al. the

placement of each sequence type appears homoplastic. These data also suggest that different LSU types exist within species of *Otidea*, a condition not previously reported in fungi. Further analysis of the origin of this polymorphism is warranted.

Combinability of data.

The question of whether or not to combine data from independent sources is often debated by systematists. There are three broad opinions on how to handle different sources of data: taxonomic congruence, total evidence, and prior agreement (Chippindale and Wiens, 1994; Bull, et al., 1993; Queiroz, 1993; Rodrigo, 1996; Barrett, Donoghue and Sober, 1991; Tehler, 1994; Nixon and Carpenter, 1996). Methods of taxonomic congruence involve three steps: (1) partitioning of data, (2) separate analysis, and (3) comparison and construction of a consensus topology (Chippindale and Wiens, 1994). The method of total evidence is simple in that all data are combined and a single simultaneous analysis is undertaken (Eernisse and Kluge, 1993). The prior agreement approach is a middle-ground between the first two approaches. First advocated by de Quiroz (1993) and elaborated upon by Bull, et al. (1993) this approach recognizes that more data (i.e., total evidence) have advantages but if the individual data sets are incongruent (e.g., strongly support conflicting phylogenies) that the combined data set has a high probability of inferring the "incorrect" phylogeny. While this approach appears to be the safest route towards phylogenetic reconstruction, data now exist that shows that tests of congruence may in fact be too conservative and therefore prevent combination of data (Cunningham, 1997; Cunningham, 1997; Lutzoni and Vilgalys, 1995; Lutzoni, 1997).

In this analysis three partitions were examined, LSU sequence data, ITS sequence data and morphological characters. The partitioning of the LSU and ITS data is seemingly artificial since these sequences represent continuous, albeit differently constrained, nucleotide data within the rDNA tandem repeat. The decision to partition these data and analyze each subunit separately is based on the expected level of sequence divergence. To answer our major question, how many species of *Otidea* are present in the Pacific Northwest, we needed gene regions that would be evolving quickly enough to resolve intrageneric relationships. The ITS region is appropriate for this type of analysis (Hibbett, et al., 1995; Liston, et al., 1996; Downie, et al., 1998). Large subunit sequence data was initially collected to confirm the appropriateness of the ITS region and to confirm the monophyly of the genus *Otidea* within the Otideaceae and Pezizales.

The phylogenetic relationships inferred in the LSU and ITS appear at least visually to be somewhat congruent (Figures 2.2 and 2.3). Each of the strict consensus trees support the monophyly of a *O. alutacea* - *O. umbrina* clade as sister to all other species and the relationship between of *O. smithii* and *O. onotica.* The major difference between the two topologies is the polyphyletic placement of species in the *O. smithii* and *O. leporina* groups. In the ITS analysis, all sampled members of these two species form monophyletic groups and are well supported (based on bootstrap values). In the LSU analysis, these species are spread throughout the consensus tree, but again their placement is well supported. The partition homogeneity test as implemented in PAUP*4d64 indicated that the LSU and ITS regions are incongruent ($p = 0.001$) and should therefore not be combined. Cunningham (1997) indicates that P-values greater than $p = 0.01$ suggest that combining data will either improve or not effect phylogenetic

accuracy, and that P-values $p < 0.001$ will cause accuracy to suffer. Also, the presence of polymorphic LSU types suggested that these data were not homogeneous. Accordingly we decided not to combine the LSU and ITS data. It is interesting to note however that if the collections representing *O. leporina* and *O. smithii* are excluded, the LSU and ITS data can be combined without causing a decrease in phylogenetic accuracy ($p = 0.029$).

Regarding the combination of the molecular data and morphological data, we analyzed the combinability of the cITS sequence data and morphological data. The partition homogeneity test used as described gave a P-value $p = 0.01$ when all spore-coding character were excluded, $p = 0.001$ and $p = 0.002$ when spore-coding characters 18 & 19 and 21 & 22 were included respectively. However, after a single round of weighting using successive approximation these values changed significantly and all combinations of ITS and morphological data were reported as sufficiently homogenous to combine (Table 2.5).

Our decision to combine ITS and morphological data, and weight these data using successive approximation is supported by our data and the work of other authors who favor combining these types of data (e.g., Hibbett and Vilgalys, 1993; Doyle, 1992; Lutzoni and Vilgalys, 1995; Tehler, 1994; Donoghue and Sanderson, 1992; Harrington, 1998). Arguments in favor of combining molecular and morphological data range from Doyle's (1992) suggestion that morphological data can root "gene trees" with independent characters and provide greater evidence for a species phylogeny that would be available with molecular data alone. Lutzoni and Vilgalys (1995), Hibbett and Vilgalys (1993) and Harrington (1998) all found that the combination of molecular and morphological characters resulted in fewer trees that were more resolved and better supported. Finally,

Donoghue and Sanderson (1992) make the case for total evidence and show quite convincingly that it is "a mistake to set morphological data aside and base phylogeny reconstruction only on molecular evidence." While they recognize limitations inherent in this type of combination -- problems of non-independence, weighting, and the potential swamping effect -- they state these are surmountable problems and *all* evidence should be examined when attempting to infer phylogeny.

In our final analysis we decided to combine the ITS sequence data with the morphological data (using Duncan's Multiple Range Test codes for spore length and width) to infer phylogeny for the genus *Otidea* as it occurs in the Pacific Northwest. In addition to the results of the partition homogeneity test, excluding LSU data from this analysis was decided for the following reasons. First, to resolve *O. smithii* and *O. leporina,* many phylogenetically informative characters would need to be excluded from the analysis. Because the sequences appeared to be free of contamination and they aligned easily with other members of the genus, we have no *a priori* reason to exclude these data. Second, due to the age of the material we failed to amplify LSU sequences for the type and paratypes. By combining LSU and ITS data we would be forced to exclude these valuable sequences, and therefore limit our taxonomic analysis of the genus.

Morphological character coding strategy.

Much has been written regarding the validity of transforming continuous data into discreet units that can be used in cladistic analysis (Pimentel and Riggins, 1987; Cranston and Humphries, 1988; Thiele and Ladiges, 1988; Archie, 1985; Goldman, 1988; Harrington, 1998; Maddison and Maddison, 1992). Archie (1985) and Goldman (1988) present a statistically-based method for coding continuous data into discreet units based on gaps in the data (gap-coding). The major criticism of this technique is the question of the cladistic significance of a mean for a taxon (Pimentel and Riggins, 1987; Cranston and Humphries, 1988). Pimentel and Riggins (1987) critically question the cladistic properties of population means and standard deviations, and recommend that cladists avoid using these types of data. Cranston and Humphries (1988) point out that, because of the questionable validity of using measurements as cladistic data, using ratios is to be further discouraged because you also lose independence of data. It is interesting to us that other authors will follow this line of reasoning and exclude valuable continuous data, while including other characters and coding character states using subjective terms such as "large" and "small" or "rarely" and "frequently" without clearly defining these terms.

Some authors suggest that continuous data need not be modified at all for use in cladistic analysis, but can be used directly without recoding (Maddison and Maddison, 1992; Farris, 1970; Maddison, 1991). In the context of this analysis, to capture important information about spore length and width, this technique seems poor at best. If a group average is used, any addition or exclusion of data from each taxon's population of spore measurements will affect the value of the population mean and thereby change the character state of that character. The gap-coding as implemented in this study minimizes

the effect of addition or removal of data. For this reason we decided not to use spore length and width measurements as continuous data proper.

In contrast to the rejection of continuous data, Thiele and Ladiges (1988), Archie (1985), Goldman (1988) and most notably Stevens (1991) make cases for inclusion of this type of data. Archie (1985) described the generalized gap-coding technique for making continuous data useful in cladistic analysis and analyzed its utility compared to other techniques. One justification for his work, and this type of analysis, is that systematists do collect and rely on continuous data in taxonomic assessments. Goldman (1988) modified Archie's methods and proposed a new method for coding the gap-subsets into discreet character states. Thiele and Ladiges (1988) base the utility of coding quantitative characters based on tree resolution. Their analysis including 18 quantitative characters and 9 qualitative characters yielded one fully-resolved most parsimonious tree, whereas the exclusion produced a little resolved tree (their Figures 2.4 and 2.5). Stevens (1991) in his review of character states, morphological variation, and phylogenetic analysis recognizes the value of including continuous data that has been coded into discreet units if this transformation is carefully undertaken. He recognizes that inclusion of continuous data in phylogenetic analyses often increases the resolution of the analysis, but states that this increase in resolution is accompanied by a decrease significance of the MPT(s) (due to an increase in homoplasy). He offers two general guidelines regarding the use of morphological data: (1) Character states used in phylogenetic analysis should be discreet and without overlap, and (2) characters and character states should be better documented and justified than is generally done. This point returns to our previous criticism of the use of ambiguous terms for character states.

Our decision to use spores coded using Duncan's Multiple Range Test as implemented in SAS Assist 6.12 was because (1) since both strategies for coding continuous data rely on the analysis of populations of measurements it was significantly less time-consuming to use the test in the available software; (2) since the addition of data results in a change in the population standard deviation, the ease-of-use of Duncan's test allowed for quick addition and removal of data that was more cumbersome with the generalized gap-coding technique; (3) each test appeared to have significant resolution, finding between 2 and 7 significant groups. Clearly, the utility of coding continuous data using Duncan's MRT is worth further study with larger data sets than available in this study.

Taxonomic implications.

Based on the final analysis of cITS and morphological data (Figures 2.4 and 2.5) our sampling supports eight species of *Otidea* in the Pacific Northwest (*O. concinna, O. leporina, O. tuomikoskii, O. rainierensis, O. smithii, O. onotica, O. alutacea,* and *O. umbrina*). Each was supported by strong bootstrap support at the terminal or subterminal node, except in the case of *O. alutacea* (discussed below), and morphological characters including ascomal color and ascospore size. Spore measurements are useful in differentiating species of *Otidea* (Raitviir, 1972; Kanouse, 1949; Nannfeldt, 1966). Two major groups of species were observed, the clade formed by *O. umbrina* - *O. alutacea,* and of all other species examined. Within the larger assemblage, two groups were observed. One, *O. concinna* and *O. leporina,* is supported by a 200 b.p. insertion event in the ITS1 region that is absent in all other members of the genus sampled (box *A*, Figure

2.4). The other, *O. rainierensis, O. microspora, O. kauffmanii*, and *O. grandis,* is supported by the presence of subglobose or globose apical cells in the paraphyses (box *C*, Figure 2.4). Each is strongly supported by bootstrap values. Additionally, one reversal is observed regarding the presence of copious white or cream-colored mycelia on the lower portion of the fruiting body in four species. This character state appears to have either arisen twice, once in the *O. onotica* - *O. smithii* clade and again in the *O. concinna* - *O. leporina* - *O. tuomikoskii* clade (boxes *B* and *D*, Figure 2.4) or has been lost twice. Other members of the genus have occasional lightening of the lower portion of the fruiting body, but the five listed are the only members of the genus known to have copious white or cream-colored mycelium on the stipe (or an obvious stipe for that matter, with the exception of *O. tuomikoskii*).

Evidence for each species group is supported as well by individual morphological characters, including those listed above. To further support each phylogenetic species we have listed each species or closely related species group below along with characters that support the clade and differentiate it from sister clades. Additional information is provided in Peterson, et al. (Chapter Three).

O. concinna - *O. leporina:* The fruiting bodies vary in shape and color but are generally short-eared with a truncate or pointed apex, and are some shade of orange, tan or brown. Colors in *O. concinna* tend to be the same inside and out whereas the fresh hymenium in *O. leporina* is generally darker than the abhymenium. The spores of *O. concinna* are significantly smaller than those of *O. leporina*. The clade containing these two species is supported by 100% bootstrap support and these are the only species that contain the 200 b.p. insertion event in the ITS1 region (box *A*, Figure 2.4). Additionally,

as stated previously, both species are generally stipitate and have white or cream-colored hyphae covering the stipe and extending down into the substrate below.

O. tuomikoskii: The fruiting body of *O. tuomikoskii* differs from the previously discussed species by being generally long-eared and slender and yellow or yellowish-brown. It is generally substipitate to astipitate, although the fruiting body may be somewhat constricted towards the base and covered with the previously described white to cream-colored hyphae. *O. tuomikoskii* lacks the 200 b.p. insertion event present in *O. concinna* and *O. leporina*. Workers in the Pacific Northwest have considered *O. tuomikoskii* to be *O. leporina* var. *minor* based on colors and spore sizes described in Kanouse (1949).

O. rainierensis - O. microspora - O. kauffmanii - O. grandis: This entire clade is supported by a 94% bootstrap value and the presence of globose to subglobose apical cells of the paraphyses. *O. grandis* is separated from the remaining three species by its primarily Eastern North American distribution (although reported once from Eastern Washington by E.B. Cooke ca 1955), a darker ascoma, and larger spores that are minutely roughened at maturity. The remaining three species, *O. rainierensis*, *O. microspora* and *O. kauffmanii*, are grouped in a strongly supported clade (100% bootstrap support, box *?*, Figure 2.4) and are very closely related (Figure 2.5). Morphologically the three species are quite similar; all have a fruiting body that split down one side, generally cupulate, sessile and often gregarious, with colors some shade of tan or brown. Whereas *O. rainierensis* and *O. microspora* are known and described from Washington, *O. kauffmanii* was described from Michigan collections (Kanouse, 1949). Microscopically the three have spores that overlap in size a great deal. *O. microspora* (Kanouse) Harmaja

(= *O. alutacea* var. *microspora* Kanouse) has spores 9 - 11 x 5.5 - 6.5 µm, *O. rainierensis* has spores 10 - 12 x 6 - 7 (8) µm and *O. kauffmanii* has spores 8 - 10 (12) x 5 -6 (7) µm. The generalized gap-coding technique grouped collections of these three species together (character 21, group 1, Table 2.3 and 2.4). The Duncan's MRT spore-coding technique grouped *O. rainierensis* and *O. microspora* in group 5 (character 18, Table 2.4 and 2.4) but placed *O. kauffmanii* in group 4 (character 18, Table 2.3 and 2.4). Analysis of morphological characters only with either spore-coding technique found a single unresolved polytomy with these three taxa and another collection identified as *O. rainierensis* (OSC 56829) (Figure 2.1a and b).

Based on cladistic support and morphological similarity one could group these three species into a single species complex and expand the description to cover increased geographical range and morphological variation. Fewer steps separate these differently named taxa than separate morphologically and nomenclaturally identical species (compare this clade to *O. concinna*, Figure 2.5). However, bootstrap support for the *O. microspora* - *O. kauffmanii* clade is high in both the ITS only and combined analysis (84% and 70% respectively), and the sample analyzed is poor, so we suggest that further investigation is warranted. We can conclude that Harmaja's elevation of *O alutacea* var. *microspora* from variety to species was warranted, based on the phylogenetic distance between this taxon and the clade representing *O. alutacea* var. *alutacea.*

O. smithii - *O. onotica*: These two species, marginally supported in the bootstrap analysis (71%) are well supported individually (100% each). Morphologically they are quite distinct in color and stature. The fruiting body of *O. smithii* is generally firm in texture and purplish-brown inside and out. Conversely, *O. onotica* is generally somewhat

brittle and yellow to bright-yellow (often having pinkish spots or a similar cast in the hymenium). Both species have similarly sized spores, 10 - 12 (14) x 6 - 7 μm vs. 12 - 14 x 6 - 7 (8) μm respectively, as well as a long-eared shape and light colored hyphae covering the stipe (if present).

O. alutacea - *O. umbrina*: These species are also closely related and differ in few morphological characters. Both are darker in color than most other species examined in this study (with the exception of *O. smithii*) and have relatively large spores. *O. alutacea* tends to be lighter in color and more brittle than *O. umbrina* and has slightly larger spores. The abhymenium of *O. umbrina* loses moisture quickly upon collection, especially when young, and often appears quite different in color when compared to the hymenium. This character is observed in *O. alutacea* but generally happens more slowly (slow hygrophanous effect). The major support for separating these species is cladistic. Both the ITS-only analysis and the combined analysis strongly support the monophyly of each terminal clade (99% and 98%, 100% and 97% respectively), as well as the sequence similarity within each species and differences between species (Figure 2.5).

The sampling of *O. alutacea* is larger (5 collections) because collections OSC 56747 and OSC 56754 were lighter in color and more commonly singular in occurrence than other collections described as *O. alutacea.* Initially these were thought to be cryptic or perhaps new species of *Otidea*, however in the LSU-only, ITS-only and combined analysis these two collections grouped with other collections fitting the description of *O. alutacea* with moderate to high bootstrap support (82 - 100%, Figures 2.2, 2.3 and 2.4). Because the LSU-only and ITS-only analyses supported the monophyly of these collections with little or no resolution, we accept the monophyly of the group in the

combined analysis and highlighted the deepest node that was inclusive of these collections as being the point of support for the species (100%, Figure 2.4).

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References

Archie, J. W. 1985. Methods for coding variable morphological features for numerical taxonomic analysis. *Systematic Zoology* 34 (3): 326-345.

Baral, H. O. 1992. Vital versus herbarium taxonomy: Morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* XLIV (2): 333-390.

Barrett, M., M. J. Donoghue, E. Sober. 1991. Against consensus. *Systematic Zoology* 40 (4): 486-493.

Bruns, T. D., R. Fogel, J. W. Taylor. 1990. Amplification and sequencing of DNA from fungal herbarium specimens. *Mycologia* 82 (2): 174-184.

Bruns, T. D., T. M. Szaro. 1992. Rate and mode differences between nuclear and mitochondrial small-subunit rRNA genes in mushrooms. *Molecular Biology and Evolution* 9 (5): 836-855.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42 (3): 384-397.

Bult, C., M. Kallersjo, Y. Suh. 1992. Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. *Plant Molecular Biology Reporter* 10 : 273-284.

Chippindale, P. T., J. J. Wiens. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. *Systematic Biology* 43 (2): 278-287.

Cranston, P. S., C. J. Humphries. 1988. Cladistics and computers: A chironomid conundrum? *Cladistics* 4 : 72-92.

Cunningham, C. W. 1997a. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14 (7): 733-740.

Cunningham, C. W. 1997b. Is congruence between data partitions a reliable predictor of phylogentic accuracy? Empirically testing an iterative proceedure for choosing among phylogenetic methods. *Systematic Biology* 46 (3): 464-478.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 88 : 172-181.

Donoghue, M. J., M. J. Sanderson. 1992. *The suitability of molecular and morphological evidence in reconstructing plant phylogeny.* In: Soltis, Soltis, Doyle (eds) *Molecular Systematics of Plants*, pp. 340-368. Chapman and Hall, New York, New York.

Downie, S. R., S. Ramanath, D. S. Katz-Downie, E. LLanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *RPOC1* intron sequences. *American Journal of Botany* 85 (4): 563-591.

Doyle, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. *Systematic Botany* 17 (1): 144-163.

Eernisse, D. J., A. G. Kluge. 1993. Taxonomic congruence versus total evidence, and Amniote phylogeny inferred from fossils, molecules, and morphology. *Molecular Biology and Evolution* 10 (6): 1170-1195.

Farris, J. S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18 : 374-385.

Farris, J. S. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19 : 83-92.

Farris, J. S., M. Kallersjo, A. G. Kluge, C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10 : 315-319.

Felsenstein, J. 1985. Confidence intervals on phylogenies: An approach using the bootstrap. *Evolution* 39 : 783-791.

Goldman, N. 1988. Methods for discrete coding of morphological characters for numerical analysis. *Cladistics* 4 : 59-71.

Harrington, F. A. 1998. Relationships among *Sarcoscypha* species: evidence from molecular and morphological characters. *Mycologia* 90 (2): 235-243.

Hibbett, D. S., Y. Fukumasa-Nakai, A. Tsuneda, M. J. Donoghue. 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. *Mycologia* 87 (5): 618-638.

Hibbett, D. S., R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Systematic Botany* 18 (3): 409-433.

Higgins, D. G., A. J. Bleasby, R. Fuchs. 1991. CLUSTAL V: Improved software for multiple sequence alignment. *CABIOS* (cited as submitted) .

Holmgren, P. K., N. H. Holmgren, L. C. Barnett. 1990. *Index Herbariorum.* New York Botanical Gardens, New York.

Kanouse, B. B. 1949. Studies in the genus *Otidea*. *Mycologia* 41 : 660-677.

Landvik, S., K. N. Egger, T. Schumacher. 1997. *Towards a subordinal classification of the Pezizales (Ascomycota)*, pp. 1-29.

Liston, A., W. A. Robinson, J. M. Oliphant, E. R. Alvarez-Buylla. 1996. Length variation in the nuclear ribosomal DNA internal transcribed spacer region of non-flowering seed plants. *Systematic Botany* 21 (2): 109-120.

Lutzoni, F., R. Vilgalys. 1995. Integration of morphological and molecular data sets in estimating fungal phylogenies. *Canadian Journal of Botany* 73 (Supplement 1): S649-S659.

Lutzoni, F. M. 1997. Phylogeny of lichen- and non-lichen-forming Omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Systematic Biology* 46 (3): 373-406.

Maddison, W. P. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Zoology* 40 (3): 304-314.

Maddison, W. P., D. R. Maddison. 1992. *MacClade. Analysis of phylogeny and character evolution.* Sinauer Associates, Inc., Sunderland, Massachussets.

Montgomery, D. C. 1997. *Design and Analysis of Experiments.* John Wiley & Sons, New York.

Mullis, K. B. and F. A. Fallona. 1987. Specific synthesis of DNA in vitro via polymerase-catalyzed chain reaction. *Methods of Enzymology* 155: 335-350.

Nannfeldt, J. A. 1966. On *Otidea caligata, O. indivisa*, and *O. platyspora* (Discomycetes, Operculatae). *Annales Botanici Fennici* 3 : 309-318.

Nixon, K. C., J. M. Carpenter. 1996. On simultaneous analysis. *Cladistics* 12 : 221-241.

O'Donnell, K., E. Cigelnik, H. I. Nirenberg. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90 (3): 465-493.

Pimentel, R. A., R. Riggins. 1987. The nature of cladistic data. *Cladistics* 3 (3): 201-209.

de Queiroz, A. 1993. For consensus (sometimes). *Systematic Biology* 42 (3): 368-372.

Raitviir, A. 1972. Statistical methods and species delimitation in the genus *Otidea*. *Persoonia* 6 (4): 415-423.

Rodrigo, A. G. 1996. On combining cladograms. *Taxon* 45 : 267-274.

Saccardo, P. A. 1882-1972. *Sylloge Fungorum.* Volumes VIII, X, XI, XIV, XVI, XVIII, XX, XXII. Many publishers, Many cities.

Saiki, R., D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. Horn, K. Mullis and H. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 : 487-494.

Samuelson, D. A. 1978. Asci of the Pezizales. II. The apical apparatus of representatives in the *Otidea-Aleuria* complex. *Canadian Journal of Botany* 56 : 1876-1904.

Stevens, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: A review. *Systematic Botany* 16 (3): 553-583.

Swofford, D. 1997. *Paup**. Sinauer and Associated, Sunderland, Massachussets.

Tehler, A. 1994. Morphological data, molecular data, and total evidence in phylogenetic analysis. *Canadian Journal of Botany* 73 (Supplement 1): S667-S676.

Thiele, K., P. Y. Ladiges. 1988. A cladistic analysis of *Angophora* cav. (Myrtaceae). *Cladistics* 4 : 23-42.

U.S.D.A. F.S., U.S.D.O.I. B.L.M. 1994. *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents within the Range of the Northern Spotted Owl*, U.S. Government Printing office. 74p. Plus Attachment A: Standards and Guidelines.

Vilgalys, R., M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 : 4238-4246.

Weber, N. S., J. M. Trappe, W. C. Denison. 1997. Studies on Western American Pezizales. Collecting and describing ascomata -- Macroscopic features. *Mycotaxon* 61 : 153-176.

White, T. J., T. D. Bruns, S. B. Lee, J. W. Taylor. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis, Gelfand, Sninsky, White (eds) *PCR Protocols: A Guide to Methods and Applications*, pp. 315-322. Academic Press, New York, New York.

Chapter 3

REVISIONAL MONOGRAPH OF THE GENUS *OTIDEA* IN THE PACIFIC NORTHWEST

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Abstract

The genus *Otidea* as it occurs in the Pacific Northwest contains three members considered rare throughout the range of the Northern Spotted Owl. *Otidea* has been studied by several authors; the most recent work on North American representatives dates from 1949. This taxonomic reassessment was undertaken due to new information and techinques currently available allowing for the re-evaluation of species concepts. In a separate analysis it was determined that there are eight phylogenetic species of the genus *Otidea* that occur in this range. These species are described in detail and keys to both fresh and dried materials are provided. *O. tuomikoskii* Harmaja and *O. umbrina* (Persoon) Bresadola are reported from North America for the first time.

Introduction

Otidea has long been recognized as a natural genus (Nannfeldt, 1966). It was first erected as a subgenus of *Peziza* by Persoon (1822) for species having an ear-shaped (*otideoid*) apothecium and then elevated to generic status by Bonorden (1851) (although no species were included at that time) According to Kanouse (1949), Cao, Fan and Liu (1990) and Nannfeldt (1966), Fuckel (1869) ascribed four species to the genus, *O. leporina, O. onotica, O. cochleata,* and *O. abietina*. Boudier (1907) regarded *Peziza abietina* (Persoon : Fries) Fuckel as representing a distinct genus based (presumably) on larger spores and a more regularly cup-like appearance than other members of *Otidea* and made it the type for his new genus *Pseudotis*. Eckblad (1968) reports that a close examination of ascospores, paraphyses, and the medullary and ectal excipula support this species as an *Otidea*, and the *Dictionary of the Fungi* (Hawksworth, et al., 1995) lists *Pseudotis* as a synonym of *Otidea*. Additionally, Kanouse (1949) reported that *O. abietina* is observed to have a split apothecium, and I have seen Canadian material that fits the description of *O. abietina* (*sensu* Kanouse and Cao, et al.) with a split, ear-shaped apothecium (*ETP 002*, collected near Ontario, Canada by Teresa Lebel).

Boudier considered the straight paraphyses of *O. auricula* to be unique and established the genus *Wynnella* with *W. auricula* as the type species (= *W. silvicola* (Beck in Saccardo) Nannfeldt) (Kanouse, 1949; Harmaja, 1972). Kanouse (1949) did not recognize *Wynnella*. In contrast, Nannfeldt (1966) agreed with Boudier because of the "horny consistency when dry" of the apothecium; spores with one, not two, guttules; straight clavate paraphyses, and differences in the outermost layer of the ectal excipulum.

Harmaja (1972) proposed that *Wynnella* differs from *Helvella* only in form and color, and phylogenetic analysis of nucleotide sequence data (O'Donnell, et al., 1997) support a close relationship between *Helvella* and *Wynnella*.

Rifai (1968), Seaver (1928) and Groves and Hoare (1954), considered the generic name *Scodellina* Gray to be appropriate for *Otidea*. Rifai pointed out that *Scodellina* was originally described as "Thallus fleshy, membranaceous, brittle, sessile, hemisphaerical, spreading; outside scaly or mealy;" but no unequal split in the apothecium was mentioned. Because of this Rifai (1968) designated "*Peziza vesiculosa* Bull. ex St-Amans" as the lectotype of *Scodellina*, which is now recognized as a synonym of *Peziza*.

Harmaja (1974) moved *O. cantharella* (Fr.) Sacc. and *O. phlebophora* (Berk. & Br.) Saccardo from *Otidea* to his new genus *Flavoscypha* Harmaja with *Peziza phlebophora* Berk. & Br. as the type. He based the change on two minor microscopic and two macroscopic differences, primarily in the outermost layer of the ectal excipulum. He indicates the abhymenium of the apothecium is more or less bright yellow in *Flavoscypha* but this condition is absent in *Otidea sensu stricto*. Additionally he states that the basal parts of the cup are either pitted or ribbed somehow in *Flavoscypha*. Our work indicates that *O. cantharella* and *O. concinna* are closely related, and more information regarding this relationship is presented under the description of *O. concinna*. Cao, et al. (1990) do not mention *Flavoscypha*. Ellis and Ellis (1998) recognize *Flavoscypha* presumably based on the "flesh of ectal excipulum made up of rows of cells at right-angles to the surface."

Originally, apothecial morphology was the primary basis for delimiting *Otidea*. The irregularly split apothecium may either be elongated on one side, giving the fruiting

body a long-eared (donkey-eared) appearance, or symmetrical, giving the apothecium a more short-eared (mouse-eared) appearance (Nannfeldt, 1966). Microscopic and anatomical characters, such as the smooth-walled, ellipsoid, biguttulate ascospores, the slender, hyaline, usually hooked paraphyses, and the structure of the ectal and medullary excipula further reinforce the similarity of taxa within *Otidea* (Nannfeldt, 1966; Kanouse, 1949; Cao, Fan and Liu, 1990). The most recent *Dictionary of the Fungi* includes 15 species in the genus, and describes its distribution as North Temperate (Hawksworth, et al., 1995). The works of Nannfeldt (1966), Kanouse (1949), Cao, et al. (1990), Ellis and Ellis (1998) and Harmaja (1976) yield an extensive list of names proposed for taxa in the genus (Table 3.1).

Table 3.1: Specific epithets used within the genus by Nannfeldt, Kanouse, Cao *et al.*, Ellis and Ellis, and Harmaja since 1949. Authorities are listed verbatim from the listed source, as are past interpretations of the ICBN code (such as var. *typica* in Kanouse, 1949 and the use of "ex" in authority citations) and orthographic errors (such as *Otidea kauffmaniana* in Nannfeldt, 1966).

Specific Epithet	Source(s)
Otidea abietina (Pers. ex Fr.) Fuckel	Kanouse, 1949; Harmaja, 1976; Cao, et al., 1990
Otidea alutacea (Fr.) Bres. var. typica	Kanouse, 1949
Otidea alutacea (Pers. ex Fr.) Bres.	Nannfeldt, 1966
Otidea alutacea var. alutacea (Pers.) Mass.	Cao, et al., 1990; Ellis and Ellis, 1998
Otidea alutacea var. microspora Kanouse	Kanouse, 1949; Nannfeldt, 1966; Cao, et al., 1990
Otidea apophysata (Cooke & Phil.) Sacc.	Nannfeldt, 1966; Cao, et al., 1990
Otidea auricula (Cke.) Mass.	Kanouse, 1949
Otidea bufonia (Pers.) Boud.	Nannfeldt, 1966; Cao, et al., 1990; Ellis and Ellis, 1998
Otidea caligata (Myl.) Sacc.	Nannfeldt, 1966; Cao, et al., 1990
Otidea cantharella var. minor Boud.	Kanouse, 1949
Otidea cochleata (L. ex Fr.) Fuckel	Nannfeldt, 1966; Ellis and Ellis, 1998
Otidea cochleata (L. ex St-Amans) Fuckel	Cao, et al., 1990
Otidea concinna (Pers. ex Fr.) Sacc.	Kanouse, 1949
Otidea felina (Pers.) Bres.	Nannfeldt, 1966
Otidea formicarum Harmaja	Harmaja, 1976
Otidea grandis (Pers.) Boud.	Nannfeldt, 1966
Otidea grandis (Pers.) Rehm.	Kanouse, 1949; Cao, et al., 1990; Ellis and Ellis, 1998
Otidea indivisa (B. & Br.) Sacc.	Nannfeldt, 1966
Otidea indivisa Vel.	Nannfeldt, 1966; Cao, et al., 1990
Otidea kauffmaniana Kanouse	Nannfeldt, 1966
Otidea kauffmanii Kanouse	Kanouse, 1949; Cao, et al., 1990
Otidea lactea Cao et Fan	Cao, et al., 1990
Otidea leporina var. minor (Rehm) Sacc.	Cao, et al., 1990
Otidea microspora (Kanouse) Harmaja	Harmaja, 1976
Otidea myosotis Harmaja	Harmaja, 1976
Otidea nannfeldtii Harmaja	Harmaja, 1976; Cao, et al., 1990
Otidea olivacea Cao et Fan	Cao, et al., 1990
Otidea onotica (Fr.) Fuckel	Kanouse, 1949
Otidea onotica (Pers. ex. Fr.) Fuckel	Nannfeldt, 1966
Otidea onotica (Pers.) Fuckel	Cao, et al., 1990; Ellis and Ellis, 1998
Otidea papillata Harmaja	Harmaja, 1976
Otidea phlebophora (B. & Br.) Sacc.	Nannfeldt, 1966
Otidea platyspora Nannf.	Nannfeldt, 1966
Otidea propinquata (Karst.) Harmaja	Harmaja, 1976; Cao, et al., 1990
Otidea purpurea (Zang) Korf et Zhuang	Cao, et al., 1990
Otidea rainierensis Kanouse	Kanouse, 1949; Harmaja, 1976; Cao, et al., 1990
Otidea sinensis Cao et Fan	Cao, et al., 1990
Otidea smithii Kanouse	Kanouse, 1949; Harmaja, 1976; Cao, et al., 1990
Otidea tianshuiensis Cao et Fan	Cao, et al., 1990
Otidea tuomikoskii Harmaja	Harmaja, 1976; Cao, et al., 1990

Table 3.1.

In the Pacific Northwest, *Otidea* fruits mostly on the duff of coniferous forests, especially under Douglas-fir (*Pseudotsuga menziesii*), Pine (*Pinus* spp.) and hemlock (*Tsuga* spp.), but occasionally under cottonwood (*Populus trichocarpa*). *O. tuomikoskii* usually fruits from well-decayed conifer logs, but is also reported from ant hills (Harmaja, 1976). In several species, including *O. tuomikoskii* and *O. concinna*, the hyphae covering the stipe often bind the apothecium to the woody substrate. Whether *Otidea* is mycorrhizal is an open question. The constant association with ectomycorrhizal trees and phenology suggests at least some species may be mycorrhizal. Additionally, a preliminary analysis of carbon-13 signatures of several genera of fungi being conducted at the Environmental Protection Agency (E.P.A.) in conjunction with Oregon State University indicates that *Otidea* may be mycorrhizal (Erik Hobbie, pers. comm.). Little information has been provided by past collectors regarding *Otidea* habitats.

The work presented here is based on phylogenetic analyses of primary nucleotide sequence characters from nuclear ribosomal DNA (rDNA) and morphological characters (Peterson, et al., Chapter Two). Numerous collections and divergent morphotypes were included in the cladistic analysis to examine the morphological variation within a single phylogenetic species. Descriptions presented here complement the work done by Kanouse (1949) and great care has been taken to present accurate descriptions of members of the genus as they occur in the Pacific Northwest of North America.

Materials and Methods

Materials and methods follow Weber, Trappe, and Denison (1997) for fresh material and Baral (1992) for dried material. Unless otherwise noted, all microscopic measurements were on fresh material in water and taken with a Zeiss compound light microscope. Sections were made from innermost layer to outermost layer of the apothecium. Twenty-five spores released from asci were randomly chosen for measurement of length and width. Ten paired ascus length and width measurements were taken from randomly selected asci. Length was measured from the "hooks" at the base of the ascus to the tip, width was measured at the widest point. The ascus in *Otidea* is apically operculate, and the apex is slightly rounded. The spores are ellipsoid, generally 1.5 to 2.7 times longer than wide, and uni- to biguttulate. Most members of the genus have smooth spores, but *O. grandis* (not treated here) has ornamented spores. The terminal cell of the paraphyses in *Otidea* is generally hooked at maturity but may be straight and filiform, clavate, subglobose or globose. The inside of the "hook" of the paraphyses often has small protuberances, often imparting an irregular look to the terminal cell (Figure 3.2). Ten randomly selected paraphyses were measured just below the apex at the broadest point for apical cell width and below the first septa from the apex for "stem" width.

For each ascomata studied, five measurements of the ectal excipulum were taken from the outside of the fruiting body to the point where there appeared to be a significant change in the cell type of the layer (from *textura globosa* or *t. angularis* to *t. intricata* as found in the medullary excipulum). For each ascomata studied, five measurements of the medullary excipulum were made from the previous end point to the often distinct

subhymenium or the base of asci if the subhymenium was indistinct. When dried material was used, samples were taken from the margin to roughly 10 mm towards the center of the ascomata, and rehydrated for 10 minutes in distilled water before measuring. All excipulum measurements were made as near the center of the section as possible from fresh material mounted in distilled water. Squash mounts were used for taking measurements except when measuring the ectal and medullary excipulum, in which case great care was taken not to expand the thickness of the section. In each case the section was made perpendicular to the margin of the apothecium, and three to five apothecia were studied from each collection.

Overall ascomatal measurements are made from the base of the stipe to the top of the fruiting body for height, and at the widest cross-sectional dimension for width and depth. In the following text "in face view" refers to the "front" of the fruiting body, the side having the split in the apothecium. "From above" denotes that the fruiting body is held in face view and then rotated 90° *towards* the viewer, who would then be examining the top of the apothecium. "In side view" or "in profile" denotes that the fruiting body would be held in face view, then rotated 90° *clockwise*. Spore measurements are reported following Huhtinen (1989), giving the 90% confidence interval for each population of measurements as well as the minimum, maximum, and median values. Ascus and paraphysis measurements are reported as minimum and maximum values.

Apothecial shape is referred to as irregular, short-eared, long-eared, spathulate, windswept, or cupulate (Figure 3.1). The apex of the apothecium is either pointed or truncate (Figure 3.1 B & C). In all species presented here the apothecium is split on one side, either to the base or the top of the stipe. The margin of the apothecium is most often

smooth, but occasionally cracks with age and is often inrolled towards the fertile surface when young. The stipe, if present, is often covered with obvious white or cream-colored mycelium. This mycelium may extend into the substrate and bind it together. If the stipe is absent there may still be light-colored hyphae present at the base of the fruiting body.

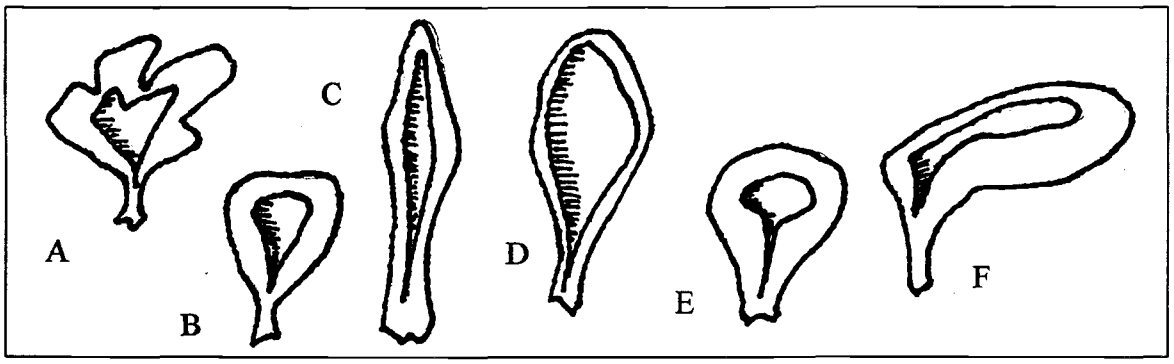

Figure 3.1: Apothecium shapes. A - Irregular; B - Short-eared, apex truncate; C - Long-eared, apex pointed; D - Spathulate; E – Cupulate; F – Windswept. All shapes presented in face-view except windswept, which is presented in profile.

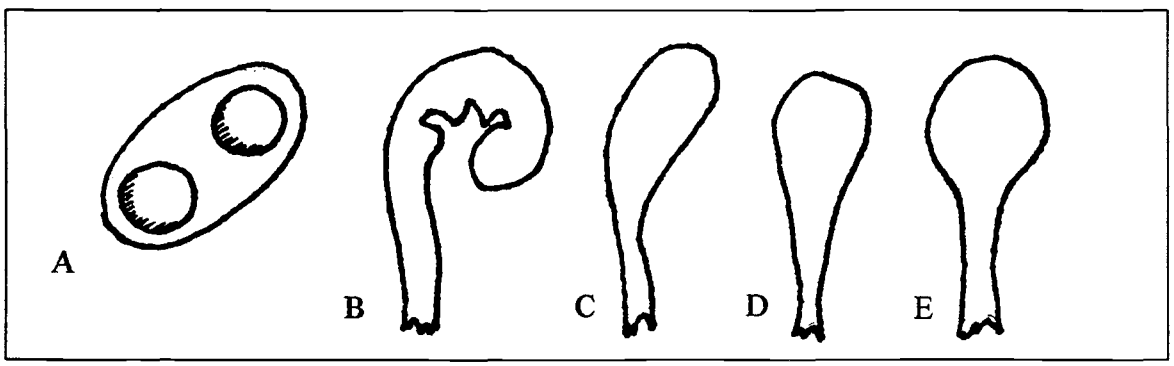

Figure 3.2: Shapes of ascospores and paraphyses apical cells. A - Mature ascospore; B - Hooked paraphysis; C - Clavate paraphysis; D - Subglobose paraphysis; E - Globose paraphysis.

Color names followed by a (Mnnn) designation follow Methuen (Kornerup and Wanscher, 1967) and those followed by a (Snnn) designation follow Smithe (1975) (where nnn is a number that matches plates or pages in each respective color guide). Every attempt has been made to use both sources for color nomenclature, except in the key to dried collections, which cites only the Smithe colors.

Specimens examined in this study were either collected in the course of the study, from the personal collection of Nancy Smith Weber, or loaned by Oregon State University (OSC) or the University of Michigan (MICH). No attempt was made to develop cultures. Specimens have been deposited at Oregon State University.

Generic description

Otidea (Persoon) Bonorden, *Handbuch der allgemeinen Mykologie als Anleitung zum Studium derselben*: 205. 1851.

Apothecia sessile to stipitate, variously shaped, split to base or top of stipe on one side to impart an "ear-shaped" appearance to the fruiting body; apex of apothecium pointed or truncate; margin generally smooth or occasionally cracking in age; colors various, generally some shade of yellow, orange, brown, or tan. Hymenium smooth. Abhymenium smooth to furfuraceous or pustulate. Stipe, if present, often covered with copious light-colored mycelia.

Asci J-; operculate, operculum round, apical; 8-spored; often arising from a darker-colored band in the subhymenium; with a slender base, bearing 1 or 2 well-separated hyphal attachments, cylindric; apex rounded; walls appearing as thick as those of the surrounding paraphyses. Spores smooth to minutely roughened, biguttulate

(infrequently uniguttulate), ellipsoid, generally 1.5 to 2.7 times longer than wide. Paraphyses hyaline, hooked or straight, occasionally with small protuberances inside the hook, slender to clavate or occasionally subglobose to globose at the tip, septate, branched below usually 3 - 4 times.

Subhymenium and medullary excipulum differentiated by a band of tissue slightly darker than the adjacent medullary excipulum. Medullary excipulum of densely interwoven, hyaline hyphae (*textura intricata*); cells elongate and slightly constricted at septa; entire layer generally thicker than adjacent ectal excipulum in section (measured at thickest point). Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis*); cells slightly constricted at septa; outermost layer smooth or ornamented with irregularily spaced clusters of globose cells and short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Type species: *Otidea onotica* (Persoon : Fries) Fuckel

No type species was designated for *Otidea* by Fuckel when he assigned *O. onotica, O. leporina, O. abietina* and *O. cochleata* to the genus. Kanouse (1949) designated *O. leporina* as the type of the genus, but Saccardo (Saccardo, 1882-1972) indicated *Peziza onotica* as the basionym for *Peziza* subgenus *Otidea*. Eckblad (1968) agreed with Saccardo on this point, as did Rifai (1968), indicating *P. onotica* (S. F. Gray) Persoon as the lectotype species. Neither Cao, et al. (1990) nor Otani (1969) provide any information about the typification of the genus. For the purposes of this work we follow Saccardo, Eckblad and Rifai and accept *O. onotica* as the type of the genus.

Key to *Otidea* (Fresh Material)

1. Ascoma typically light in color, some shade of yellow, orange or tan, or if darker (some shade of brown) then stipitate, stipe covered with white mycelium *2*
1. Ascoma dark in color, some shade of brown or purplish brown, typically astipitate or short stipitate *6*

2. Ascoma typically astipitate or substipitate; cupulate or obviously long eared in face view *3*
2. Ascoma typically stipitate; spathulate, windswept, short-eared, or irregular in face view *4*

3. Ascoma typically cupulate in face view; lower portion of ascoma may be lighter in color than apothecium, but not covered with copious white mycelium; spores (4.9--)5.6--5.7(--6.5) µm broad .....***O. rainierensis***
3. Ascoma typically long-eared; lower portion of the ascoma covered with copious white mycelium (4.9--)6.6--6.7(--6.7) µm .....***O. tuomikoskii***

4. Ascoma typically much taller than broad, often windswept or spathulate in face view; often with a pinkish cast or pinkish spots on the hymenium; spores (9.2--)11.6--11.9(--13.4) x (5.5--)6.3--6.4(--8.5) µm .....***O. onotica***
4. Ascoma not much taller than broad, often short-eared or irregular in face view; rarely with pink spots in the hymenium; spores of different length or width *5*

5. Hymenium concolorous with abhymenium or nearly so; apothecium generally short-eared or irregular in face view; spores (7.9--)10.3--10.6(--12.2) x (5.5--)6.2--6.4(--7.3) .....***O. concinna***
5. Hymenium typically darker than abhymenium; apothecium short-eared to approaching long-eared in face view; spores (12.2--)12.8--13.1(--14.6) x (6.7--)7.2--7.3(--7.9) µm .....***O. leporina***

6. Ascoma purplish-brown, drying brown; apex generally pointed but occasionally truncate; spores (12.2--)13.4--13.6(--15.5) x (6.0--)6.7--6.8(--8.0) µm....***O. smithii***
6. Ascoma brown but not purplish-brown, drying tan; apex generally truncate; spores of different length or width 7

7. Spores (11.6--)12.7--13.1(--14.0) x (6.1--)6.6--6.8(--7.3) µm; abhymenium loses color quickly upon drying .....***O. umbrina***
7. Spores (13.4--)15.4--15.7(--17.7) x (6.1--)7.1--7.2(--7.9) µm; abhymenium loses color slowly upon drying .....***O. alutacea***

Key to *Otidea* (Dried Material)

1. Spores small, (7.9--)10.3--11.9(--13.4) x (4.9--)5.6--6.7(--7.3) µm **2**
1. Spores large (11.6--)12.7--15.7(--17.7) x (6.0--)6.6--7.2(--7.9) µm **5**

2. Ascoma small, typically less than 2 cm tall, stipe (if present) lighter in color than hymenium; outside near "Raw Umber" (S23), "Cinnamon" (S39), "Brussels Brown" (S121B) or "Mikado Brown" (S121C); inside slightly lighter than "Clay Color" (S26) to "Cinnamon" (S39), "Cream Color" (S54), "Buff" (S124) or "Tawny Olive" (S223D); spores (7.9--)10.3--10.6(--12.2) x (5.5--)6.2--6.4(--7.3) .....***O. concinna***
2. Ascoma not as above, typically taller than 2 cm or otherwise different **3**

3. Ascoma medium to large, stipe (if present) lighter in color than above; concolorous inside and out, hymenium often with pink spots or patches; outside near "Clay Color" (S123B) or "Cinnamon" (S123A); inside near "Yellow Ocher" (S123C) or "Chamois" (S123D); spores (9.2--)11.6--11.9(--13.4) x (5.5--)6.3--6.4(--8.5) µm .....***O. onotica***
3. Hymenium without pink spots or patches, spores not as above **4**

4. Ascoma short-ear with apex truncate, typically sub- to astipitate but lower half typically light in color; concolorous inside and out or nearly so; outside "Cream Color" (S54), "Pale Pinkish Buff" (S121D), "Yellow Ocher" (S123C) or "Buff" (S124); inside "Cream Color" (S54), "Buff-Yellow" (S53) or "Buff" (S124); spores (9.2--)10.5--10.7(--12.2) x (4.9--)5.6--5.7(--6.5) µm .....***O. rainierensis***
4. Ascoma long-eared, base of ascoma typically lighter than above; concolorous inside and out to slightly lighter inside; outside "Cinnamon" (S123A), "Clay Color" (S123B) or "Verona Brown" (S223B); inside "Cinnamon" (S123A), "Clay Color" (S123B), "Yellow Ocher" (S123C) or "Chamois" (S123D); spores (9.2--)10.4--10.6(--11.6) x (4.9--)6.6--6.7(--6.7) µm .....***O. tuomikoskii***

5. Ascoma small, stipe (if present) lighter in color than above; outside "Cinnamon" (S123A), "Clay Color" (S123B), "Yellow Ocher" (S123C) or "Verona Brown" (S223B); inside "Raw Umber" (S223), "Mars Brown" (S223A), near "Vinaceous Pink" (S221C) or lighter to "Light Russet Vinaceous" (S121D); spores (12.2--)12.8--13.1(--14.6) x (6.7--)7.2--7.3(--7.9) µm .....***O. leporina***
5. Ascoma medium to large, not stipitate and otherwise different than above **6**

6. Ascoma large, long-eared with apex pointed or short-ear with apex truncate in shape; outside "Cinnamon" (S121A), "Clay Color" (S121B), near "Raw Umber" (S121), "Mars Brown" (S223A) or lighter to "Sayal Brown" (S223C), purplish cast not obvious in dried collections; inside "Vandyke Brown" (S121), "Prout's Brown" (S121A), "Chamois" (S123D) or "Tawny Olive" (S223D); spores (12.2--) 13.4--13.6(--15.5) x (6.0--)6.7--6.8(--8.0) µm ..... ***O. smithii***

6. Dried ascoma lighter in color than above, otherwise similar in shape and size 7

7. Abhymenium "Tawny Olive" (S223D) or "Sayal Brown" (S223C); hymenium "Pale Horn Color" (S92), "Drab-Gray" (S119D), lighter than "Chamois" (S123D) or "Tawny Olive" (S223D); spores (11.6--)12.7--13.1(--14.0) x (6.1--)6.6--6.8(--7.3) µm ..... ***O. umbrina***

7. Abhymenium "Tawny Olive" (S223D) or "Sayal Brown" (S223C); hymenium "Tawny Olive" (S223D), "Sayal Brown" (S223C) or "Raw Umber" (S223); spores (13.4--)15.4--15.7(--17.7) x (6.1--)7.1--7.2(--7.9) µm ..... ***O. alutacea***

Descriptions

Otidea alutacea (Persoon : Fries) Massee, *British Fungus Flora IV*: 446. 1895.

≡ *Peziza alutacea* Persoon, *Observationes Mycologicae II*: 78. 1796.
≡ *P. alutacea* Persoon : Fries, *Systema Mycologicum II*: 50. 1822.

Ascoma taller than broad, typically truncate at apex in face view; margins in-rolled towards hymenium when young, shape short-eared to cupulate in face view at maturity. Apothecia split to base, 2.0--6.0 cm tall in face view, 1.5--4.5 x 1.5--4.5 from above; flesh rubbery to firm, without taste. Hymenium smooth, near "Olive-Brown" (S28) or "Walnut-Brown" (S221B) when young to "Drab" (S27) when mature, occasionally with a hint of lilac, towards "Fawn Color" (S25). Margin entire, occasionally cracked at maturity. Abhymenium concolorous with the hymenium or lighter when fresh, near "Olive-Brown" (S28) or "Walnut-Brown" (S221B) when young to "Drab" (S27) when mature, occasionally with a hint of lilac, towards "Fawn Color" (S25); surface sparsely pustulate and minutely tomentose, wrinkled when dried especially

in mature specimens, quickly hygrophanous, and appearing to bruise when handled. Base of ascoma substipitate to astipitate, often lighter in color than the apothecium.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (13.4--)15.4--15.7(--17.7) x (6.1--)7.1--7.2(--7.9) µm, **x** = 15.5 x 7.2 µm (n = 125), hyaline. Asci J-; operculate, operculum round, apical; 8-spored; 150--207 x 10.5--12.9 µm. Paraphyses (Figure 3.2) hooked or straight, occasionally having small protuberances inside the hook, hyaline; often clavate at the tip, the clava 3.7--5.5 µm in diameter, below clava 2.4--3.1 µm thick; septate; branched below, usually 3--4 times.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata*); entire layer to 400--560 µm thick in section (measured at thickest point); cells elongate and slightly constricted at septa; hyphae 2.4--11.0 µm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis*), entire layer to 105--135 µm thick in section; cells slightly constricted at septa, 6.1--67.0 µm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells and short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious or caespitose on exposed soil, duff, or in moss under 80-year-old to 150-year-old *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Calocedrus decurrens* inland; in duff under young *Pinus contorta*, and *Picea sitchensis* on the coast; Oregon, Washington; September to March.

Specimens Examined. U.S.A. OREGON: Lincoln County, Devil's Punchbowl State Park, off Highway 101, 13 Mar. 1997, *T. O'Dell, ETP 039* (OSC 56770); same locality, 18 Mar. 1997, *E. T. Peterson, ETP 040* (OSC 56771). WASHINGTON: Clallam County, Olympic National Park, Elwha River, 27 Sept. 1941, *A. H. Smith, AHS 17338* (MICH); Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, 31 July 1948, *A. H. Smith, AHS 29714* (MICH); same locality, 28 July 1948, *A. H. Smith, AHS 29507* (MICH); same locality, 29 Oct. 1996, *E. T. Peterson, ETP 016* (OSC 56747); same locality, 29 Oct. 1996, *E. T. Peterson, ETP 023* (OSC 56754); same locality, 19 Sept. 1997, *E. T. Peterson, ETP 203* (OSC 56774); same locality, 19 Sept. 1997, *E. T. Peterson, ETP 205* (OSC 56776); same locality, 18 Oct. 1997, *E. T. Peterson, ETP 227* (OSC 56798); Snohomish County, Sloan Creek Trail, 24 Sept. 1997, *M. Madsen, ETP 206* (OSC 56777).

Observations. Kanouse (1949) referred to the type variety as *O. alutacea* var. *typica* in accordance with the ICBN (Camp, Rickett and Weatherby, 1947) in place at the time when she described the new variety *O. alutacea* var. *microspora*. Cao, et al. (1990) and Otani (1969) refer to this taxon as *O. alutacea* var. *alutacea*, whereas Britenbach and Kränzlin (1984), Tylutki (1993) and Dennis (1981) refer to this species as *O. alutacea* (Tylutki does however recognize *O. alutacea* var. *microspora*). Harmaja (1976) elevated *O. alutacea* var. *microspora* Kanouse to *O. microspora* (Kanouse) Harmaja based on the differences in spore size and ascoma color. Cao, et al. explicitly state that they follow Kanouse (1949) based on a lack of material available for examination. Britenbach and

Kränzlin include *O. alutacea* in their work with no mention of variety nor *O. microspora.* Preliminary molecular analysis by Peterson, et al. (Chapter Two) indicates the two varieties differ significantly phylogenetically, therefore we support Harmaja's conclusions.

This species occurs in two slightly different forms, perhaps depending upon habitat. The gregarious, caespitose, and slightly darker form tends to occur in coastal habitats under pine and spruce. The solitary, substipitate, lighter form tends to occur in inland habitats dominated by 80- to 150-year-old Douglas-fir, hemlock, and cedar. Collections from these divergent habitats have similar microscopic features and form a monophyletic group when analyzed phylogenetically (Peterson, et al., Chapter Two). Dermek (1977) includes a color plate of *O. alutacea* from Europe that resembles the solitary form and has slightly smaller spores than we report here.

Otidea concinna (Persoon : Fries) Saccardo, *Sylloge Fungorum VIII*: 96. 1889.

≡ *Peziza concinna* Persoon, *Mycologia Europea I*: 221. 1822.
≡ *P. concinna* Persoon : Fries, *Systema Mycologicum II*: 49. 1822.

Ascoma stipitate, often as tall as broad, truncate to pointed at apex in face view; margins in-rolled towards hymenium when young, short-eared to cupulate and split to irregular in face view at maturity. Apothecia split to top of stipe or nearly so, 1.0--4.5 cm tall in face view, 1.0--6.5 x 1.0--6.5 cm from above; flesh thin, without taste. Hymenium smooth, "Topaz" (M5C5) to "Clay" (M5D5), "Apricot (Yellow)" (M5C6) to "Oak Brown" (M5D6), or "Linoleum Brown" (M5E7), or "Clay Color" (S123B) to "Yellow Ocher" (S123C), occasionally with a pinkish cast or pink spots. Margin entire, occasionally cracked at maturity. Abhymenium concolorous with the hymenium when

fresh, "Topaz" (M5C5) to "Clay" (M5D5), "Apricot (Yellow)" (M5C6) or "Butter Yellow" (M4A5) to "Oak Brown" (M5D6), or "Bronze (Brown)" (M5E6), or "Cinnamon" (S123A); hygophanous towards "Light Yellow" (M4A4) or "Trogon Yellow" (S153); abhymenial pustulate and minutely tomentose, concolorous with hymenium when moist, often appearing wrinkled when dried. Stipe 3.0--1.2 mm tall, it and the lower portion of the ascoma covered with white or cream-colored hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (7.9--)10.3--10.6(--12.2) x (5.5--)6.2--6.4(--7.3) μm, **x** = 10.4 x 6.4 μm (n = 124), hyaline. Asci 150--212 x 7.5--13.5 μm. Paraphyses (Figure 3.2) hyaline, hooked at the apex, rarely with protuberances inside the hook; some apical cells clavate, the clava 2.4--3.7 μm thick, below clava 2.4--3.1 μm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata*), entire layer to 400--720 μm thick in section (measured at thickest point); cells elongate, slightly constricted at septa, hyphae 3.9--17.6 μm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis*), the entire layer to 150--300 μm thick in section; cells slightly constricted at septa, 9.8--30.3 μm in diameter at widest point; outer surface with irregularly spaced clusters of globose cells, or short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious or clustered on exposed soil, duff or moss under *Pseudotsuga menziesii, Tsuga heterophylla*; Oregon, Washington, Idaho, California; September to March.

Specimens Examined. U.S.A. CALIFORNIA: Del Norte County, Lake Earl, end of Sand Hill road, 15 Dec 1997, *J. Stockman, ETP 256* (OSC 56827). IDAHO: Idaho County, Seven Devils Mountains, Papoose Creek, 3 Sept 1954, *A. H. Smith* and *H. E. Bigelow, AHS 47346* (MICH). OREGON: Lane County, Hobbit Trail Coastal Area, East side of road, 11 Mar 1996, *E. T. Peterson, ETP 001* (OSC 56732); same locality, 16 Oct. 1996, *E. T. Peterson, ETP 004* (OSC 56735); Lane County, H.G. Andrews Experimental Forest, Lookout Creek Old-growth trail, 20 Oct 1996, *E. T. Peterson, ETP 005* (OSC 56736); same locality, 15 Oct 1997, *J. W. Spatafora, ETP 218* (OSC 56789); Linn County, Hackleman Old-growth trail, Highway 20, just East of Tombstone summit, 6 Oct. 1997, *E. T. Peterson, ETP 209* (OSC 56780); Marion County, Breitenbush Hot Springs Community, near Detroit Reservoir, in woods, 8 Nov. 1997, *J. W. Spatafora, ETP 237* (OSC 56808); same locality, same stand, 8 Nov. 1997, *J. W. Spatafora, ETP 238* (OSC 56809). WASHINGTON: Clallam County, Olympic National Park, Whiskey Bend trailhead, 26 Nov. 1996, *E. T. Peterson, ETP 029* (OSC 56760); Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, 29 Oct 1996, *E. T. Peterson, ETP 018* (OSC 56749); same locality, 22 Sept 1997, *E. T. Peterson, ETP 204* (OSC 56775); same locality, 18 Oct 1997, *E. T. Peterson, ETP 232* (OSC 56803).

Observations. *O. concinna* is the most common species that occurs in the Pacific Northwest. Fruiting in various habitats but most commonly 30 to 80-year-old Douglas-fir stands, it was collected nearly five times more often over this two-year study than the next most common species, *O. tuomikoskii*. Our species concept matches that of

Bresadola (1933), Boudier (1907), Kanouse (1949), Britenbach and Kränzlin (1984) and Cao, et al. (1990). The colors in Bresadola's Tab. 1226 appear lighter than we observed. Dermek (1977) includes *O. concinna* in his work but the color plate resembles *O. rainierensis sensu* Peterson, et al. and has spores much larger than expected for either fungus (19 – 21 x 9 – 12 μm).

Regarding the relationship between *O. concinna* and *O. cantharella sensu* Harmaja (= *O. cantharella* var. *minor sensu* Kanouse), we examined three collections of Alexander Smith and colleagues determined as *O. cantharella* var. *minor* by Kanouse, and in none did we observe the character states that Harmaja describes for *O. cantharella* (= *Flavoscypha cantharella* (Fr.) Harmaja) (AHS 27064, AHS 31033, AHS 56168; MICH). The ectal excipulum was of *textura globosa* and *t. angularis* and had only few short chains and clusters of cells. The dried color of the ascomata was in the range common to the genus, especially in *O. concinna* and *O. tuomikoskii*. No ribs or pits were observed, but the base of the fruiting body had white mycelium present in all three collections. Additionally, spore sizes were 9.8--11.0 x 5.5--6.1 μm. In these three collections it appears that either A) Kanouse misidentified light colored *O. concinna* as *O. cantharella* var. *minor* or B) the two species are synonyms, in which case the appropriate name would be *O. concinna*. It should also be noted that Kanouse (1949) refers to this taxon as *O. cantharella* var. *minor* Boudier and Harmaja (1974) refers to it as *O. cantharella* Fries, although both authors give very similar measurements for spore length and width (10--11(--12) x 6--7 μm in Kanouse, 10--12 x 5--6 μm in Harmaja).

Otidea leporina (Batsch : Fries) Fuckel, *Symbolae Mycologicae*: 329. 1870.

≡ *Peziza leporina* Batsch, *Elenchus Fungorum*: 117. 1783.
≡ *P. leporina* Batsch : Fries, *Systema Mycologicum II*: 47. 1822.

Ascoma stipitate to substipitate, taller than broad, pointed to truncate at apex in face view, short-ear shaped to slightly irregular or occasionally long-eared shaped in face view at maturity. Apothecia split to base or top of stipe, 2.0--6.0 cm tall in face view, 0.5--2.5 x 0.6--2.5 cm from above; taste slightly peppery. Hymenium smooth, "Golden Blonde" (M5C4) to "Topaz" (M5C5), "Oak Brown" (M5D6) to "Golden Brown" (M5D7), "Linoleum Brown" (M5E7) to "Yellowish Brown" (M5E8), or "Yellow Ocher" (S123C) to "Verona Brown" (S223B). Margin entire, in-rolled towards hymenium when young and occasionally cracked at maturity. Abhymenium concolorous with the hymenium or lighter when fresh, near "(Golden) Wheat" (M4B5), or "Pompeian Yellow" (M5C6) to "Oak Brown" (M5D6), very near "Cinnamon" (S123A) or "Clay Color" (S123B); abhymenial surface pustulate and minutely tomentose, not hygrophanous. Stipe, when present, covered with copious light colored hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (12.2--)12.8--13.1(--14.6) x (6.7--)7.2--7.3(--7.9) μm, **x** = 13.0 x 7.3 μm (n = 75), hyaline. Asci 165--12 x 9--10.5 μm. Paraphyses (Figure 3.2) hyaline, hooked, occasionally having 2--3 small protuberances inside the hook; often slightly clavate at the tip, the clava 3.7--4.9 μm thick, below clava 2.4--3.1 μm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata*), entire layer to 320 -- 400 μm thick in section (measured at thickest point); cells elongate and slightly constricted at septa, 2.9--13.7 μm in diameter at widest point. Ectal excipulum of

short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis*), the entire layer to 60--90 μm thick in section; cells slightly constricted at septa; 9.8--23.5 μm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells and short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious on exposed soil or in needle duff under *Pseudotsuga menziesii*, *Tsuga heterophylla*, or *Picea* spp.; Oregon, California; October to December.

Specimens Examined. U.S.A. CALIFORNIA: Del Norte County, Lake Earl Wildlife Area, 15 Dec. 1997, *J. Stockman ETP 254* (OSC 56825); Humboldt County, Big Lagoon State Park, 16 Dec. 1956, *A. H. Smith, AHS 56799* (MICH); same locality, 23 Dec. 1956, *A. H. Smith, AHS 56954* (MICH); same locality, 14 Dec. 1997, *M. Madsen, ETP 253* (OSC 56824). OREGON: Douglas County, Douglas County Bureau of Land Management, North Bank Habitat Management Unit, 4 Nov. 1997, *J. M. Trappe, JMT 21805* (OSC 60414); Lincoln County, Fogarty Creek State Park, Highway 101 near Depoe Bay, 15 Oct. 1997, *E. T. Peterson, ETP 213* (OSC 56784); same locality, 17 Oct 1997, *E. T. Peterson, ETP 221* (OSC 56792); same locality, 8 Nov 1997, *E. T. Peterson, ETP 235* (OSC 56806).

Observations. *O. leporina* is easily confused with *O. concinna* macroscopically. Both are typically short-eared or irregular in shape and occur in similar habitats. The two species differ slightly in color. *O. concinna* tends to be more orange or brownish-orange

(but displays a wide range of color variation throughout its range and is lighter when faded) and concolorous throughout, whereas *O. leporina* appears to have a lighter colored abhymenium and darker colored hymenial layer than *O. concinna*. An example of this confusion is the description of *O. leporina* in Tylutki (1993). He reports morphological characters that match both fungi, but smaller spore measurements (10 - 14 x 6 - 8 μm) than those attributed to *O. leporina,* which lead us to believe that perhaps Tylutki identified *O. concinna* as *O. leporina* (which he does point out is similar if not identical). The single best character to differentiate the two species is spore size, *O. leporina* has significantly longer spores than *O. concinna*.

O. leporina f. ***minor*** was proposed by Rehm (1881) and described as follows:

"f. minor.

Sporen elliptisch, stumpf, glatt, 1 zellig, mit meist 2 Kernen, -- 12/7; 8 1 reihig in cylindrischen Schlauchen, 120/9; Paraphysen fadig, hackig, Oben kaum etwas dicker, C. 3 mikr. Jod -."

The key and description in Kanouse (1949) will lead one to *O. leporina* var. ***minor*** (Rehm) Saccardo when trying to identify all light-colored, small spored, ear-shaped members of the genus having hooked apical cells of the paraphyses. Cao, et al. (1990) recognize this variety of *O. leporina* but state that they have not seen an authentic specimen and have viewed only one collection from China. In our work, collections that key to *O. leporina* var. ***minor*** with Kanouse (1949) are easily identified as *O. tuomikoskii* using the key in Cao, et al. (1990). Additionally, collections that were thought to be *O.*

leporina var. *minor* also keyed to *O. concinna*, which is the older of the two names. With the above in mind, we propose that *O. leporina* var. *minor* be regarded as a *nomen dubium*. Our interpretation of this species agrees visually with that of Boudier (1907) and Bresadola (1933). Dahncke and Dahncke (1979) include a photo of *O. leporina* from Europe that is closer to *O. concinna sensu* Peterson, et al. but has spores as expected for *O. leporina*. Lincoff's (1981) *O. leporina* fits our concept very well, and the color difference from hymenium to abhymenium is visible in the smaller fruiting body included in his work. Peter's (1964) photo of *O. leporina* from Europe again resembles *O. concinna* but has spores that fit our concept (12 – 15 x 6 – 8 μm).

Otidea onotica (Persoon : Fries) Fuckel, *Symbolae Mycologicae*: 329. 1870.

≡ *Peziza onotica* Persoon, *Synopsis Methodica Fungorum*: 637. 1801.
≡ *P. onotica* Persoon : Fries, *Systema Mycologicum II*: 48. 1822.

Ascoma stipitate, taller than broad, truncate to pointed at apex in face view; often semi-spathulate in face view or windswept in profile at maturity, split to top of stipe or nearly so, 1.0--7.0 cm tall in face view, 1.0--3.0 x 1.0--3.0 cm from above; flesh thin, without taste. Hymenium smooth, "Butter yellow" (M4A5) to "Maize" (M4A6) to "Sunflower" (M4A7) or "Saffron" (M4A8), slightly lighter than "Orange Yellow" (S18), often having a pinkish cast or pink spots throughout the layer at maturity. Margin entire, in-rolled towards hymenium when young and occasionally cracked at maturity. Abhymenium concolorous with the hymenium when fresh, to slightly darker, towards "Indian Yellow" (M5B7) to "Golden Brown" (M5D7), or slightly lighter than "Orange Yellow" (S18), slightly darker than "Buff-Yellow" (S53), very near "Trogon Yellow" (S153) when fresh, fading quickly when drying, especially in young specimens and then

"Pastel Yellow" (M3A4) to "Yellow" (M3A6) or "Buff-Yellow" (S53); abhymenial surface pustulate and minutely tomentose, concolorous with hymenium when moist, often appearing wrinkled when dried, hygrophanous. Stipe covered with white to cream colored hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (9.2--)11.6--11.9(--13.4) x (5.5--)6.3--6.4(--8.5) μm, **x** = 11.8 x 6.3 μm (n = 100), hyaline. Asci 150--180 x 9.0--10.5 μm. Paraphyses (Figure 3.2) hyaline, hooked at the apex, rarely having protuberances inside the hook; some apical cells clavate, the clava 2.4--3.1 μm thick, below clava 2.0--2.4 μm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata),* entire layer to 400 μm thick in section (measured at thickest point); cells elongate and slightly constricted at septa; hyphae 2.5--15.7 μm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis),* the entire layer to 120 - 160 μm thick in section; cells slightly constricted at septa, 11.8--40.0 μm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells, or short chains of globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to scattered or gregarious on exposed soil, duff or moss under 40-year-old to 150-year-old *Pseudotsuga menziesii*-dominated stands; Oregon, Washington; August to December.

Specimens Examined. U.S.A. OREGON: Benton County, Philomath, Woods Creek road, 18 Nov. 1996, *E. T. Peterson, ETP 028* (OSC 56759); Josephine County, Oregon Caves road, 1 Dec. 1937, *A. H. Smith, AHS 9322* (MICH); Lane County, Lamb Butte, 30 Sept. 1997, *T. O'Dell, ETP 208 (TOD 4273)* (OSC 56779). WASHINGTON: Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, Westside road, near highway 709, 27 Aug. 1948, *A. H. Smith, AHS 30674* (MICH); same locality, 8 Sept. 1948, *A. H. Smith, AHS 30990* (MICH); same locality, 18 Oct. 1997, *E. T. Peterson, ETP 230* (OSC 56801); same locality, 18 Oct. 1996, *E. T. Peterson, ETP 229* (OSC 56800); same locality, 1 Oct. 1996, *E. T. Peterson, ETP 003* (OSC 56734); same locality, 29 Oct. 1996, *Thom O'Dell, ETP 019* (OSC 56750); same locality, 30 Oct. 1996, *E. T. Peterson, ETP 024* (OSC 56755); same locality, 22 Sept. 1997, *E. T. Peterson, ETP 201* (OSC 56772); same locality, 22 Sept. 1997, *E. T. Peterson, ETP 202* (OSC 56773);

Observations. *O. onotica* has been considered easy to identify since the time of Fries and Persoon because of the characteristic pink hue often observed in the hymenium. This character can be absent or present either as spots in the hymenium or as large patches appearing pink throughout the hymenium. We have observed specimens with a slight pink hue in the hymenium that otherwise clearly match *O. concinna*. Careful examination differentiates the two based primarily on apothecium color and size and spore length. *O. concinna* is generally smaller and rarely as yellow, and has shorter spores than *O. onotica*. Our species concept is in general agreement with most previous authors, including Dahncke and Dahncke (1979), Dermek (1977) and Maublanc (1971). Exceptions include Romagnesi (1962) who includes a photo that resembles *O. leporina*

but has spores that fit our concept, and Schalkwijk-Barendsen (1991) who includes a fungus that resembles *O. tuomikoskii* and is described as a "lopsided cup, like donkey ears, edges inrolled; up to 10 cm tall."

Otidea rainierensis Kanouse, *Mycologia* 41: 674. 1949.

Ascoma substipitate to astipitate, taller than broad, usually truncate at apex in face view; split-cupulate in face view at maturity. Apothecia split to base, 1.0--5.0 cm tall in face view, 1.0--5.0 x 1.0--5.0 cm from above; flesh thin, without taste. Hymenium smooth, "Drab" (S27) to "Drab-Gray" (S119D) or lighter, approaching "Fawn Color" (S25). Margin entire and occasionally cracked and darker colored than the abhymenium proper at maturity. Abhymenium concolorous with the hymenium or lighter when fresh, "Drab" (S27) to "Drab-Gray" (S119D) or lighter, approaching "Fawn Color" (S25); abhymenial surface smooth, apparently lacking pustules. Lower portion of ascoma lacking light colored superficial hyphae but occasionally is lighter in color than the apothecium.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (9.2 --) 10.5 -- 10.7 (--12.2) x (4.9--) 5.6 -- 5.7 (--6.5) µm, **x** = 10.6 x 5.7 µm (n = 100) hyaline. Asci 140 -- 160 x 10.0 µm. Paraphyses (Figure 3.2) hyaline, straight or slightly hooked, often globose to subglobose (Figure 3.2) or clavate at the tip; the clava 3.7--7.3 µm thick, below clava 2.4--3.1 µm thick; septate; branched below, usually 3--4 times.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata),* entire layer to 560--800 µm thick in section (measured at thickest point); cells elongate and slightly constricted at septa, hyphae 2.4--11.0 µm in diameter at widest point. Ectal

excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis),* the entire layer to 200--400 µm thick in section; cells slightly constricted at septa, 6.1--67.0 µm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells and short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious and caespitose on exposed soil, duff or moss under 80-year-old to 150-year-old *Pseudotsuga menziesii*, *Tsuga heterophylla*; Oregon, Washington, Idaho; August to October.

Specimens Examined. U.S.A. IDAHO: Bonner County, Priest River Experimental Forest, 26 Sept. 1968, *N. J. Smith, NJS 2067* (MICH). OREGON: Jackson County, Rogue River National Forest, off Forest Service road 37, 27 Oct. 1990, *D. McKay, NSW 6354*; Marion County, near Breitenbush Hot Springs Community, 27 Oct. 1996, *J. M. Trappe, ETP 014* (OSC 56745). WASHINGTON: Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, 23 Aug. 1948, *A. H. Smith, AHS 30553* (MICH, HOLOTYPE); same locality, 5 Sept. 1948, *E. G. Simmons, EGS 2179* (MICH, PARATYPE); Lewis County, Gifford-Pinchot National Forest, Camp Creek Falls Trail, 6 Nov. 1997, *M. Castellano, ODELL 4680* (OSC 56829).

Observations. Kanouse (1949) based *O. rainierensis* primarily on subglobose to globose apical cells of paraphyses, a character state not previously reported for the genus. Often an equal or greater number of regularly hooked apical cells occur on the

paraphyses. We speculate that there are two modes of apical cell formation present in the genus. One, observed here, has apical cells subglobose to globose or clavate when young and become hooked or bent at maturity. Another has apical cells that are slender (filiform) to slightly clavate when young becoming regularly hooked or bent at maturity. Careful examination of a large number of paraphyses is necessary to observe the states of this character. One may question the value of this character, especially considering that Kanouse described two new taxa based primarily on this feature and minor color variations that she did not observe directly (notes of C. H. Kauffman and A. H. Smith). However, the molecular analysis by Peterson, et al. (Chapter Two) supports the monophyly of this species, and the two character states map onto two distinct clades in rDNA ITS analysis (their Figure 4). *O. rainierensis, O. kauffmanii, O. microspora*, and *O. grandis* form a monophyletic, basal lineage within *Otidea*, and all have subglobose to globose apical cells in the paraphyses.

Otidea smithii Kanouse, *Papers of the Michigan Academy of Science, Arts & Letters* 24: 28. 1939.

Ascoma stipitate to substipitate, taller than broad, usually pointed at apex in face view; short-ear or long-ear shaped in face view at maturity. Apothecia always split to top of stipe, 3.0--9.0 cm tall in face view, 3.0--5.0 x 3.0--5.0 cm from above; flesh thick, without taste. Hymenium smooth, "Brown" (M6E4) to "Dark Brown" (M7F7 to M7F8), or "Dark Greyish Brown" (S20) or "Drab" (S27), often with a purplish cast. Margin entire and occasionally cracked at maturity. Abhymenium concolorous with the hymenium or lighter when fresh, "Brown" (M6E4) to "Chestnut Brown" (M6F7) or "Brownish Grey" (M6F8) or "Burnt Umber" (S22) to "Raw Umber" (S23), hygrophanous

to "Reddish Golden" (M6C7) or "Raw Sienna" (M6D7); abhymenial surface pustulate and minutely tomentose, wrinkled in appearance when dried especially in mature specimens, hygrophanous. Stipe, when present, covered with light colored hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (12.2--)13.4--13.6(--15.5) x (6.0--)6.7--6.8(--8.0) µm, **x** = 13.5 x 6.7 µm (n = 100), hyaline. Asci 170--203 x 10.5 µm. Paraphyses (Figure 3.2) hyaline, hooked, occasionally having 2--3 small protuberances inside the hook; often slightly clavate at the tip, the clava 3.1--4.9 µm thick, below clava 2.4--3.7 µm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata*), entire layer to 480--560 µm thick in section (measured at thickest point); cells elongate and slightly constricted at septa, hyphae 5.0--7.3 µm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis*), the entire layer to 90--120 µm thick in section; cells slightly constricted at septa, 6.1--18.4 µm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells and short chains of 3 to 4 globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious on exposed soil, duff or moss under 80-year-old to 150-year-old *Pseudotsuga menziesii*, 100-year-old *Populus trichocarpa*, 150-year-old *Tsuga heterophylla*; California, Idaho, Oregon, Washington; September to December.

Specimens Examined. U.S.A. CALIFORNIA: Del Norte County, Crescent City, 18 Nov. 1937, *A. H. Smith, AHS 8843* (MICH, HOLOTYPE); Earl Lake State Park, 15 Dec. 1997, *M. Madsen* and *R. Davis, ETP 252* (OSC 56823). IDAHO: Bonner County, Priest Lake area, 27 Sept. 1968, *N. J. Smith, NJS 2076* (MICH); Priest River Experimental Forest, 27 Sept. 1995, *T. Lebel, NSW 7536*. OREGON: Benton County, Philomath, Woods Creek road, 15 Nov. 1997, *E. T. Peterson* and *T. Lebel, ETP 240* (OSC 56811). WASHINGTON: Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, 8 Sept. 1948, *A. H. Smith, AHS 30994* (MICH); same locality, 30 Oct. 1996, *E. T. Peterson, ETP 022* (OSC 56753); same locality, 18 Oct. 1997, *E. T. Peterson, ETP 228* (OSC 56799); same locality, 18 Oct. 1997, *E. T. Peterson, ETP 226* (OSC 56797); Lewis County, Gifford-Pinchot National Forest, Camp Creek Falls Trail, 6 Nov. 1997, *E. Hathaway and E. Milliman, ODELL 4455* (OSC 56830).

Observations. *O. smithii* is relatively easy to identify when fresh by its overall shape, size, and color (purplish-brown hymenium, unique within the genus). Habitat for this species is quite variable as observed in the course of this study. Kanouse (1939) described the species as under pine in Crescent City, California. In this study we collected *O. smithii* under pine, Douglas-fir, cottonwood, and hemlock. *O. smithii* does appear to have an association with older forests, although one collection (ETP 240) was made in a 60- to 80-year-old Douglas-fir stand in Oregon. Miller (1977) includes a photo of a fungus he refers to as *O. smithii* having spores 18 – 24 x 12 – 14 μm and colors much more red than purple. We believe Miller's *O. smithii* to actually be *Wynnella silvicola*.

Otidea tuomikoskii Harmaja, *Karstenia* 15: 30. 1976.

Ascoma stipitate to substipitate, much taller than broad, usually pointed at apex in face view; narrowly long-ear shaped in face view at maturity. Apothecia always split to the base or nearly so, 6.0--8.5 cm tall in face view, 1.0--2.5 x 1.0--2.5 cm from above; flesh thick, without taste. Hymenium smooth, slightly lighter than "Butter Yellow" (M4A5) or "Buff-Yellow" (S53) to "Yellow-Ocher" (S123C). Margin entire, in-rolled towards hymenium when young and occasionally cracked at maturity. Abhymenium concolorous with the hymenium when fresh, "Butter Yellow" (M4A5) to slightly darker, "Maize (Yellow)" (M4A6) or "(Golden) Wheat" (M4B5), or "Buff-Yellow" (S53) or "Cream Color" (S54) to "Yellow-Ocher" (S123C), slowly hygrophanous to "Butter Yellow" (M4A5) or lighter; abhymenial surface pustulate and minutely tomentose, concolorous with hymenium when moist, hygrophanous. Lower portion of the apothecium often covered with light colored superficial hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (9.2--)10.4--10.6(--11.6) x (4.9--)6.6--6.7(--6.7) µm, **x** = 10.5 x 5.7 µm (n = 100), hyaline. Asci 150--201 x 9.0--10.5 µm, arising from a band in the subhymenium slightly darker than adjacent tissue (approximately 80 µm thick in section). Paraphyses (Figure 3.2) hyaline, hooked at the apex, often having 2-3 protuberances inside the hook; the tip 1.8--3.7 µm thick, below tip 1.8--2.4 µm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata),* entire layer to 400--480 µm thick in section (measured at thickest point); cells elongate and slightly constricted at septa, hyphae 6.7--11.6 µm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or

± irregular (*textura globosa* to *t. angularis*), the entire layer to 120--150 µm thick in section; cells slightly constricted at septa; 9.8--49.0 µm in diameter at widest point; outermost layer with irregularily spaced clusters of globose cells, or short chains of globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious on exposed soil, duff or well-decayed conifer logs, under 40-year-old to 80-year-old *Pseudotsuga menziesii*; Oregon, Washington, California; September to December.

Specimens Examined. U.S.A. CALIFORNIA: Del Norte County, Earl Lake State Park, off Sand Hill road, 15 Dec. 1997, *M. Madsen & R. Davis, ETP 255* (OSC 56826); Humboldt County, Big Lagoon Park, 16 Dec. 1956, *A. H. Smith, AHS 56799* (MICH); same locality, 23 Dec. 1956, *A. H. Smith, AHS 56954* (MICH). OREGON: Benton County, Corvallis, MacDonald-Dunn Research Forest, Road 580, stand 050907, 1 Nov. 1996, *E. T. Peterson, ETP 025* (OSC 56756); same locality, 2 Dec. 1996, *E. T. Peterson, ETP 030* (OSC 56761); same locality, 23 Oct. 1996, *E. T. Peterson, ETP 011* (OSC 56742); same locality, 15 Oct. 1997, *E. T. Peterson, ETP 212* (OSC 56783); same locality, 11 Oct. 1997, *E. T. Peterson, ETP 222* (OSC 56793); same locality, stand 060510, 17 Oct. 1997, *L. Grubisha, ETP 219* (OSC 56790); same locality, stand 080801 near intersection of roads 680 and 700, 25 Nov. 1997, *E. T. Peterson, ETP 247* (OSC 56818); Columbia County, Tillamook BLM Resource Area, Pig's Puzzle Timber sale, 10 Dec. 1997, *M. Madsen* and *L. R. Scofield, ETP 251* (OSC 56822). WASHINGTON: Clallam County, Olympic National Park, Elwha River, 27 Sept. 1941, *A. H. Smith, AHS*

17338 (MICH); Pierce County, Mt. Rainier National Park, Lower Tahoma Creek, 18 Oct. 1997, *E. T. Peterson, ETP 231* (OSC 56802).

Observations. *O. tuomikoskii* was described from Finnland (Harmaja, 1976); this is the first report of it from North America. Numerous collections of this taxon have been ascribed to *O. leporina* var. *minor* based loosely on color and spore size, especially when identified with descriptions in Kanouse (1949). Cao, et al. (1990) include *O. tuomikoskii* in their descriptions and differentiate it from *O. leporina sensu stricto* and *O. leporina* var. *minor* based on having a "yellowish to clear yellow" hymenium and a "brown to dull brown" exterior. Harmaja is careful to note that the apothecia of *O. tuomikoskii* are "gregarious to caespitose, pronouncedly and rather narrowly ear-shaped", this morphology being unique to the genus as it occurs in the Pacific Northwest. Additionally, Harmaja notes that under ultraviolet light (UV_{254}) there is a stark contrast between abhymenial and hymenial colors, being dark outside but bright pale yellow internally. This character was checked and his description is quite good. Considering Harmaja's work and the distinctive morphology of this taxon we now recognize *O. tuomikoskii* as occurring in the Pacific Northwest, primarily in 40- to 80-year-old Douglas-fir stands in-or-near well decayed conifer logs. Schalkwijk-Barendsen (1991) describes a fungus as *O. onotica* that we believe to be *O. tuomikoskii* and in her description states that this fungus is "said to be a good edible with an almond taste."

Otidea umbrina (Persoon) Bresadola, *Fungi Tridentini II*: 68. 1898.

≡ *Peziza umbrina* Persoon, *Observationes Mycologicae II*: 77. 1796.

Ascoma substipitate to astipitate, taller than broad, usually truncate at apex in face view; semi-spathulate, occasionally deep-cupulate in face view at maturity. Apothecia always split to the base or nearly so, 2.0--7.0 cm tall in face view, 1.0--5.5 x 1.0--5.5 cm from above; flesh thick, without taste. Hymenium smooth, milk-chocolate-brown when fresh, slightly lighter than "Mustard Brown" (M5E6) or "Linoleum Brown" (M5E7) or "Cinnamon-Brown" (S33), or "Ground-Cinnamon" (S239), or slightly lighter than "Raw Umber" (S123). Margin entire, in-rolled towards hymenium when young and occasionally cracked at maturity. Abhymenium concolorous with the hymenium when fresh, to slightly darker, towards "Snuff (Brown)" (M5F6) or "Coffee" (M5F7) or "Cinnamon-Brown" (S33), slightly darker than "Raw Umber" (S223), or slightly lighter than "Prout's Brown" (S121A), fading quickly when drying, especially in young specimens, towards "Ivory" (M4B3) or "Champagne" (M4B4); abhymenial surface pustulate and minutely tomentose, concolorous with hymenium when moist, hygrophanous. Lower portion of ascoma rarely covered with light colored superficial hyphae.

Ascospores (Figure 3.2) smooth, biguttulate, ellipsoid, (11.6--)12.7--13.1(--14.0) x (6.1--)6.6--6.8(--7.3) μm, **x** = 12.9 x 6.7 μm (n = 25), hyaline. Asci 180--245 x 9.0--12.0 μm. Paraphyses (Figure 3.2) hyaline, hooked, often irregularily so, at the apex, often having 2--3(--5) protuberances inside the hook; often slightly clavate at the tip, the clava 3.1--5.5 μm thick, below clava 2.4--3.1 μm thick; septate; branched below, usually once.

Medullary excipulum of densely woven hyaline hyphae (*textura intricata),* entire layer to 400--440 μm thick in section (measured at thickest point); cells elongate and slightly constricted at septa, hyphae 5.0--6.0 μm in diameter at widest point. Ectal excipulum of short, hyaline, versiform to globose or pyriform cells varying to angular or ± irregular (*textura globosa* to *t. angularis),* the entire layer to 120 μm thick in section; cells slightly constricted at septa, cells 6.1--24.5 μm in diameter at widest point; outer surface with irregularily spaced clusters of globose cells, or short chains of globose to subglobose cells that are slightly constricted at the septa.

Habit, Habitat and Distribution. Solitary to gregarious or occasionally caespitose on exposed soil, occasionally in duff, under 40-year-old to 80-year-old *Pseudotsuga menziesii* often mixed with *Acer* spp. and *Quercus garryana*; Oregon, Washington; October to November.

Specimens Examined. OREGON: Benton County, Corvallis, north of Corvallis in McDonald-Dunn Research Forest, stand 080801, 25 Oct. 1996, *E. T. Peterson, ETP 013* (OSC 56744); same locality, 9 Nov. 1996, *E. T. Peterson, ETP 026* (OSC 56757); same locality, 18 Nov. 1996, *E. T. Peterson, ETP 027* (OSC 56758); same locality, 13 Oct. 1997, *E. T. Peterson, ETP 210* (OSC 56781); same locality, stand 060210, 10 Oct. 1997, *S. Holmes, ETP 211* (OSC 56782); same locality, stand 060510, 8 Nov 1997, *R. Hamill, ETP 239* (OSC 56810); Corvallis, Witham Hill, back of Witham Oaks apartments, 25 Nov 1997, *L. Wilson, ETP 242* (OSC 56813). WASHINGTON: Pierce

County, Mt. Rainier National Park, Lower Tahoma Creek, 13 Sept. 1954, *A. H. Smith, AHS 47471* (MICH); same locality, 15 Sept. 1954, *A. H. Smith, AHS 47514* (MICH).

Observations. We initially thought this was a new species of *Otidea*; however careful examination of herbarium material and the works of Boudier (1907) and Bresadola (1933) reveal that this species matches *O. umbrina*. Both the description of the internal and external color, down to the perhaps unintentional description of a lighter colored exterior without noting the quick hygrophanous effect, as well as the spore size, fits Bresadola's interpretation very well. Very little has been written about *O. umbrina* regarding habitat or distribution, and in the literature it has not been noted to occur in North America. In fact, the name *O. umbrina* appears to have not been used recently.

Several names have been used when discussing dark-brown-colored members of the genus including *O. cochleata, O. bufonia,* and *O. alutacea*. Cao, et al. (1990) report that *O. alutacea* is lighter in color than *O. cochleata* and *O. bufonia* (dull brown vs. dark brown). *O. cochleata* is reported to have larger spores than *O. umbrina* (16 - 19 μm x 7.5 - 8.0 μm vs. 11.6 - 15 μm x 6.1 - 7.3 μm). The photograph of *O. cochleata* in Breitenbach and Kränzlin (1984) shows the color of *O. cochleata*, reported as reddish-brown to dark-brown by Cao, et al. (1990), and in our opinion the two species are quite different in this respect. Romagnesi (1962) includes *O. umbrina* from France and his concept very closely matches our own.

O. bufonia is considered to be a synonym of *O. umbrina* by Dennis (1981), and his color plate (VIII C.) superficially matches fresh material collected in the course of this study, except for the obvious light-colored hyphae at the base of the apothecium.

Additionally, Dennis describes the spores of *O. bufonia* as elliptic-fusiform, a character state that was not observed in the taxa in this study. Because of the lack of conspicuous light-colored hyphae near the base of the apothecium and the difference in shape of the ascospores, we choose not to recognize the synonymy of *O. umbrina* and *O. bufonia sensu* Dennis (1981).

The apparent difference between *O. umbrina* and *O. alutacea* is very slight, dull-brown versus dark-brown. Otherwise they share many characters states including those of stipe, stature, and shape. The two major differences between these two closely related taxa as observed in this study are those of ascospore size and abhymenial ornamentation. The ascospores of *O. alutacea* are slightly longer than those of *O. umbrina*, 13.4--17.7 x 6.1--7.9 μm vs. 11.6--14.0 x 6.1--7.3 μm. The exterior of *O. umbrina* becomes lighter very quickly upon drying, a visible change is obvious in under five minutes, especially in younger specimens, and this effect is easily observed in dried specimens. This quickly-hygrophanous effect was not observed and has not been reported for *O. alutacea.* Additionally, although *O. umbrina* and *O. alutacea* form a monophyletic group in the analysis of Peterson, et al. (Chapter Two), each individual species is well supported as a distinct monophyletic taxon (97%, their Figure 4).

O. umbrina has been collected quite frequently in the vicinity of Corvallis, Oregon, primarily due to the activity of Dr. J. W. Spatafora's Introductory Mycology class. Outside of Corvallis, this species is very poorly known and has been reported less than five times during this study from inadequate collections. More data needs to be collected regarding this fungus, its distribution, and habitat. To confirm the identification of this name specimens from MICH were examined, and in every case a near exact match was made.

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References

Baral, H. O. 1992. Vital versus herbarium taxonomy: Morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* XLIV (2): 333-390.

Bonorden, H. F. 1851. *Handbuch der allgemeinen Mykologie als Anleitung zum Studium derselben.* E. Schweizerbart, Stuttgart.

Boudier, E. 1907. *Histoire et Classification des Discomycetes d'Europe*. Paul Klincksieck, Paris.

Breitenbach, J., F. Kränzlin. 1984. *Fungi of Switzerland: Volume 1 Ascomycetes*, pp. 310. Verlag Mykologia, Luzern.

Bresadola, J. 1933. *Iconographia Mycologica.* Volume 25. E. J. Gilbert, Milano.

Camp, W. H., H. W. Rickett, C. A. Weatherby. 1947. International rules of botanical nomenclature. *Brittonia* 6 (1): 1-120.

Cao, J.-Z., L. Fan, B. Liu. 1990. Some species of *Otidea* from China. *Mycologia* 86 (6): 734-741.

Dahncke, R. M., S. M. Dahncke. 1979. *700 Pilze in Farbfotos*. Verlag, Aarau.

Dennis, R. W. G. 1981. *British Ascomycetes*. J. Cramer, Vaduz.

Dermek, A. 1977. *Atlas nasich hub*. Publisher unknown, Obzor.

Eckblad, F.-E. 1968. The Genera of Operculate Discomycetes. *Nytt Magasin for Botanikk* 15 (1-2): 1-191.

Ellis, R. W., P. Ellis. 1998. *Microfungi on Miscellaneous Substrates*. The Richmond Publishing Co. Ltd., Slough.

Fuckel, L. 1869-1870. *Symbolae Mycologicae. I. Fungi Perfecti*. Julius Niedner, Wiesbaden.

Groves, J. W., S. C. Hoare. 1954. Notes on fungi from Northern Canada. I. Hypocreales and Discomycetes. *The Canadian Field Naturalist* 68 (1): 1-8.

Harmaja, H. 1972. Notes on the genus *Helvella*, including the merging of the genus *Wynnella*. *Karstenia* 14 : 102-104.

Harmaja, H. 1974. *Flavoscypha*, a new genus of the Pezizales for *Otidea cantharella* and *O. phlebophora*. *Karstenia* 14 : 105-108.

Harmaja, H. 1976. New species and combinations in the genera *Gyromitra*, *Helvella*, and *Otidea*. *Karstenia* 15 : 29-32.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, P. N. Pegler. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*. CAB, London.

Kanouse, B. B. 1939. Notes on new or unusual discomycetes. *Papers of the Michigan Academy of Science, Arts & Letters* 24 : 28.

Kanouse, B. B. 1949. Studies in the genus *Otidea*. *Mycologia* 41 : 660-677.

Kornerup, A., J. H. Wanscher. 1967. *Methuen Handbook of Colour*. Methuen & Co. Ltd, London.

Lincoff, G. H. 1981. *The Audubon Society Field Guide to North American Mushrooms*. Alfred A. Knopf, New York.

Maublanc, A. 1971. *Champignons Comestibles et Veneneux*. Editions Lechevalier S.A.R.L., Paris.

Miller, O. K. 1977. *Mushrooms of North America*. E. P. Dutton, New York.

Nannfeldt, J. A. 1966. On *Otidea caligata, O. indivisa*, and *O. platyspora* (Discomycetes, Operculatae). *Annales Botanici Fennici* 3 : 309-318.

O'Donnell, K., E. Cigelnik, N. S. Weber, J. M. Trappe. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *Mycologia* 89 (1): 48-65.

Otani, Y. 1969. Some species of the genus *Otidea* collected in Japan. *Transactions of the Mycological Society of Japan* 9 (3): 101-108.

Peter, J. 1964. *Das Grosse Pilzbuch*. Safari-Verlag, Berlin.

Rifai, M. A. 1968. The Australasian Pezizales in the Herbarium of the Royal Botanic Garden Kew. *Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, afd. Natuurkunde* 57 (3): 1-295.

Romagnesi, H. 1962. *Petit Atlas des Champignons*. Bordas, unknown.

Saccardo, P. A. 1882-1972. *Sylloge Fungorum*. Volumes VIII, X, XI, XIV, XVI, XVIII, XX, XXII. Many publishers, Many cities.

Schalkwijk-Barendsen, H. M. E. 1991. *Mushrooms of Western Canada.* Lone Pine Publishing, Edmonton.

Seaver, F. J. 1928. *The North American Cup-fungi (Operculates).* Fred Jay Seaver, New York.

Smithe, F. B. 1975. *Naturalist's Color Guide.* The American Museum of Natural History, New York.

Tylutki, E. E. 1993. *Mushrooms of Idaho and the Pacific Northwest. Volume One Discomycetes.* University of Idaho Press, Moscow.

Weber, N. S., J. M. Trappe, W. C. Denison. 1997. Studies on Western American Pezizales. Collecting and describing ascomata -- Macroscopic features. *Mycotaxon* 61 : 153-176.

Chapter 4

CONCLUSIONS

Molecular analysis described in Chapter Two of this work indicates that there are eight species of *Otidea* that occur commonly in the Pacific Northwest. This number may be as high as ten with the inclusion of *O. microspora* and *O. abietina,* which were not collected in the course of this study. The information presented in Chapter Two also indicates that nucleotide data from the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) repeat unit is adequate to allow for the differentiation of phylogenetic species, especially when these data are combined with characters coded from morphology. Additionally, molecular analysis of the large subunit (LSU) of several species of *Otidea* indicates that there are at least three different LSU types present in *Otidea* as sampled in this study. The origin of these divergent types is speculated to be multiple ancient gene duplication events. The result is multiple LSU rDNA types (ancestral LSU rDNA polymorphisms) present within both *O. leporina* and *O. smithii* that have somehow not been homogenized by lineage sorting and concerted evolution (Zimmer, et al., 1980).

The phylogenetic species inferred in Chapter Two have been described in Chapter Three and new keys to both fresh and dried materials have been written. Pending additional information, the species of *Otidea* known to occur in the Pacific Northwest are as follows: *O. smithii, O. onotica, O. leporina, O. concinna, O. tuomikoskii, O. alutacea, O. umbrina* and *O. rainierensis*. Kanouse (1949) indicates that *O. abietina* is known to

occur in Washington and *O. microspora* was described from this range, although neither was found in the course of this study.

Regarding conservation of *Otidea* species and their habitat, while I have no way to make a formal statement about rarity I can comment on the number of occurrences of individual species. A graph of the total number of collections for each species examined in the course of this study is presented in Figure 4.1. The numbers on this graph represent all collections made in the course of this two-year study. Clearly, the small number of collections of the species represented on the right side of the graph indicates that *O. microspora, O. rainierensis,* and *O. smithii* are rare. For a fungus to be collected less than five times in two years, especially when a group of experts is looking for it, must surely be some indication of rarity. Castellano (1997) defines rare as "species known from 10 or less vouchered occurrences" and gives a range of 1 km for the boundary of collection. The 1996 R.E.D. list for Oregon Macrofungi only indicates that *O. onotica* is rare in Oregon (Castellano, 1997). The 1994 FEMAT list includes *O. onotica* along with *O. smithii* and *O. leporina* as rare or endangered in the range of the Northern Spotted Owl (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994). In the course of this study *O. onotica* has been found quite often and now I question whether or not this listing is warranted. *O. onotica* aside, it is clear that under Castellano's definition of rare that *O. umbrina, O. leporina, O. alutacea, O. smithii, O. rainierensis,* and *O. microspora* are quite rare.

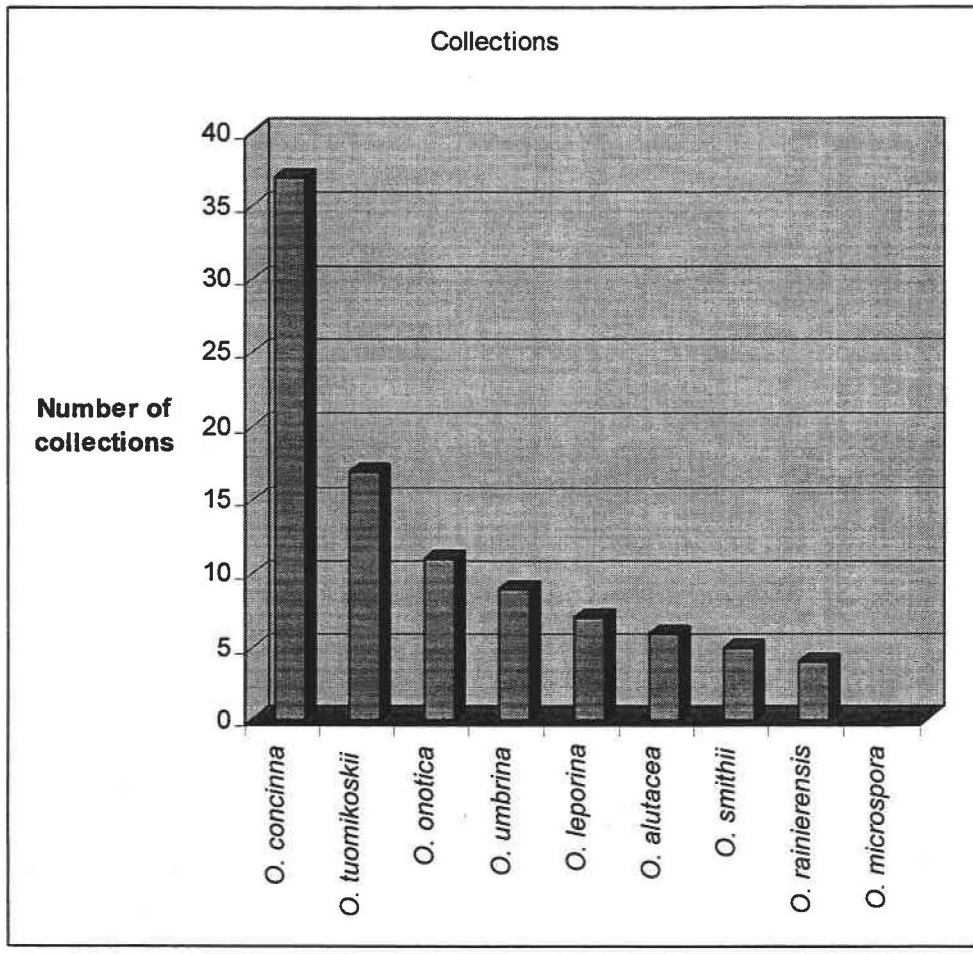

Figure 4.1: Number of collections by species made during the course of this study (1996 – 1998).

In addition to being infrequently collected, several of the aforementioned species are limited in their distribution, at least in this study. *O. leporina* is only known from two localities, *O. rainierensis* from three localities, and *O. alutacea* from four. Before this study was undertaken *O. smithii* was extremely poorly known, and still is excepting the relatively large number of collections made in 1997. *O. umbrina* is almost unknown except in and around Corvallis, Oregon, which must surely be an artifact of the

distribution of mycologists. *Otidea microspora* was not collected once in the course of this two-year study in the Pacific Northwest.

Of the species under discussion, while there is no quantifiable evidence to this point, it is clear to this author that old-growth habitat is necessary for these fungi. Few collections of any of the aforementioned species, with the exception of *O. umbrina,* were made in habitat that did not contain coniferous trees older than 150 years. A single collection of *O. smithii* was made near Corvallis, Oregon in a stand seemingly devoid of any trees older than 80 years but little is known to us about the history of this stand and it is adjacent to stands containing older trees. The remaining collections of *O. smithii* made in the course of this study were made near if not immediately underneath old-growth trees. The continued destruction of old-growth forests throughout the Pacific Northwest will surely lead to a reduction in populations of *Otidea* species.

As part of the effort to catalogue and conserve fungi known to be rare in the Pacific Northwest, under the definition of rarity defined by Castellano (1997) and the Federal Government (U.S.D.A. F.S., U.S.D.O.I. B.L.M., 1994), I recommend that the following species be added where appropriate to the FEMAT strategy one and three listings:

Table 4.1: FEMAT listing recommendations for rare *Otidea* species.

Species	Recommended FEMAT listing
O. microspora	Strategy One
O. rainierensis	Strategy One
O. smithii	Strategy One
O. leporina	Strategy One
O. alutacea	Strategy One
O. umbrina	Strategy Three

As additional information is collected regarding these species a better assessment of their rarity and the habitat relationship with the Northern Spotted Owl will be possible. Until that time I believe that it is better to be conservative and attempt to preserve the habitat of these fungi and associated organisms.

References

Castellano, M. A. 1997. *Towards a RED list for Oregon macrofungi.* In: Kaye, Liston, Love, Luoma, Meinke and M. V. Wilson (eds) *Conservation and Management of Native Flora and Fungi.* Native Plant Society of Oregon, Corvallis. 222-226.

Kanouse, B. B. 1949. Studies in the genus *Otidea. Mycologia* 41 : 660-677.

U.S.D.A. F.S., U.S.D.O.I. B.L.M. 1994. *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents within the Range of the Northern Spotted Owl,* U.S. Government Printing office. 74p. Plus Attachment A: Standards and Guidelines.

Zimmer, E. A., S. L. Martin, S. M. Beverley, Y. W. Kan and A. C. Wilson. 1980. Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. *Proceedings of the National Academy of Science* 77:2158-2162.

BIBLIOGRAPHY

Alexopolus, C. J., C. W. Mims, M. Blackwell. 1996. *Introductory Mycology*. John Wiley & Sons, Inc., New York.

Archie, J. W. 1985. Methods for coding variable morphological features for numerical taxonomic analysis. *Systematic Zoology* 34 (3): 326-345.

Arpin, N. 1968. *Les Carotenoides de Discomycetes: Essai Chimiotaxonomique*, pp. 170. University of Lyons, Villeurbanne, France.

Baral, H. O. 1992. Vital versus herbarium taxonomy: Morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* XLIV (2): 333-390.

Barrett, M., M. J. Donoghue, E. Sober. 1991. Against consensus. *Systematic Zoology* 40 (4): 486-493.

Berbee, M. L., J. W. Taylor. 1992. Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequences. *Molecular Biology and Evolution* 9 (2): 278-284.

Blackwell, M., J. W. Spatafora. 1994. *Molecular data sets and broad taxon sampling in detecting morphological convergence*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 243-248. Plenum Press, New York.

Bonorden, H. F. 1851. *Handbuch der allgemeinen Mykologie als Anleitung zum Studium derselben.* E. Schweizerbart, Stuttgart.

Boudier, E. 1907. *Histoire et Classification des Discomycetes d'Europe*. Paul Klincksieck, Paris.

Breitenbach, J., F. Kränzlin. 1984. *Fungi of Switzerland: Volume 1 Ascomycetes*, pp. 310. Verlag Mykologia, Luzern.

Bresadola, J. 1933. *Iconographia Mycologica*. Volume 25. E. J. Gilbert, Milano.

Brummelen, J. v. 1994. *Problems in the systematics of the Pezizales*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 303-314. Plenum Press, New York.

Bruns, T. D., R. Fogel, J. W. Taylor. 1990. Amplification and sequencing of DNA from fungal herbarium specimens. *Mycologia* 82 (2): 174-184.

Bruns, T. D., T. M. Szaro. 1992. Rate and mode differences between nuclear and mitochondrial small-subunit rRNA genes in mushrooms. *Molecular Biology and Evolution* 9 (5): 836-855.

Bruns, T. D., R. Vilgalys, S. M. Barns, D. Gonzales, D. S. Hibbett, D. J. Lane, L. Simon, S. Stickel, T. M. Szaro, W. G. Weisburg and M. L. Sogin. 1992. Evolutionary relationships within the fungi: Analysis of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution* 1 (3): 231-241.

Bruns, T. D., T. J. White, J. W. Taylor. 1991. Fungal Molecular Systematics. *Annual Review of Ecology and Systematics* 22 : 525-564.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42 (3): 384-397.

Bult, C., M. Kallersjo, Y. Suh. 1992. Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. *Plant Molecular Biology Reporter* 10 : 273-284.

Camp, W. H., H. W. Rickett, C. A. Weatherby. 1947. International rules of botanical nomenclature. *Brittonia* 6 (1): 1-120.

Cao, J.-Z., L. Fan, B. Liu. 1990. Some species of *Otidea* from China. *Mycologia* 86 (6): 734-741.

Castellano, M. A. 1997. *Towards a RED list for Oregon macrofungi.* In: Kaye, Liston, Love, Luoma, Meinke and M. V. Wilson (eds) *Conservation and Management of Native Flora and Fungi.* Native Plant Society of Oregon, Corvallis. 222-226.

Chippindale, P. T., J. J. Wiens. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. *Systematic Biology* 43 (2): 278-287.

Cranston, P. S., C. J. Humphries. 1988. Cladistics and computers: A chironomid conundrum? *Cladistics* 4 : 72-92.

Crouan, P. I., H. M. Crouan. 1857. Note sur queleues *Ascobolus* nouveaux et sur une espece nouvelle de *Vibrissea*. *Annales des Sciences Naturelles. A. Botanique* IV (7): 173-178.

Cunningham, C. W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14 (7): 733-740.

Cunningham, C. W. 1997. Is congruence between data partitions a reliable predictor of phylogentic accuracy? Empirically testing an iterative proceedure for choosing among phylogenetic methods. *Systematic Biology* 46 (3): 464-478.

Dahncke, R. M., S. M. Dahncke. 1979. *700 Pilze in Farbfotos*. Verlag, Aarau.

Dennis, R. W. G. 1981. *British Ascomycetes*. J. Cramer, Vaduz.

Dermek, A. 1977. *Atlas nasich hub*. unknown, Obzor.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 88 : 172-181.

Donoghue, M. J., M. J. Sanderson. 1992. *The suitability of molecular and morphological evidence in reconstructing plant phylogeny*. In: Soltis, Soltis, Doyle (eds) *Molecular Systematics of Plants*, pp. 340-368. Chapman and Hall, New York, New York.

Downie, S. R., S. Ramanath, D. S. Katz-Downie, E. Lanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *RPOC1* intron sequences. *American Journal of Botany* 85 (4): 563-591.

Doyle, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. *Systematic Botany* 17 (1): 144-163.

Eckblad, F.-E. 1968. The Genera of Operculate Discomycetes. *Nytt Magasin for Botanikk* 15 (1-2): 1-191.

Eernisse, D. J., A. G. Kluge. 1993. Taxonomic congruence versus total evidence, and Amniote phylogeny inferred from fossils, molecules, and morphology. *Molecular Biology and Evolution* 10 (6): 1170-1195.

Ellis, R. W., P. Ellis. 1998. *Microfungi on Miscellaneous Substrates*. The Richmond Publishing Co. Ltd., Slough.

Farris, J. S. 1969. A successive approximations approach to character weighting. *Systematic Zoology* 18 : 374-385.

Farris, J. S. 1970. Methods for computing Wagner trees. *Systematic Zoology* 19 : 83-92.

Farris, J. S., M. Kallersjo, A. G. Kluge, C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10 : 315-319.

Felsenstein, J. 1985. Confidence intervals on phylogenies: An approach using the bootstrap. *Evolution* 39 : 783-791.

Fries, E. M. 1822. *Systema Mycologicum*, Greifswald.

Fuckel, L. 1869-1870. *Symbolae Mycologicae. I. Fungi Perfecti.* Julius Niedner, Wiesbaden.

Le Gal, M. 1947. Recherches sur les ornamentations sporales des Discomycetes opercules. *Annales des Sciences Naturelles. A. Botanique* XI (8): 73-297.

Gargas, A., P. T. DePriest, M. Grube, A. Tehler. 1995. Multiple origins of lichen symbioses in fungi suggested from SSU rDNA phylogeny. *Science* 268 : 1492-1495.

Gargas, A., J. W. Taylor. 1995. Phylogeny of discomycetes and early radiations of the apothecial Ascomycotina inferred fron SSU rDNA sequence data. *Experimental Mycology* 9 : 7-15.

Goldman, N. 1988. Methods for discrete coding of morphological characters for numerical analysis. *Cladistics* 4 : 59-71.

Groves, J. W., S. C. Hoare. 1954. Notes on fungi from Northern Canada. I. Hypocreales and Discomycetes. *The Canadian Field Naturalist* 68 (1): 1-8.

Harmaja, H. 1972. Notes on the genus *Helvella*, including the merging of the genus *Wynnella*. *Karstenia* 14 : 102-104.

Harmaja, H. 1974. *Flavoscypha*, a new genus of the Pezizales for *Otidea cantharella* and *O. phlebophora*. *Karstenia* 14 : 105-108.

Harmaja, H. 1976. New species and combinations in the genera *Gyromitra*, *Helvella*, and *Otidea*. *Karstenia* 15 : 29-32.

Harrington, F. A. 1998. Relationships among *Sarcoscypha* species: evidence from molecular and morphological characters. *Mycologia* 90 (2): 235-243.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, P. N. Pegler. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*. CAB, London.

Hibbett, D. S. 1992. Ribosomal RNA and fungal systematics. *Transactions of the Mycological Society of Japan* 33 : 533-556.

Hibbett, D. S., M. J. Donoghue. 1998. Integrating phylogenetic analysis and classification in fungi. *Mycologia* 90 (3): 347-356.

Hibbett, D. S., Y. Fukumasa-Nakai, A. Tsuneda, M. J. Donoghue. 1995. Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences. *Mycologia* 87 (5): 618-638.

Hibbett, D. S., R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Systematic Botany* 18 (3): 409-433.

Higgins, D. G., A. J. Bleasby, R. Fuchs. 1991. CLUSTAL V: Improved software for multiple sequence alignment. *CABIOS* (cited as submitted) .

Hillis, D. M., J. P. Huelsenbeck, C. W. Cunningham. 1994. Application and accuracy of molecular phylogenies. *Science* 264 : 671-677.

Holmgren, P. K., N. H. Holmgren, L. C. Barnett. 1990. *Index Herbariorum.* New York Botanical Gardens, New York.

Kanouse, B. B. 1939. Notes on new or unusual discomycetes. *Papers of the Michigan Academy of Science, Arts & Letters* 24 : 28.

Kanouse, B. B. 1949. Studies in the genus *Otidea. Mycologia* 41 : 660-677.

Kerrigan, R. W., D. B. Carvalho, P. A. Horgen, J. B. Anderson. 1998. The indigenous coastal Californian population of the mushroom *Agaricus bisporus*, a cultivated species, may be at risk of extinction. *Molecular Ecology* 7 : 35-45.

Kimbrough, J. W. 1970. Current trends in the classification of discomycetes. *The Botanical Review* 36 (2): 92-161.

Kluge, A. G. 1989. A concern for evidence and phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Systematic Zoology* 38: 7-25.

Kohn, L. M. 1992. Developing new characters for fungal systematics: An experimental approach for determining the rank of resolution. *Mycologia* 84 (2): 139-153.

Korf, R. P. 1973. *Discomycetes and Tuberales.* In: Anisworth, Sparrow, Sussman (eds) *The Fungi IVA: An advanced treatise*, pp. 249-319. Academic Press, New York, New York.

Kornerup, A., J. H. Wanscher. 1967. *Methuen Handbook of Colour.* Methuen & Co. Ltd, London.

Kretzer, A., Y. Li, T. Szaro, T. D. Bruns. 1996. Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: Phylogenetic and taxonomic implications. *Mycologia* 88 (5): 776-785.

Landvik, S., K. N. Egger, T. Schumacher. 1997. *Towards a subordinal classification of the Pezizales (Ascomycota)* Department of Ecological Botany, pp. 1-29. Umea University, Umea, Sweden.

Lincoff, G. H. 1981. *The Audubon Society Field Guide to North American Mushrooms.* Alfred A. Knopf, New York.

Linneaus, C. 1753. *Species Plantarum.* II. Laurentii Salvii, Stockholm.

Liston, A., W. A. Robinson, J. M. Oliphant, E. R. Alvarez-Buylla. 1996. Length variation in the nuclear ribosomal DNA internal transcribed spacer region of non-flowering seed plants. *Systematic Botany* 21 (2): 109-120.

Lutzoni, F., R. Vilgalys. 1995. Integration of morphological and molecular data sets in estimating fungal phylogenies. *Canadian Journal of Botany* 73 (Supplement 1): S649-S659.

Lutzoni, F., R. Vilgalys. 1995. *Omphalina* (Basidiomycota, Agaricales) as a model system for the study of coevolution in lichens. *Cryptogamic Botany* 5 : 71-81.

Lutzoni, F. M. 1997. Phylogeny of lichen- and non-lichen-forming Omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Systematic Biology* 46 (3): 373-406.

Maddison, W. P. 1991. Squared-change parsimony reconstructions of ancestral states for continuous-valued characters on a phylogenetic tree. *Systematic Zoology* 40 (3): 304-314.

Maddison, W. P., D. R. Maddison. 1992. *MacClade. Analysis of phylogeny and character evolution.* Sinauer Associates, Inc., Sunderland, Massachussets.

Maublanc, A. 1971. *Champignons Comestibles et Veneneux.* Editions Lechevalier S.A.R.L., Paris.

Miller, O. K. 1977. *Mushrooms of North America.* E. P. Dutton, New York.

Montgomery, D. C. 1997. *Design and Analysis of Experiments.* John Wiley & Sons, New York.

Mullis, K. B. and F. A. Fallona. 1987. Specific synthesis of DNA in vitro via polymerase-catalyzed chain reaction. *Methods of Enzymology* 155: 335-350.

Nannfeldt, J. A. 1966. On *Otidea caligata, O. indivisa,* and *O. platyspora* (Discomycetes, Operculatae). *Annales Botanici Fennici* 3 : 309-318.

Nixon, K. C., J. M. Carpenter. 1996. On simultaneous analysis. *Cladistics* 12 : 221-241.

Nylander, W. 1869. Observationes circa Pezizas Fenniae. *Notiser ur Saellskapets Pro Fauna et Flora Fennica Foerhandlinger* 10 : 1-97.

O'Donnell, K., E. Cigelnik, H. I. Nirenberg. 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90 (3): 465-493.

O'Donnell, K., E. Cigelnik, N. S. Weber, J. M. Trappe. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. *Mycologia* 89 (1): 48-65.

Otani, Y. 1969. Some species of the genus Otidea collected in Japan. *Transactions of the Mycological Society of Japan* 9 (3): 101-108.

Persoon, C. H. 1796. *Observationes mycologicae*. Petrum Phillippum [sic] Wolf, Leizig.

Persoon, C. H. 1801. *Synopsis Methodica Fungorum*. Henricum Dieterich, Gottingen.

Persoon, C. H. 1822. *Mycologia Europaea*. Joanni Jacobi Palmii, Erlangae.

Peter, J. 1964. *Das Grosse Pilzbuch*. Safari-Verlag, Berlin.

Pimentel, R. A., R. Riggins. 1987. The nature of cladistic data. *Cladistics* 3 (3): 201-209.

de Queiroz, A. 1993. For consensus (sometimes). *Systematic Biology* 42 (3): 368-372.

Raitviir, A. 1972. Statistical methods and species delimitation in the genus *Otidea*. *Persoonia* 6 (4): 415-423.

Rifai, M. A. 1968. The Australasian Pezizales in the Herbarium of the Royal Botanic Garden Kew. *Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, afd. Natuurkunde* 57 (3): 1-295.

Rodrigo, A. G. 1996. On combining cladograms. *Taxon* 45 : 267-274.

Romagnesi, H. 1962. *Petit Atlas des Champignons*. Bordas, unknown.

Saccardo, P. A. 1882-1972. *Sylloge Fungorum*. Volumes VIII, X, XI, XIV, XVI, XVIII, XX, XXII. Many publishers, Many cities.

Saiki, R., D. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G. Horn, K. Mullis and H. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 : 487-494.

Samuelson, D. A. 1978. Asci of the Pezizales. II. The apical apparatus of representatives in the *Otidea-Aleuria* complex. *Canadian Journal of Botany* 56 : 1876-1904.

Schaffer, H. B., P. Meylan, M. L. McKnight. 1997. Tests of turtle phylogeny: Molecular, morpholgical, and paleontological approaches. *Systematic Biology* 46 (2): 235-268.

Schalkwijk-Barendsen, H. M. E. 1991. *Mushrooms of Western Canada*. Lone Pine Publishing, Edmonton.

Seaver, F. J. 1928. *The North American Cup-fungi (Operculates)*. Fred Jay Seaver, New York.

Smithe, F. B. 1975. *Naturalist's Color Guide*. The American Museum of Natural History, New York.

Spatafora, J. W. 1995. Ascomal evolution of filamentous ascomycetes: Evidence from molecular data. *Canadian Journal of Botany* 73 (Supplement 1): S811-S815.

Spatafora, J. W., M. Blackwell. 1994. *Cladistic analysis of partial ssrDNA sequences among unitunicate perithecial ascomycetes and its implacations on the evolution of centrum development*. In: Hawksworth (ed) *Ascomycete Systematics: Problems and Perspectives in the Nineties*, pp. 233-242. Plenum Press, New York.

Stevens, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: A review. *Systematic Botany* 16 (3): 553-583.

Swofford, D. 1997. *Paup**. Sinauer and Associated, Sunderland, Massachussets.

Swofford, D. L., G. J. Olsen, P. J. Waddell, D. M. Hillis. 1996. *Phylogenetic Inference*. Sinauer Associates, Inc, Sunderland, MA.

Tehler, A. 1994. Morphological data, molecular data, and total evidence in phylogenetic analysis. *Canadian Journal of Botany* 73 (Supplement 1): S667-S676.

Thiele, K., P. Y. Ladiges. 1988. A cladistic analysis of *Angophora* cav. (Myrtaceae). *Cladistics* 4 : 23-42.

Trappe, J. M. 1979. The orders, families, and genera of hypogeous ascomycotina (truffles and their relatives). *Mycotaxon* 9 (1): 297-340.

Tylutki, E. E. 1993. *Mushrooms of Idaho and the Pacific Northwest. Volume One Discomycetes*. University of Idaho Press, Moscow.

U.S.D.A. F.S., U.S.D.O.I. B.L.M. 1994. *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents within the Range of the Northern Spotted Owl*, U.S. Government Printing office. 74p. Plus Attachment A: Standards and Guidelines.

Vilgalys, R., M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 : 4238-4246.

Vilgalys, R., B. L. Sun. 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. *Proceedings of the National Academy of Science* 91 : 4599-4603.

Weber, N. S., J. M. Trappe, W. C. Denison. 1997. Studies on Western American Pezizales. Collecting and describing ascomata -- Macroscopic features. *Mycotaxon* 61 : 153-176.

Wheeler, W. C., J. Gatesy, R. DeSalle. 1995. Elision: A method for accomodating multiple molecular sequence alignments with alignment-ambiguous sites. *Molecular Phylogenetics and Evolution* 4 (1): 1-9.

White, T. J., T. D. Bruns, S. B. Lee, J. W. Taylor. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis, Gelfand, Sninsky, White (eds) *PCR Protocols: A Guide to Methods and Applications*, pp. 315-322. Academic Press, New York, New York.

Zimmer, E. A., S. L. Martin, S. M. Beverley, Y. W. Kan and A. C. Wilson. 1980. Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. *Proceedings of the National Academy of Science* 77:2158-2162.

APPENDIX

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149  GAGAATCCCG TGCCTGGATA GCTGTCCTGC TCCATGTGAA GTGTCTCCGA   198 OSC 56745
150  GAGAATCCCG TGCCTGGATG GCTGTCCATC TCCATGTGAA GTGTCTCCGA   199 OSC 56831
149  GAGAATCCCG TGCCTGGATG GCTGTCCATC TCCATGTGAA NTGTCTCCGA   198 Scutellinia
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 AHS 8843
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 OSC 56830
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 EGS 2179
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 AHS 30502
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 AHS 21147
151  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   200 ML 941947
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     201         211         221         231         241       250
     |           |           |           |           |         |
199  CGaGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56811
197  CGAGTCGAGT T-TTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   245 OSC 56823
197  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   246 OSC 56753
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56799
198  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 NSW 7536
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56734
199  CGANTCGANT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56801
198  CgAgTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 OSC 56759
200  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56754
198  CgAgTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 OSC 56747
198  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 OSC 56758
200  CNAGTCNAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56777
200  CNAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56798
199  CNAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56813
200  CAANTCAAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56770
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56782
199  CGAGTCGA-T TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 OSC 56784
198  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   247 OSC 56825
197  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   246 OSC 56824
197  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   246 OSC 56809
194  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   243 OSC 56760
195  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   244 OSC 56749
197  CgAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   246 NSW 7574
199  CgAGTCGAGT TGTTTGGGAA TGCAcCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56756
200  CgAGTCGAGT TGTTTGGGAA TGCAgCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56761
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56826
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56829
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 NSW 6354
199  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   248 OSC 56745
200  CGAGTCGAGT TGTTTGGGAA TGCAGCTCAA AATGGGTGGT AAATTTCATC   249 OSC 56831
199  CNANTCNAGT TGTTTGGGAA TGCANCTCAA AATGGGTGGT AAATTTCATC   248 Scutellinia
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   250 AHS 8843
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   250 OSC 56830
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   25  EGS 2179
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   250 AHS 30502
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   250 AHS 21147
201  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   250 ML 941947
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     251         261         271         281         291       300
     |           |           |           |           |         |
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56811
246  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   295 OSC 56823
247  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   296 OSC 56753
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56799
248  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA NAGTGATCGA   297 NSW 7536
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56734
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56801
248  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   297 OSC 56759
250  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56754
248  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   297 OSC 56747
248  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   297 OSC 56758
250  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56777
250  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56798
249  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56813
250  TAAAGCTAAA TACTGGCGAN AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56770
249  TAAAGCTAAA TACTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56782
248  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   297 OSC 56784
248  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAANTA GAGTGATCGA   297 OSC 56825
247  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   296 OSC 56824
247  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   296 OSC 56809
244  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   293 OSC 56760
245  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   294 OSC 56749
247  TAAAGCTAAA TATTGGCGAG AGACCGAT-G CGCACAAGTA GAGTGATCGA   295 NSW 7574
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56756
250  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56761
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAgTGATCGA   298 OSC 56826
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56829
249  TAAAGCTAAA TATTGGCGAG AGACCGAT-G CGCACAAGTA GAGTGATCGA   297 NSW 6354
249  TAAAGCTAAA TATTGGCGAG AGACCGATAG CGCACAAGTA GAGTGATCGA   298 OSC 56745
250  TAAAGCTAAA TATTGGCGAA AGACCGATAG CGCACAAGTA GAGTGATCGA   299 OSC 56831
249  TAAAGCTAAA TATTGGCGAN AGACCGATAG CGCACAATTA GAGTGATCGA   298 Scutellinia
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 AHS 8843
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 OSC 56830
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 EGS 2179
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 AHS 30502
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 AHS 21147
251  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   300 ML 941947
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     301         311         321         331         341       350
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299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AACAGTACGT GAAATTGTTG   348 OSC 56811
296  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATANTACGT GAAATTGTTG   345 OSC 56823
297  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AACAGTACGT GAAATT-TTG   345 OSC 56753
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AACAGTACGT GAAATTGTTG   348 OSC 56799
298  AAGATGAAAA GCACTTTGAN AAGAAAGTTA AATNTTACNT GAAATTGTTG   347 NSW 7536
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AACAGTACGT GAAATTGTTG   348 OSC 56734
299  AAGATGAAAA GCACTTTGAA AAGANAGTTA AACAGTACGT GAAATTGTTG   348 OSC 56801
298  AAGATGAAAA GCACTTTGAA AAGAGAgTTA AACAGTACGT GAAATTGTTG   347 OSC 56759
300  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   349 OSC 56754
298  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   347 OSC 56747
298  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   347 OSC 56758
300  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   349 OSC 56777
300  AAGATGAAAA GCACTTTGAA AAGANAGTTA AAAAGTACGT GAAATTGTTG   349 OSC 56798
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   348 OSC 56813
300  AAGATGAAAA GCACTTTGAA AAGANAGTTA AAAAGTACGT GAAATTGTTG   349 OSC 56770
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   348 OSC 56782
298  AAGATGAAAA GCACTTTGAA AAGAGAGTTA A-CAGTACGT GAAATTGTTG   346 OSC 56784
298  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   347 OSC 56825
297  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   346 OSC 56824
297  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   346 OSC 56809
294  AAGATGAAAA GCACTTTGAA AAGAgAgTTA AATAGTACGT GAAATTGTTG   343 OSC 56760
295  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   344 OSC 56749
296  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   345 NSW 7574
299  AAGATGAaAA GCaCTTTGAA AAgAGAGTTA AATAgTACGT GAAATTGTTG   348 OSC 56756
300  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   349 OSC 56761
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   348 OSC 56826
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   348 OSC 56829
298  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   347 NSW 6354
299  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AATAGTACGT GAAATTGTTG   348 OSC 56745
300  AAGATGAAAA GCACTTTGAA AAGAGAGTTA AAAAGTACGT GAAATTGTTG   349 OSC 56831
299  AANATGAAAA NCACTTTGAA AAGAAAGTTA AAAANTACNT GAAATTGTTG   348 Scutellinia
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 AHS 8843
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 OSC 56830
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 EGS 2179
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 AHS 30502
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 AHS 21147
301  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   350 ML 941947
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     351         361         371         381         391       400
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349  AAAGGGAAGC GCTTGAGACT AGATTCGACC GGCGGTCATC AGCTGCGTTT   398 OSC 56811
346  AAAGGGAAGC GCTTGAGACT ACATTCAGCC GGCGGTGATC AGCTATGCTT   395 OSC 56823
346  AAAGGGAAGC GCTTGAGACT AGATTCGACC GGCGGTCATC AGCTGCGTTT   395 OSC 56753
349  AAAGGGAAGC GCTTGAGACT AGATNCGACC GGCGGTCATC AGCTGCGTTT   398 OSC 56799
348  AAATGGAACG C-NNGACACT AANATCGGAC CGTGGTGANC GNCCCAGCTC   396 NSW 7536
349  AAAGGGAAGC GCTTGAGACC AGATTCAGCC GGCGGTCATC AGCTACATTT   398 OSC 56734
349  AAAGGGAAGC GCTTGAGACC AGATTCAGCC GGCGGTCATC AGCTACATTT   398 OSC 56801
348  AAAGGGAAGC GCTTGAGACC AGATTCAGCC GGCGGTCATC AGCTACATTT   397 OSC 56759
350  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AgCCGTGCTT   399 OSC 56754
348  AAAGGGAAGC GCTTGAgACC AGACTCAACC TTCGGTGATC AGCCGTGCTT   397 OSC 56747
348  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AGCTGCGCTT   397 OSC 56758
350  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AGCCGTGCTT   399 OSC 56777
350  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AGCCGTGCTT   399 OSC 56798
349  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AGCTGCGCTT   398 OSC 56813
350  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC ACCG-TGCTT   398 OSC 56770
349  AAAGGGAAGC GCTTGAGACC AGACTCAACC TTCGGTGATC AGCTGCGCTT   398 OSC 56782
347  AAAGGGAAGC GCTTGAGACT AGATTCGACC GGCGGTCATC AGCTGCGTTT   396 OSC 56784
348  AAAGGGAAGC GCTTGAGACT AGAT-CGGCC GGTGGTGATC AGCCATGCTT   396 OSC 56825
347  AAAGGGAAGC GCTTGAGACT AGATTCAGCC GGCGGTGATC AGCTATGCTT   396 OSC 56824
347  AAAGGGAAGC GCTTGAGACT AGATTCAGTC GGCGGTGATC AGCTATGCTT   396 OSC 56809
344  AAAGGGAAGC GCTTGAGACT AGATTCAGTC GGCGGTGATC AGCTATGCTT   393 OSC 56760
345  AAAGGGAAGC GCTTGAGACT AGATTCAGTC GGCGGTGATC AGCTATGCTT   394 OSC 56749
346  AAAGGGAAGC GCTTGAGACT AGATTCAGTC GGCGGTGATC AGCTATGCTT   395 NSW 7574
349  AAAGGGAAtC GCTTGATACT AgATTCGGCC GGTGGTGATC AGCCATGCTT   398 OSC 56756
350  AAAGGGAAGC GCTTGAGACT AGATTCGGCC GGTGGTGATC AGCCATGCTT   399 OSC 56761
349  AAAGGGAAGC GCTTGAGACT AGATTCGGCC GGTGGTGATC AGCCATGCTT   398 OSC 56826
349  AAAGGGAAGC GCTTGGGACT ATATACAGCC GGCTGTGATC AGCCACGTTT   398 OSC 56829
348  AAAGGGAAGC GCTTGGGACT ATATACAGCC GGCTGTGATC AGCCACGTTT   397 NSW 6354
349  AAAGGGAAGC GCTTGGGACT ATATACAGCC GGCTGTGATC AGCCACGTTT   398 OSC 56745
350  AAAGGGAAGC GCTTGAGACC AGACTCAATC TTTGGTGATC AGCTGTGCTT   399 OSC 56831
349  AAAGGGAAGC GCTTGCNACC AGACTCGATC TTTGGTGATC AGCCGGGCTT   398 Scutellinia
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 AHS 8843
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 OSC 56830
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 EGS 2179
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 AHS 30502
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 AHS 21147
351  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   400 ML 941947
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     401         411         421         431         441       450
     |           |           |           |           |         |
399  TTACGTGGTG CACTTGCCGT CGGTTGAACC AGCATCGGTT TCGATGGCAG   448 OSC 56811
396  CTGCGTGGTG CACTTGCCGT CGGTTGAACC ANCATCGGTT TTGATGGCGG   445 OSC 56823
396  TTACGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT TCGATGGCAG   445 OSC 56753
399  TTACGTGGTG CACTTGCCGT CGGTTGAACC ANCATCCGTT TCTATGGCAG   448 OSC 56799
397  CGTCTTGNNG TNCTTGCCTT CCGNTCAGNC -G-ACCCCNN TTCTGATTCT   444 NSW 7536
399  CTATGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT TTGATGGTGG   448 OSC 56734
399  CTATGTGGTG CACTTGCCGT CGGTTGANCC AGCATCGGTT TTGATGGTGG   448 OSC 56801
398  CTATGTGGTG CACTTGCCGT CGGTTGAgCC AGCATCGGTT TTGATGGTGG   447 OSC 56759
400  TTGTGCGGTG CACTTGCCGG GGGTTGGGCC AACATCgGTT TTGACGGTGG   449 OSC 56754
398  TTGTGCGGTG CACTTGCCGG GGGTTGGGCC AGCATCgGTT TTGACGGTGG   447 OSC 56747
398  CTGTGCGGTG CACTTGCCGT GGGTTGGGCC AGCATCGGTT TTGACGGTGG   447 OSC 56758
400  TTGTGCGGTG CACTTGCCGG GGGTTGGGCC AGCATCGGTT TTGACGGTGG   449 OSC 56777
400  TTGTGCGGTG CACTTGCCGG GGGTTGGGCC AGCATCCGTT TTGACGGTGG   449 OSC 56798
399  CTGTGCGGTG CACTTGCCGT GGGTTGGGCC ANCATCGGTT TTGACGGTGG   448 OSC 56813
399  TTGTGCGGTG CACTTGCCGG GGGTTGGGCC ANCATCNGTT TTGACGGTGG   448 OSC 56770
399  CTGTGCGGTG CACTTGCCGT GGGTTGGGCC AGCATCGGTT TTGACGGTGG   448 OSC 56782
397  TTACGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT TCGATGGCAG   446 OSC 56784
397  TTGCGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT CTGATGGTGG   446 OSC 56825
397  CTGCGTGGTG CACTTGCCGT CGGTTGANCC AGCATCNGTT TTGATGGCGG   446 OSC 56824
397  CTGCGTGGTG CACTTGCCGT CGATTGAGCC AGCATCGGTT TTGATGGCGG   446 OSC 56809
394  CTGCGTGGTG CACTTGCCGT CGATTGAGCC AGCATCGGTT TTGAtGGCGG   443 OSC 56760
395  CTGCGTGGTG CACTTGCCGT CGATTGAGCC AGCATCGGTT TTGATGGCGG   444 OSC 56749
396  CTGCGTGGTG CACTTGCCGT CGATTGAGCC AGCATCGGTT TTGATGGCGG   445 NSW 7574
399  TTGCGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT CTGATGGTGG   448 OSC 56756
400  TTGCGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT CTGATGGTGG   449 OSC 56761
399  TTGCGTGGTG CACTTGCCGT CGGTTGAGCC AGCATCGGTT CTGATGGTGG   448 OSC 56826
399  TTACGTGGTG CACTACAAGT CGGTTGAGCC AGCATCGGTT CTGACGGCGG   448 OSC 56829
398  TTACGTGGTG CACTACAAGT CGGTTGAgCC AGCATCGGTT CTGACGGCGG   447 NSW 6354
399  TTACGTGGTG CACTACAAGT CGGTTGAGCC AGCATCGGTT CTGACGGCGG   448 OSC 56745
400  CTGTACAGTG CACTTGCCAT TGATTGGGCC AGCATCGGTT CTGACGGTGG   449 OSC 56831
399  CTGTCCGGTG CACTTGCCAC TGATCGGGCC AGCATCGGTT CTGANGGCGG   448 Scutellinia
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 AHS 8843
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 OSC 56830
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 EGS 2179
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 AHS 30502
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 AHS 21147
401  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   450 ML 941947
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     451         461         471         481         491       500
     |           |           |           |           |         |
449  GATAAAGGTT TCAGGAATGT GGCNCCTCAA TTT-CGAGGA GTGTTATAGC   497 OSC 56811
446  GATAAAGGTT TGAGGAATGT GGCTCCCCG- TTT-CGGGGA ATGTTATANC   493 OSC 56823
446  GATAAAGGTT TCAGGAATGT GGCTCCTCAA TTT-CGAAGA gTGTTATAgC   494 OSC 56753
449  GATAAAAGTT -CAGGAATGT GGCTCCTCCN NT--CGANGA GTNTTATA-C   494 OSC 56799
445  NNGNNANGAG TNCGGTGACN GNAGTGTCCA TT-GCGGGGA GTGTTATANC   493 NSW 7536
449  GATAAAGGTT TCAGGAATGT GGgTCTTCGA TTT-CGAGGA GTGTTATAGC   497 OSC 56734
449  GATAAANGTT C-AGGAATGT GGCTCCTCCA TTT-CGAAGA ATGTTATT-C   495 OSC 56801
448  GATAAAGGTT TCAGGAATGT GGCTCCTCgA TTT-CGAAGA GTGTTATAGC   496 OSC 56759
450  GATAAAGGTT CGAAGAATGT GGCTCCCCGA TTT-CGGGGA GTGTTATAGC   498 OSC 56754
448  GATAAAGGTT CGAAGAATGT GGCTCCCCGA TTT-CGGGGA gTGTTATAGC   496 OSC 56747
448  GATAAAGGTT CGAGGAATGT GGCTCCCCGA TT-CCGGGGA gTGTTATAgC   496 OSC 56758
450  GATAAAG-TT CGAGGAATGT GGCTCCCCGA TTT-CGGGGA GTGTTATAGC   497 OSC 56777
450  GATAAANGTT CNAAGAATGT GGCTCCCCNA TTT-CGGGGA ATGTTATANC   498 OSC 56798
449  GATAAANGTT CGAAGAATGT GGCTCCCCGA TT-CCGGGGA NTGTTATANC   497 OSC 56813
449  GATAAANGTT CGAAGAATGT GGCTCCCN-A TTTCCGGG-A ATGTTATA-C   495 OSC 56770
449  GATAAAGGTT CGAAGAATGT GGCTCCCCGA T--CCGGGGA GTGT-ATA-C   494 OSC 56782
447  GATAAAGGTT TCAGGAATGT GGCTCCTCAT TTT-CGAGGA GTGTTATAAC   495 OSC 56784
447  GATAAAGGTT CNANGAATGT GGCTCCCCAA TT-GCGGGGA GTGTTATA-C   494 OSC 56825
447  GATAAANGTT TGAAGAATGT GGCTCCCCG- TTT-CGGGGA ATGTTATA-C   493 OSC 56824
447  GATAAAGGTT TGAGGAATGT GGCTCCCCGA TT-CTGGGGA GTGTTATAGC   495 OSC 56809
444  GATAAAGGTT TGAGGAATGT GGCTCCCCGA TT-CTGGGGA GTGTTATAGC   492 OSC 56760
445  GATAAAGGTT TGAGGAATGT GGCTCCCCGA TT-CTGGGGA GTGTTATAGC   493 OSC 56749
446  GATAAAGGTT TGAAGAATGT TGCTCCCCGA TT-CTGGGGA GTGTTATAGC   494 NSW 7574
449  GATAAAGGTT CGAGGAATGT GGCTCCCCAA TT-GCGGGGA GTGTTATAGC   497 OSC 56756
450  GATAAAGGTT CGAGGAATGT GGCTCCCCAA TT-GCGGGGA GTGTTATAGC   498 OSC 56761
449  GATAAAGGTT CGAGGAATGT GGCCCCCCAA TT-GCGGGGA GTGTTATAGC   497 OSC 56826
449  GATAAAGGTT TCAGGAATGT GGCTCCCCAA TTATC-GGGA GTGTTAAAGC   497 OSC 56829
448  GATAAAGGTT TCAGGAATGT GGCTCCCCAA TTATC-GGGA GTGTTAAAGC   496 NSW 6354
449  GATAAAGGTT TCAGGAATGT GGCTCCCCAA TTATC-GGGA GTGTTAAAGC   497 OSC 56745
450  GATAAAATTT -GAGGAATGT GGCTCTC--- TT--CGGGGA GTGTTATANC   493 OSC 56831
449  GATAAAAGGT TGGGGAATGT ACCTTCTCT- ----CGGGGA ATGTTATACC   493 Scutellinia
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 AHS 8843
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 OSC 56830
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 EGS 2179
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 AHS 30502
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 AHS 21147
451  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn   500 ML 941947
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     501         511         521         531         541       550
     |           |           |           |           |         |
498  CTGGAGCGCA ATGCTGCCTG TTGGGACCCG AGGACCGCGC ---TTNTGCT    544 OSC 56811
494  CTCNAACGTA ATGCCGCCTG TTGGGACC-G AAGAACGCGC ---TTCNGCT    539 OSC 56823
495  CTGGAgCGCA ATGCTGCCTG TTGGGACC-G AAGACCGCGC ---TTTTGCT    540 OSC 56753
495  CTNGAACGCA ATGCTGCCTG TTGGGAAC-G AGGAACGCGC ---TTTTGCT    540 OSC 56799
494  CTGGAACGCA ATNCCACCTG TTGGGAAC-G NGGACCGCGA C--NTCCGCT    540 NSW 7536
498  CTGGAGCGCA ATGCCGCCTG TCGGGACC-G AGGACCGCGC ---TTTTGCT    543 OSC 56734
496  CTGGANCGCA ATGCCGCCTG TCCGGAACCA AGGACCCCCC ----TTTGCT    541 OSC 56801
497  CTGGAgCGCA ATGCCGCCTG TCGGGAAC-G AAGACCGCGC ---TTTTGCT    542 OSC 56759
499  CCTGAGCGCA ATGCCACCTG TTGGGACC-G AGGACCGCGC ---TTCCGCT    544 OSC 56754
497  CCTGAgCGCA ATGCCACCTG TTGGGACC-G AgGACCGCGC ---TTCCGCT    542 OSC 56747
497  CCTGAgCGCA ATGCCACCTG TTGGGACC-G AGGACCGCGC ---TTCCGCT    542 OSC 56758
498  CCTGAGCGCA ATGCCACCTG TTGGGACC-G AAGGACCGCG ---CTCCGCT    543 OSC 56777
499  C-TGANCCCA ATGCC-CCTG TTNGGACC-A AGGACCGCCC ---TCCCCCT    542 OSC 56798
498  C-TGANCGCA ATGCC-CCTG TTGGGACC-A AGGACCGCCC ----TCCCCT    540 OSC 56813
496  CCTGAACGCA ATGCC-NCTG TTNGGACC-N AGGACCCNC- ----TCCCCT    538 OSC 56770
495  CCTGANCGCA ATGCCCCCTG TTGGGAAC-G AAGAACGCCC C--TCCCCCT    541 OSC 56782
496  CTGGAGCGCA ATGCT-CCTG TTGGGACC-N AGGACCNCGC ---TTTTGCT    540 OSC 56784
495  CTGGAACGCA ATGCC-CCTG TTGGGACC-N AGGACCGCCC ----TCCGCT    538 OSC 56825
494  CTCCAACGTT ATGCCGCCTG TTNGGAAC-G AAGACCCCCC ----TCCGCT    538 OSC 56824
496  CTTGAGCGTA ATGCCGCCTG TCGGGACCCG AGGACCGCGC ---TTCGGCT    542 OSC 56809
493  CTTGAGCGTA ATGCCgCCTG TCGGGACC-G AGGACCGCGC ---TTCGGCT    538 OSC 56760
494  CTTGAgCGTA ATGCCGCCTG TCGGGACC-G AgGACCGCGC ---TTCGGCT    539 OSC 56749
495  CTTGAGCGTA ATGCCGCCTG TCGGGACC-G AGGACCGCGC ---TTCGGCT    540 NSW 7574
498  CTGGAGCGCA ATGCCACCTG TTGGGACC-G AGGACCGCGC ---TTCGGCT    543 OSC 56756
499  CTGGAGCGCA ATGCCACCTG TTGGGACC-G AGGACCGCGC ---TTCGGCT    544 OSC 56761
498  CTGGAGCGCA ATGCCNCCTG TTGGGACC-G AGGACCGCGC --TTTCGGCT    544 OSC 56826
498  CTGGAGCGCA ATGCCGCCAG TTGGGACC-G AGGCTAGCGC GGATTCGCAT    546 OSC 56829
497  CTGGAGCGCA ATGCCGCCAG TTGGGACC-G AGGCTAGCGC GGATTCGCAT    545 NSW 6354
498  CTGGAGCGCA ATGCCGCCAG TTGGGACC-G AGGCTAGCGC GGATTCGCAT    546 OSC 56745
494  CTCGATCATA ATGCTGCCAG TTGGGACC-G AAGACCGCGC ---TTCGGCT    539 OSC 56831
494  C-CAGTCATA ATGCC-CCA- TCGGGACC-N AGGACCGCCC ---TNCGC-T    535 Scutellinia
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 AHS 8843
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 OSC 56830
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 EGS 2179
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 AHS 30502
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 AHS 21147
501  nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn    550 ML 941947
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     551         561         571         581         591       600
     |           |           |           |           |         |
545  AGGATGCTGG CTAAAA-GTC TCAATTCTAA CACACTCTGC GTACCTTTCC   593 OSC 56811
540  AAGATGCTGG CNAAAANGTC TCAATTcTAA CACAcTcTGC GTACCTTTCC   589 OSC 56823
541  AGGATGCTGG CTAAAAGGTC TCAATTCTAA CACACTCTGC GTACCTTTCC   590 OSC 56753
541  AGGAT-CTGG CTAAAAAGTC TCAATTcTAA CACAcTcTGC GTACCTTTCC   589 OSC 56799
541  ANGAT-CTGG CCAAGAGGTC TCAATTCTAA CACACTCTGC GTACCTTTCC   589 NSW 7536
544  AGGATGCTGG CTAAAAGGTC TCAATTCCAA CCCATTCAGC GAATCATTCC   593 OSC 56734
542  ANGATGCTGG CTAAAAAGTC TCAATTCCAA CCCATTCTGC GTATCATTCC   591 OSC 56801
543  AGGATGCTGG CTAAAAGGTC TCAATTCCAA CCCATTCTGC GTATCATTCC   592 OSC 56759
545  AGGATGCTGG CGCAATGGTC TCAATTCAAA CACACTCTGC GTACCTTTCC   594 OSC 56754
543  AGGATGCTGG CGCAATGGTC TCAATTCAAA CACACTCTGC GTACCTTTCC   592 OSC 56747
543  AGGATGCTGG CGCAATGGTC TCAATTCAAA C-CACTCTGC GTACCTTTCC   591 OSC 56758
544  AGGATGCTGG CGCAATGGTC TCAATTCAAA CACACTCTGC GTACCTTTCC   593 OSC 56777
543  ANGATGCTNG CGCCATNGTC TCAATTCAAA CACACTCTGC GTACCTTTCC   592 OSC 56798
541  AGGAT-CTGG CGCCATNGTC TCAATTCAAA CCCACTCTGC GTACCTTTCC   589 OSC 56813
539  AGGATGCTGG CC-CATGGTC TNNNNNNNNN NNNNNTCTGN GTGCCTTACC   587 OSC 56770
542  AGGATGCTGG CGCCATGGTC TCAATTCAAA CCCACTCTGC GTACCTTTCC   591 OSC 56782
541  AGGATGCTGG CTTNAAGGTC TCAATTCTAA CACACACTGT GTACCATTCC   590 OSC 56784
539  AGGAT-CTGG CGAAAANGTC TCAaTTcTAA cAcACAcTGt GTACCATTCC   587 OSC 56825
539  ANGANGCTNG CCAAAAAGTC TCAATTCTAA CACACACTGT GTACCATTCC   588 OSC 56824
543  AGGATGCTGG CGAAAA-GTC TCAATTCTAA CACACACTGC GTACCATTCC   591 OSC 56809
539  AGGATGCTGG CGAAAAGGTC TCAATTCTAA CACACACTGC GTACCATTCC   588 OSC 56760
540  AGGATGCTGG CGAAAAGGTC TCAATTCTAA CACACACTGC GTACCATTCC   589 OSC 56749
541  AGGATGCTGG CGAAAAGGTC TCAATTCTAA CACACACTGC GTACCATTCC   590 NSW 7574
544  AGGATGCTGG CGAAAAGGTC TCAATTCTAA CACATTCTGC GTACCATTCC   593 OSC 56756
545  AGGATGCTGG CGAAAAGGTC TCAATTCTAA CACATTCTGC GTACCATTCC   594 OSC 56761
545  AGGATGCTGG CGAAAA-GTC TCAATTcTAA CACATTcTGC GTACCATTCC   593 OSC 56826
547  -GGATGCTGG CTAAT--GTA TCAGTTCAAA CACACCcTGC GTACCCTTCC   593 OSC 56829
546  -GGATGCTGG CTAAATGGTC TCAGTTCAAA CACACCCTGC GTACCCTTCC   594 NSW 6354
547  -GGATGCTGG CTAAATGGTC TCAGTTCAAA CACACCCTGC GAACCCTTCC   595 OSC 56745
540  ANGATGCTGG CGTAATGGTC TACATTAAAA GATAT---GC ATACTCT-CC   585 OSC 56831
536  ANGAT-CTGG CGTA-TGGTC TACATTATAA ACCCATCTGT GTATCTTACC   583 Scutellinia
551  nnnnnnnnnn nnnnnnnnnn nCAATTCTAA CACACTCTGC GTACCTTTCC   600 AHS 8843
551  nnnnnnnnnn nnnnnnnnnn nCAATTCTAA CACACTCTGC GNACCTTTCC   600 OSC 56830
551  nnnnnnnnnn nnnnnnnnnn nCAGTTCAAA CACACCCTGC GTACCCTTCC   600 EGS 2179
551  nnnnnnnnnn nnnnnnnnnn nCAGTTCAAA CACACCCTGT GTACCCTTCC   600 AHS 30502
551  nnnnnnnnnn nnnnnnnnnn nCAGTTCAAA CACACCCTGT GtACCCTTCC   600 AHS 21147
551  nnnnnnnnnn nnnnnnnnnn nCAATTCTAA CACACTCTGC GTATCATTCC   600 ML 941947
```

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     601        611        621        631        641       650
     |          |          |          |          |         |
594  TGTTGCTTCC ---------- ---------- ---------- ----------   603 OSC 56811
590  TGTTGCTTCC ---------- ---------- ---------- ----------   599 OSC 56823
591  TGTTGCTTCC ---------- ---------- ---------- ----------   600 OSC 56753
590  TGTTGCTTCc ---------- ---------- ---------- ----------   599 OSC 56799
590  TGTTGCTTCC ---------- ---------- ---------- ----------   599 NSW 7536
594  TGATGCTTCC ---------- ---------- ---------- ----------   603 OSC 56734
592  TGTTGCTTCC ---------- ---------- ---------- ----------   601 OSC 56801
593  TGTTGCTTCC ---------- ---------- ---------- ----------   602 OSC 56759
595  TCTTGCTTCC ---------- ---------- ---------- ----------   604 OSC 56754
593  TGTTGCTTCC ---------- ---------- ---------- ----------   602 OSC 56747
592  CGTTGCTTCC ---------- ---------- ---------- ----------   601 OSC 56758
594  TGTTGCTTCC ---------- ---------- ---------- ----------   603 OSC 56777
593  TGTTGCTTCC ---------- ---------- ---------- ----------   602 OSC 56798
590  CGTTGCTTCC ---------- ---------- ---------- ----------   599 OSC 56813
588  TGTTGCTNCC ---------- ---------- ---------- ----------   597 OSC 56770
592  CGTTGCTTCC ---------- ---------- ---------- ----------   601 OSC 56782
591  TGTCGCTTCC GTGGGGTGGA GCTGCTGTCT TGGGAAGCTT CAACCGTAGC   640 OSC 56784
588  TGTtGcTTCC GTGGgGtGGA GCTGCTgTCT TGGGAaGCTT CAaCCGTAGc   637 OSC 56825
589  TGTTGCTTCC GTGGGGTGGA GCTGCTGTCT TGGGAAGCTT CAACCGTAGC   638 OSC 56824
592  TGTTGCTTCC GTGGGGTGGG GCTGCTGTCT TGGGAATCTT CAACCGTAGC   641 OSC 56809
589  TGTTGCTTCC GTGGGGTGGG GCTGCTGTCT TGGGAATCTT CAACCGTAGC   638 OSC 56760
590  TGTTGCTTCC GTGGGGTGGG GCTGCTGTCT TGGGAATCTT CAACCGTAGC   639 OSC 56749
591  TGTTGCTTCC GTGGGGCGGG GCTGCTGTCT TGGGAATCTT CAACCGTAGC   640 NSW 7574
594  TGTTGCTTCC ---------- ---------- ---------- ----------   603 OSC 56756
595  TGTTGCTTCC ---------- ---------- ---------- ----------   604 OSC 56761
594  TGTTGCTTCC ---------- ---------- ---------- ----------   603 OSC 56826
594  TGTTGCTTCC ---------- ---------- ---------- ----------   603 OSC 56829
595  TGTTGCTTCC ---------- ---------- ---------- ----------   604 NSW 6354
596  TGTTGCTTCC ---------- ---------- ---------- ----------   605 OSC 56745
586  GGA-GC---- ---------- ---------- ---------- ----------   590 OSC 56831
584  CGTTGCTTCC ---------- ---------- ---------- ----------   593 Scutellinia
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 AHS 8843
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 OSC 56830
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 EGS 2179
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 AHS 30502
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 AHS 21147
601  TGTTGCTTCC ---------- ---------- ---------- ----------   610 ML 941947
```

```
     651         661         671         681         691       700
     |           |           |           |           |         |
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56811
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56823
601  ---------- ---------- ---------- ---------- ----------   600 OSC 56753
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56799
600  ---------- ---------- ---------- ---------- ----------   599 NSW 7536
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56734
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56801
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56759
605  ---------- ---------- ---------- ---------- ----------   604 OSC 56754
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56747
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56758
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56777
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56798
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56813
598  ---------- ---------- ---------- ---------- ----------   597 OSC 56770
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56782
641  TGGCTGCCTT CTTTTTCGTT GGCGGCTGCC TTCCTTTTGT TGGTGGCTAC   690 OSC 56784
638  TGGcTGCCTT CcTTTTcGtT GGCGGctGCC TTCcTTTTGT TGGTGGcTAC   687 OSC 56825
639  TGGCTGCCTT CCTTTTCGTT GGCGGCTGCC TTCCTTTTGT TGGTGGCTAC   688 OSC 56824
642  TGGCTGCCTT CCTTGT-GTT GGTGGCTGCC TTCCTTTTGT TGGTGGCTAC   690 OSC 56809
639  TGGCTGCCTT CCTTGT-GTT GGTGGCTGCC TTCCTTTTGT TGGTGGCTAC   687 OSC 56760
640  TGGCTGCCTT CCTTGT-GTT GGTGGCTGCC TTCCTTTTGT TGGTGGCTAC   688 OSC 56749
641  TGGCTGCCTT CCTTGT-GTT GGTGGCTGCC TTCCTTTTGT TGGTGGCTAC   689 NSW 7574
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56756
605  ---------- ---------- ---------- ---------- ----------   604 OSC 56761
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56826
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56829
605  ---------- ---------- ---------- ---------- ----------   604 NSW 6354
606  ---------- ---------- ---------- ---------- ----------   605 OSC 56745
591  ---------- ---------- ---------- ---------- ----------   590 OSC 56831
594  ---------- ---------- ---------- ---------- ----------   593 Scutellinia
611  ---------- ---------- ---------- ---------- ----------   610 AHS 8843
611  ---------- ---------- ---------- ---------- ----------   610 OSC 56830
611  ---------- ---------- ---------- ---------- ----------   610 EGS 2179
611  ---------- ---------- ---------- ---------- ----------   610 AHS 30502
611  ---------- ---------- ---------- ---------- ----------   610 AHS 21147
611  ---------- ---------- ---------- ---------- ----------   610 ML 941947
```

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     701         711         721         731         741       750
     |           |           |           |           |         |
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56811
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56823
601  ---------- ---------- ---------- ---------- ----------   600 OSC 56753
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56799
600  ---------- ---------- ---------- ---------- ----------   599 NSW 7536
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56734
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56801
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56759
605  ---------- ---------- ---------- ---------- ----------   604 OSC 56754
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56747
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56758
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56777
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56798
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56813
598  ---------- ---------- ---------- ---------- ----------   597 OSC 56770
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56782
691  CATCTTTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   740 OSC 56784
688  CATcTTTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   737 OSC 56825
689  CATCTTTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   738 OSC 56824
691  CATCTCTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   740 OSC 56809
688  CATCTCTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   737 OSC 56760
689  CATCTCTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   738 OSC 56749
690  CATCTCTTAG TTGGTGGCTG CCTTCCGTTT GTTGGTGGCT GCCTTCATTT   739 NSW 7574
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56756
605  ---------- ---------- ---------- ---------- ----------   604 OSC 56761
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56826
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56829
605  ---------- ---------- ---------- ---------- ----------   604 NSW 6354
606  ---------- ---------- ---------- ---------- ----------   605 OSC 56745
591  ---------- ---------- ---------- ---------- ----------   590 OSC 56831
594  ---------- ---------- ---------- ---------- ----------   593 Scutellinia
611  ---------- ---------- ---------- ---------- ----------   610 AHS 8843
611  ---------- ---------- ---------- ---------- ----------   610 OSC 56830
611  ---------- ---------- ---------- ---------- ----------   610 EGS 2179
611  ---------- ---------- ---------- ---------- ----------   610 AHS 30502
611  ---------- ---------- ---------- ---------- ----------   610 AHS 21147
611  ---------- ---------- ---------- ---------- ----------   610 ML 941947
```

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     751         761         771         781         791       800
     |           |           |           |           |         |
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56811
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56823
601  ---------- ---------- ---------- ---------- ----------   600 OSC 56753
600  ---------- ---------- ---------- ---------- ----------   599 OSC 56799
600  ---------- ---------- ---------- ---------- ----------   599 NSW 7536
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56734
602  ---------- ---------- ---------- ---------- ----------   601 OSC 56801
603  ---------- ---------- ---------- ---------- ----------   602 OSC 56759
605  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   627 OSC 56754
603  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   625 OSC 56747
602  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   624 OSC 56758
604  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   626 OSC 56777
603  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   625 OSC 56798
600  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   622 OSC 56813
598  ---------- ---------- --ANGG---- GAGCGG-CTA ACCTCCCTCC   620 OSC 56770
602  ---------- ---------- --ATGG---- GAGCGG-CTA ACCTCCCTCC   624 OSC 56782
741  TGTTGGCGGC TGTCGCCCGT TTATGGTGGG TGGCGGTCGC TGCTTTGTTT   790 OSC 56784
738  TGTTGGCGGC TGTCGCCCGT TTATGGTGGG TGGCGGTCGC TGCTTTGTTT   787 OSC 56825
739  TGTTGGCGGC TGTCGCCCGT TTATGGTGGG TGGCGGTCGC TGCTTTGTTT   788 OSC 56824
741  TGTTGGCGGC TGTCACCCGT TTATGGTGGG TGGCGGTCGC TGCTTTATTT   790 OSC 56809
738  TGTTGGCGGC TGTCACCCGT TTATGGTGGG TGGCGGTCGC TGCTTTATTT   787 OSC 56760
739  TGTTGGCGGC TGTCACCCGT TTATGGTGGG TGGCGGTCGC TGCTTTATTT   788 OSC 56749
740  TGTTGGCGGC TGTCACCCGT TTATGGTGGG TGGCGGTCGC TGCTTTATTT   789 NSW 7574
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56756
605  ---------- ---------- ---------- ---------- ----------   604 OSC 56761
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56826
604  ---------- ---------- ---------- ---------- ----------   603 OSC 56829
605  ---------- ---------- ---------- ---------- ----------   604 NSW 6354
606  ---------- ---------- --------TG TGCGGGGGAG G--TCCC-CC   624 OSC 56745
591  ---------- ---------- --ATAT---- ---------T TATACCCCTT   605 OSC 56831
594  ---------- ---------- ---------- ---------- ----------   593 Scutellinia
611  ---------- ---------- ---------- ---------- ----------   610 AHS 8843
611  ---------- ---------- ---------- ---------- ----------   610 OSC 56830
611  ---------- ---------- ---------- ---------- ----------   610 EGS 2179
611  ---------- ---------- ---------- ---------- ----------   610 AHS 30502
611  ---------- ---------- ---------- ---------- ----------   610 AHS 21147
611  ---------- ---------- ---------- ---------- ----------   610 ML 941947
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     801         811         821         831         841       850
     |           |           |           |           |         |
604  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA GATGCTTCTT    652 OSC 56811
600  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA GATGcTTcTT    648 OSC 56823
601  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA GATGCTTCTT    649 OSC 56753
600  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA GATGcTTcTT    648 OSC 56799
600  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA GATGCTTCTT    648 NSW 7536
604  -GNGG-GGAG GGGATCGAGC ATGATTCGGN TCGCTCCGAC TAGN-CAGTT    650 OSC 56734
602  -GTGG-GGTG GAGTTCGAGC ATGTTTCGGA TCGCTCCGAC TAGT-CAGTT    648 OSC 56801
603  -GTGG-GGTG GAGTTCGAGC ATGTTTCGGA TCGCTCCGAC TAGT-CAGTT    649 OSC 56759
628  TGTGCGGGGG AGGTCCCCCG AGACAAGCCC CTGCCAT-GG TGTAGGCGGT    676 OSC 56754
626  ---------- ---------- ------GCCC CTGCCATCGG TGTAGGCGGT    649 OSC 56747
625  TGTG-TGGGG AGGTCCCCCG AGACAAGCCC CTGCCATCGT TGCAGGCGGT    673 OSC 56758
627  TGTGCGGGGG AGGTCCCCCG AGACAAGCCC CTGCCATCGG TGTAGGCGGT    676 OSC 56777
626  TGTGCGGGGG AGGTCCCCCG AGACAAGCCC CTGCCATCGG TGTAGGCGGT    675 OSC 56798
623  TGTG-TGGGG AGGTCCCC-G ANACAAGCCC CTGCCATCGT TGCAGGCGGT    670 OSC 56813
621  TGTGCGGGGG AGGTCCCCCG AGACAAGCCC CTGCCATCGG TGTAGGCGGT    670 OSC 56770
625  TGTGTGGGG- AGGTCCCCCG AGACAAGCCC CTGCCATCGT TGCAGGCGGT    673 OSC 56782
791  -GTTGGTGGC TGGC-----G ACG------- --GTT-GAGT GTTCTGGGGA    824 OSC 56784
788  -GTTGGTGGC TGGC-----G ACG------- --GTT-GAGG GTTCTGGGGA    821 OSC 56825
789  -GTTGGTGGC TGGC-----G ACG------- --GTT-GAGG GTTCTGGGGA    822 OSC 56824
791  -GTTGGTGGC TGGC-----A ACG------- --GTT-GANG ATTCTGGGGA    824 OSC 56809
788  -GTTGGTGGC TGGC-----A ACG------- --GTT-GAGG ATTCTGGGGA    821 OSC 56760
789  -GTTGGTGGC TGGC-----G ACG------- --GTT-GAGG ATTCTGGGGA    822 OSC 56749
790  -GTTGGTGGC TGGC-----G ACG------- --GTT-GAGG ATTCTGGGGA    823 NSW 7574
604  -GTGGGGTAT -GGA-----G CT-GT----C CTG---GACA G--CTTCGG-    635 OSC 56756
605  -GTGGGGTAT -GGA-----G CT-GT----C CTG---GACA G--CTTCGG-    636 OSC 56761
604  -GTGGGGTAT -GGA-----G CT-GT----C CTG---GACA G--CTTCGG-    635 OSC 56826
604  --------GC -GGTTGGGGG CTTGT----C ACT---GACA GTCCT----A    633 OSC 56829
605  --------GC -GGTTGGGGG CTTGT----C ACT---GACA GTCCT----A    634 NSW 6354
625  -GAGACAAGC -GGTTGGGGG CTTGT----C ACT---GACA GTCCT----A    661 OSC 56745
606  T-CCGAGTAC C--TTACCTG -TTGC----T TCC---GTAN AnCANTAAC-    643 OSC 56831
594  --------GC GAGGCAG-TG -ATCTCGG-- -------TCA CCTCCGAAC-    623 Scutellinia
611  -GTGGTGGTC GGGTTCGTGC AAGTTAGGCC ACGTTCCCCA NATGCTTCTT    659 AHS 8843
611  -GTGGTGGTC GGGNTCGTGC AAGTTAG-CC ACGTTCCCCA GNTGCTTCTT    658 OSC 56830
611  --------GC -GGTTGGGCG CTTGTCACT- ------GACA GTCCT----A    640 EGS 2179
611  --------GC -GGTTGGGGG CTCGTCACT- ------GACA GTCCT----A    640 AHS 30502
611  --------GC -GGTTGGGGG CTCGTCACT- ------GACA GTCCT----A    640 AHS 21147
611  -GTGGGGT-- GGAAGC---T GTCCTG---- ------GACA G-CCT----C    639 ML 941947
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     851         861         871         881         891       900
     |           |           |           |           |         |
653  TATTGCTACC TG-CCAGCCC CTACTGGC-T TG-TCCCTAC -GGGAGG---    695 OSC 56811
649  TATTGcTACc TG-CCAGCCC cTAcTGGC-T TG-TCCcTAC -GGGAGG---    691 OSC 56823
650  TATTGCTACC TG-CCAGCCC CTACTGGC-T TG-TCCCTAC -GGGAGG---    692 OSC 56753
649  TATTGcTACC TG-CCAGCCC cTAcTGGC-T TG-TCCcTAC -GGGAGG---    691 OSC 56799
649  TATTGCTACC TG-CCAGCCC CTACTGGC-T TG-TCCCTAC -GGGAGG---    691 NSW 7536
651  T-GG-CT-GG TC-CC----- ---------- ----CGC--G -GGGAGA---    671 OSC 56734
649  T-GG-CT-GG TC-CC----- ---------- ----CGC--- -GGGAGG---    668 OSC 56801
650  T-GG-CT-GG TC-CC----- ---------- ----CGC--- -GGGAGG---    669 OSC 56759
677  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    715 OSC 56754
650  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    688 OSC 56747
674  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    712 OSC 56758
677  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    715 OSC 56777
676  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    714 OSC 56798
671  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    709 OSC 56813
671  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    709 OSC 56770
674  GTGGTCTTGG T--CCATGAA -GAC--GCCT T----CCTGC -GG-AGGGTG    712 OSC 56782
825  GCGG--TTCA GA-CCAGCCA --TTG-CGCT GGGCT-CTGC -GGGAGG---    863 OSC 56784
822  GTGG--TTCA GA-CCAGCCA --TTGTGC-T GGGCT-CTGC -GGGAGG---    860 OSC 56825
823  ACGG--TTCA GA-CCAGCCA --TTGCGC-T GGGCT-CTGC -GGGAGG---    861 OSC 56824
825  GCGG--TTCC GA-CCAGCCA --TTGTGCCT GGGCT-CTGC -GGGAG----    863 OSC 56809
822  GCGG--TTCC GA-CCAGCCA --TTGTGCCT GGGCT-CTGC -GGGAGG---    861 OSC 56760
823  GCGG--TTCC GA-CCAGCCA --TTGTGCCT GGGCT-CTGC -GGGAGG---    862 OSC 56749
824  GCGG--TTCC GA-CCAGCCA --TTGTGCCT GGGCT-CTGC -GGGAAG---    863 NSW 7574
636  ---------- ---CCGGCCA --GCAGGC-T GG-TTCCCAC -GGGAGG---    664 OSC 56756
637  ---------- ---CCGGCCA --GCAGGC-T GG-TTCCCAC -GGGAG----    664 OSC 56761
636  ---------- ---CCGGCCA --GCAGGC-T GG-TTCCCAC -GGGAGG---    664 OSC 56826
634  GCTGA----- ---CCAAGAA -----GGT-T GGCTTCCGT- ----------    658 OSC 56829
635  GCTGA----- ---CCAAGAA -----GGT-T GGCTTCCGT- ----------    659 NSW 6354
662  GCTGA----- ---CCAAGAA -----GGN-T GGCTTCCGT- ----------    686 OSC 56745
644  TCTGA-TTAC CTCTGATCAT GGTCTTGATC ATCTTCA--- -GGGAGTCTC    688 OSC 56831
624  GCTGG--CGA CAGCCGT--- ---------- -----CCGAC GGGGAGCACT    653 Scutellinia
660  TATTGCTACC TG-CCAGCCC CTACTGGC-T TG-TCCCTAC -GGGAGG---    702 AHS 8843
659  TATTGCTACC TG-CCAGCCC CTACTGGC-T TG-TCCCTAC -GGGNGG---    701 OSC 56830
641  GCTGA----- ---CCAAGAA -----GGT-T GGCTTCCGT- ----------    665 EGS 2179
641  GCTGG----- ---CCAAGAA -----GGT-T GGCTTCTGT- ----------    665 AHS 30502
641  GCTGG----- ---CCTAGAA -----GgT-T GGCTTCCGT- ----------    665 AHS 21147
640  AGCCA----- --GCC-GGCA -----GGT-T GG-TTCC-TG C---------    664 ML 941947
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     901        911        921        931        941       950
     |          |          |          |          |         |
696  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   735 OSC 56811
692  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   731 OSC 56823
693  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   732 OSC 56753
692  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   731 OSC 56799
692  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   731 NSW 7536
672  ---------- ACCCT-ACAA ACCATTTGCA TTTATTTTGC CTTCTGACTG   710 OSC 56734
669  ---------T ACCCT-ACAA ACCATTTGCA TTTATTTTGC CTTCTGTCTG   708 OSC 56801
670  ---------T ACCCT-ACAA ACCATTTGCA TTTATTTTGC CTTCTGTCTG   709 OSC 56759
716  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   752 OSC 56754
689  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   725 OSC 56747
713  T--GAA-GA- --------AA A-CATTTGCA TT-ATCTTGC CATCAGTCTG   748 OSC 56758
716  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   752 OSC 56777
715  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   751 OSC 56798
710  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   746 OSC 56813
710  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   746 OSC 56770
713  T--GAA-GA- --------AA ACCATTTGCA TT-ATCTTGC CATCAGTCTG   749 OSC 56782
864  ---------T ATCCTGT-AA ACATTGAGCA TTTATTTTAC CTTCCGTCTG   903 OSC 56784
861  ---------T ATCCTGT-AA ACATTGAGCA TTTATTTTAC CTTCCGTCTG   900 OSC 56825
862  ---------T ATCCTGT-AA ACATTGAGCA TTTATTTTAC CTTCCGTCTG   901 OSC 56824
864  ---------T ATCCTGT-AA ACATTGAGCT TTTATTTAAN CTTCTGTCTG   903 OSC 56809
862  ---------T ATCCTGT-AA ACATTGAGCT TTTATTTTAC CTTCTGTCTG   901 OSC 56760
863  ---------T ATCCTGT-AA ACATTGAGCT TTTATTTTAC CTTCTGTCTG   902 OSC 56749
864  ---------T ATCCTGT-AA ACATTGAGCT TTTATTTTAC CTTCTGTCTG   903 NSW 7574
665  ---------T ATTCT-ACAA ACCATTTGTA TATAT-TTGC CTTCTGTCTG   703 OSC 56756
665  ---------T ATTCT-ACAA ACCATTTGTA TATAT-TTGC CTTCTGTCTG   703 OSC 56761
665  ---------T ATTCT-ACAA ACCATTTGTA TATAT-TTGC CTTCTGTCTG   703 OSC 56826
659  ---GAAAGAT ATTTT-ACAA ACTATTTGCA TTTGTCTTGC cTTCTGTCTG   704 OSC 56829
660  ---GAAAGAT ATTTT-ACAA ACTATTTGCA TTTGTCTTGC CTTCTGTCTG   705 NSW 6354
687  ---GAAAGAT ATTTT-ACAA ACTATTTGCA TTTGTCTTGC CTTCTGTCTG   732 OSC 56745
689  TGCGGGAGGT ATACATT-AA ACTC-TTGCA TT-ACCATGT CATCTGTCTG   735 OSC 56831
654  CGCGGGAGGT ATACAT-CAA ACTC-TTGCA TTT-TTATGT CATCTGTCTG   700 Scutellinia
703  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   742 AHS 8843
702  ---------T CCCCC-GCAA ACCATTTGTA TTTATTTTGC CTTCTGTCTG   741 OSC 56830
666  ---GAAAGAT ATTTT-ACAA ACTATTTGCA TTTGTCTTGC CTTCTGTCTG   711 EGS 2179
666  ---GGAAGAT ATTTTTACAA ACCATTTGCA TTTGTCTTGC CTTCTGTCTG   712 AHS 30502
666  ---GGAAGAT ATTTTTACAA ACCATTTGCA TTTGTCTTGC CTTCTGTCTG   712 AHS 21147
665  ---GGGAGGT ATTTT-ACAA ATCATTTGCA TTCATTTTGC CTTCTGTCTG   710 ML 941947
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     951         961         971         981         991       1000
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736  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   785 OSC 56811
732  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   781 OSC 56823
733  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   782 OSC 56753
732  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   781 OSC 56799
732  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   781 NSW 7536
711  AATAATGATT ATAACAATTG CTAAAACTTT CAACAACGGA TCTCTTGGTT   760 OSC 56734
709  AATAATGTTT ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   758 OSC 56801
710  AATAATGTTT ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   759 OSC 56759
753  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   802 OSC 56754
726  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   775 OSC 56747
749  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   798 OSC 56758
753  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   802 OSC 56777
752  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   801 OSC 56798
747  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   796 OSC 56813
747  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   796 OSC 56770
750  AATTTTGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   799 OSC 56782
904  AATTATATCA ATAACATTTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   953 OSC 56784
901  AATTATATCA ATAACATTTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   950 OSC 56825
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904  AATTATATCA ATAANANTTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   953 OSC 56809
902  AATTATATCA ATAACATTTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   951 OSC 56760
903  AATTATATCA ATAACATTTG TTAAAAATTT CAACAACGGA TCTCTTGGTT   952 OSC 56749
904  AATTATATCA ATAACATTTG TTAAAAATTT CAACAACGGA TTTTTTGGTT   953 NSW 7574
704  AATTATGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   753 OSC 56756
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704  AATTATGTTA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   753 OSC 56826
705  AATCATGTTT ATAACAAATG TTAAAaCTTT CAACAACGGA TCTCTTGGTT   754 OSC 56829
706  AATCATGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   755 NSW 6354
733  AATCATGATT ATAACAAATG ATAAAACTTT CAACAACGGA TCTCTTGGTT   782 OSC 56745
736  AATC-TGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   784 OSC 56831
701  AA-C-TGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   748 Scutellinia
743  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   792 AHS 8843
742  AATCATGTTA ATAACAATCG TTAAAACTTT CAACAACGGA TCTCTTGGTT   791 OSC 56830
712  AATCATGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   761 EGS 2179
713  AATCATGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   762 AHS 30502
713  AATCATGTTT ATAACAAATG TTAAAACTTT CAACAACGGA TCTCTTGGTT   762 AHS 21147
711  AATCTGTTAA ATAACAATTG TTAAAACTTT CAACAACGGA TCTCTTGGTT   760 ML 941947
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	1001	1011	1021	1031	1041	1050		
786	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	835	OSC	56811
782	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	831	OSC	56823
783	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	832	OSC	56753
782	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	831	OSC	56799
782	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	831	NSW	7536
761	CTCGCATCGA	TGAAGGACGC	AGCGAAATGC	GATAAGAAGT	GTGAATTGCA	810	OSC	56734
759	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	808	OSC	56801
760	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	809	OSC	56759
803	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	852	OSC	56754
776	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	825	OSC	56747
799	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	848	OSC	56758
803	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	852	OSC	56777
802	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	851	OSC	56798
797	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	846	OSC	56813
797	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	846	OSC	56770
800	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	849	OSC	56782
954	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	1003	OSC	56784
951	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	1000	OSC	56825
952	CTCGCATCNA	TAAAAAACGC	ACCGAAATGC	GATAAGTANT	GTGAATTGCA	1001	OSC	56824
954	CTC-CATCGA	TCAAGAACGC	ANCNAAATGC	GANAAGTACT	GTGAATNGCA	1002	OSC	56809
952	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	1001	OSC	56760
953	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	1002	OSC	56749
954	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	1003	NSW	7574
754	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	803	OSC	56756
754	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	803	OSC	56761
754	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	803	OSC	56826
755	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	804	OSC	56829
756	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	805	NSW	6354
783	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	832	OSC	56745
785	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	834	OSC	56831
749	CTCGCATCGA	TGAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	798	*Scutellinia*	
793	CTCGCATCGA	TGAAGAAcgc	agcGAAAtgc	gataagtagt	gtgaaTTGCA	842	AHS	*8843*
792	CTCGCATCGA	TNAAGAACGC	AGCGAAATGC	GATAAGTAGT	GTGAATTGCA	841	OSC	*56830*
762	CTCGCATCGA	TGAAGAACGC	nnnnnnnnnn	nnnnnnnnnn	nnnAATTGCA	811	EGS	*2179*
763	CTCGCATCGA	TGAAGAACgc	agcgaaatgc	gataagtagt	gtGAATTGCA	812	AHS	*30502*
763	CTCGCATCGA	TAAAGAACGc	nnnnnnnnnn	nnnnnnnnnn	nnGAAtTGCA	812	AHS	*21147*
761	CTCGCATCGA	TGAAGAACGC	AGCAGNNNNN	NNNNNNNNNN	NNNNNNNGCA	810	ML	*941947*

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     1051        1061        1071        1081        1091      1100
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 836 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   885 OSC 56811
 832 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   881 OSC 56823
 833 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   882 OSC 56753
 832 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   881 OSC 56799
 832 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   881 NSW 7536
 811 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   860 OSC 56734
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 810 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   859 OSC 56759
 853 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGCAT   902 OSC 56754
 826 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGCAT   875 OSC 56747
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 850 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGCAT   899 OSC 56782
1004 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCATGGTAT  1053 OSC 56784
1001 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCATGGTAT  1050 OSC 56825
1002 NAAT-CAGTG AATCATCNAA TCTTN-AACN CACATTGCCC CTCATGGTAT  1049 OSC 56824
1003 CAATTCATTN AATCATCCAA TCTTTGAACG C-CATTNCGC CCCATGGTAT  1051 OSC 56809
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1003 GAATTCAGTG AATCATCGAA TTTTTGAACG CACATTGCGC CCCATGGTAT  1052 OSC 56749
1004 GAATTCAGTG AATCATCGAA TTTTTGAACG CACATTGCGC CCCATGGTAT  1053 NSW 7574
 804 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGCAT   853 OSC 56756
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 805 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   854 OSC 56829
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 833 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   882 OSC 56745
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 799 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   848 Scutellinia
 843 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   892 AHS 8843
 842 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   891 OSC 56830
 812 GAnTTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   861 EGS 2179
 813 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC CTCCTGGTAT   862 AHS 30502
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 882 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AGGAGATATT TCAAGCTCTT   931 OSC 56823
 883 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AGGAGATATT TCAAGCTCTT   932 OSC 56753
 882 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AGGAGATATT TCAAGCTCTT   931 OSC 56799
 882 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AGGAGATATT TCAAGCTCTT   931 NSW 7536
 861 TCCGGGAAGC ATGCCTGTTC GAGCGTCATG AAGAAAATTT -CAAGCTCTT   909 OSC 56734
 859 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AAGACAATTT -CAAGCTCTT   907 OSC 56801
 860 -CCGGGAAGC ATGCCTGTTC GAGCGTCATG AAGAACAATT TCAAGCTCTT   908 OSC 56759
 903 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CAAGCTCTT   951 OSC 56754
 876 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CAAGCTCTT   924 OSC 56747
 899 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CA-GCTCTT   946 OSC 56758
 903 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CAAGCTCTT   951 OSC 56777
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 897 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CAAGCTCTT   945 OSC 56813
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 900 TCCGGGAGGC ATGCCTGTTC GAGCGTCATA AAAACCCACT -CAAGCTCTT   948 OSC 56782
1054 CCCATGGGGC ATGCCTTCCG GA-CGTCGGG AAAACCCCCT TCAAGCATTT  1102 OSC 56784
1051 CCCATGGGGC ATGCCTTCCG GAgCGTCgGG AAAACCCCcT TCAAGCATTT  1100 OSC 56825
1050 CCCATGGGGC ATCCNTN-CG GAACGTCCGG AAAACCCCTT -CAANCATTT  1097 OSC 56824
1052 CCCCTGGGGC ATGCCTTCCG GAACGTCTGG AAAAACCTTT -CAANCTTTA  1100 OSC 56809
1051 CCCGTGGGGC ATGCCTTCCG GAGCGTCCGG AAAACCCCT- -CAAGCTTTT  1098 OSC 56760
1053 CCCGTGGGGC ATGCCTTCCG GAGCGTCGGG AAAACCCCTT -CAAGCTTTT  1101 OSC 56749
1054 CCCGTGGGGC ATGCCTTCCG GAGCGTCGGG AAAACCCCTT -CAAGCTTTT  1102 NSW 7574
 854 TCCGGGAGGC ATGCCTGTTC GAGCGTCATG AAGACCATTT -CAAGCTCTT   902 OSC 56756
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 855 TCCGGGAGGC ATGCCTGTCC GCGC-ACAAA ATGGCTCATT -CAGGGTGAC   902 OSC 56829
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 883 TCCGGGAAGC ATGCCTGTCC GCGC-ACAAA ATGGCTCATT -CAGGGTGAC   930 OSC 56745
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 849 TCCGGGAGGC ATGCCTGTTC GAGCGTCATT AAAAACCACT -CAAGCTCTT   897 Scutellinia
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 932 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGCTCTTGT A-CTCATTCA    976 OSC 56823
 933 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGCTCTTGT A-CTCATTCA    977 OSC 56753
 932 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGCTCTTGT A-CTCATTCA    976 OSC 56799
 932 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGT-CTTGT A-CTNANTCA    975 NSW 7536
 910 TT-----GCT TGGTTTT-GA AGGTTGAGTG TTGCGCTTGC G-CTCGTTCA    952 OSC 56734
 908 TT-----GCT TGGTTTT-GA AGGTTGAGTG TTGCGCTTGC G-CTCGTTCA    950 OSC 56801
 909 TT-----GCT TGGTTTT-GA AGGTTGAGTG TTGCGCTTGC G-CTCGTTCA    951 OSC 56759
 952 T-----GGCT TGGTTAT-GG AGGTTGAGCT ACGTCCTTCG GTGACC-TCA    994 OSC 56754
 925 T-----GGCT TGGTTAT-GG AGGTTGAGCT ACGTCCTTCG GTGACC-TCA    967 OSC 56747
 947 T-----GGCT TGGTTAT-GG AGGTTGAGCT ATGCCCTTCG GTAACC-TCA    989 OSC 56758
 952 T-----GGCT TGGTTAT-GG AGGTTGAGCT ACGTCCTTCG GTGACC-TCA    994 OSC 56777
 951 T-----GGCT TGGTTAT-GG AGGTTGAGCT ACGTCCTTCG GTGACC-TCA    993 OSC 56798
 946 T-----GGCT TGGTTAT-GG AGGTTGAGCT ATGCCCTTCG GTAACC-TCA    988 OSC 56813
 946 T-----GGCT TGGTTAT-GG AGGTTGAGCT ACGTCCTTCG GTGACC-TCA    988 OSC 56770
 949 T-----GGCT TGGTTAT-GG AGGTTGAGCT ATGCCCTTCG GTAACC-TCA    991 OSC 56782
1103 TTTT---GCT TGGTTGT-GG AGGCTGAGCG TTGCCTGCGT G-CACGTTTG   1147 OSC 56784
1101 TTTT---GCT TGGTTGT-GG AGGCTGAGCG TTGCCTGCGT G-CACGTTTG   1145 OSC 56825
1098 TTTT---GCT TGGTTGTTGA AGCT-GAACN TTGCCTCCTT --CNCCTTTG   1141 OSC 56824
1101 TTNT---GTC -GGAGCT-GG AGCT-GAACC TTGCTTGCTT --CACTTTCC   1142 OSC 56809
1099 TTTTTT-GCT TGgTTCT-GG AgGT-GAACg TTtCTTGCTT TTCACTTTCg   1145 OSC 56760
1102 TTTTTT-GCT TGGTTCT-GG AGGCTGAGCG TTGCTTGCGT G-CACGTTCG   1148 OSC 56749
1103 TTTTTT-GCT TGGTTCT-GG AGGCTGAGCG TTGCTTGCGT G-CACGTTCG   1149 NSW 7574
 903 TT-----GCT TGGTTGT-GG AGGTTGAGCG TTGCCCGCGT G-CTCGTTCC    945 OSC 56756
 903 TT-----GCT TGGTTGT-GG AGGTTGAGCG TTGCCCGCGT G-CTCGTTCC    945 OSC 56761
 903 TT-----GCT TGGTTGT-GG AGGTTGAGCG TTGCCCGCGT G-CTCGTTCC    945 OSC 56826
 903 CAGGGTTGCT TTGGTACTGA AGGTCGAATG TTGCTCATAT G-TTTGTTCG    951 OSC 56829
 904 CA----GGCT TTGGTACTGA AGGTCGAATG TTGCTCATAT G-TTTGTTCG    948 NSW 6354
 931 CAGGGTTGCT TTGGTACTGA AGGTCGAATG TTGCTCATAT G-TTTGTTCG    979 OSC 56745
 934 TT-----GCT TGGTCATGGA AGAG-GAGGG T-GC--CTGT GTACTC-TCC    973 OSC 56831
 898 TT-----GCT TGGTATTGGG AGAG-GAGTG CACTCGTTGC CCTCCCTTCC    941 Scutellinia
 943 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGCTCTTGT A-CTCATTCA    987 AHS 8843
 942 TTTT---GCT TGGTCTT-GG AGGTTGAGTG TTGCTCTTGT A-CTCATTCA    986 OSC 56830
 910 CAGGGTTGCT TTGGTACTGA AGGTCAAATG TTGCTCATAT G-TTTGTTCG    958 EGS 2179
 911 CAGGGTTGCC TTGGTATTGA AGGTCGAATG TTGCTCATAT G-TTTGTTCG    959 AHS 30502
 911 CAGGGTTGCC TTGGTATTGA AgGTCGAATG TTGCTCATAT G-TTTGTTCG    959 AHS 21147
 910 TTGCTTGGTT CTGAAGGCT- -GATGTAAT- ----CCATAT A-CTCATTCA    951 ML 941947
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        1201        1211        1221        1231        1241      1250
        |           |           |           |           |         |
 981    CCTTTGAAAT  TTACAGGCGG  GATTGCGTCG  TGTTCCTTTT  --GGCGTTAT   1028  OSC 56811
 977    CCTTTGAAAT  TTACAGGCGG  -ATTGCGTCG  TGTTCCTTTT  --GGCGTTAT   1023  OSC 56823
 978    CCTTTGAAAT  TTACAGGCGG  -ATTGCGTCG  TGTTCCTTTT  --GGCGTTAT   1024  OSC 56753
 977    CCTTTGAAAT  TTACAGGCGG  -ATTG-GTCG  TGTTCCTTTT  --GGCGTTAT   1022  OSC 56799
 976    CCTTTGAAAT  TTACAGGCGG  -ATTGCGTCG  TGTTCCTTTT  --GGCGTTAT   1022  NSW 7536
 953    CCATTGAAAT  TCACAGGCGG  -ACGGTTTCG  TGTTCCTTCT  --GGCGTTAT    999  OSC 56734
 951    CCATTGAAAT  TCACAGGCGG  -ACGGTTTCG  TGTTCCTTCT  --GGCGTTAT    997  OSC 56801
 952    CCATTGAAAT  TCACAGGCGG  -ACGGTTTCG  TGTTCCTTCT  --GGCGTTAT    998  OSC 56759
 995    CCTTCGAAAT  TCAGAGGCGG  -ATCGTCCAT  TGTTGCCCT-  --GGCGTTAT   1040  OSC 56754
 968    CCTTCGAAAT  TCAGAGGCGG  -ATCGTCCAT  TGTTGCCCT-  --GGCGTTAT   1013  OSC 56747
 990    CCTTCGAAAT  TCAGAGGCGG  -ATTGCCCCG  TGTTGCCCT-  --GGCGTTAT   1035  OSC 56758
 995    CCTTCGAAAT  TCAGAGGCGG  -ATCGTCCAT  TGTTGCCCT-  --GGCGTTAT   1040  OSC 56777
 994    CCTTCGAAAT  TCAGAGGCGG  -ATCGTCCAT  TGTTGCCCT-  --GGCGTTAT   1039  OSC 56798
 989    CCTTCGAAAT  TCAGAGGCGG  -ATTGCCCCG  TGTTGCCCT-  --GGCGTTAT   1034  OSC 56813
 989    CCTTCGAAAT  TCAGAGGCGG  -ATCGTCCAT  TGTTGCCCT-  --GGCGTTAT   1034  OSC 56770
 992    CCTTCGAAAT  TCAGAGGCGG  -ATTGCCCCG  TGTTGCCCT-  --GGCGTTAT   1037  OSC 56782
1148    CCTTCGAAAT  TCATTGGCGG  -ACTGTTTCA  TGTTCCATTT  --GGCGTTAT   1194  OSC 56784
1146    CCTTCGAAAT  TCATTGGCGG  -ACTGTTTCA  TGTTCCATTT  --GGCGTTAT   1192  OSC 56825
1142    CCTTCCAAAA  TCAT-GGCCG  GANTGTTTNN  TGTTCCATTT  --GGCGTTAT   1188  OSC 56824
1143    CCNCCNNANA  TCCATGGCGG  -AC-GTTTCA  TTTTCCATTT  --GGCGTTAT   1188  OSC 56809
1146    CCtCCAAAAT  TCATTGGCGG  -AgTGTTTCA  TNTTCCATTT  --GGCGTTAT   1192  OSC 56760
1149    CCTCCGAAAT  TCATTGGCGG  -ACTGTTTCA  TGTTCCATTT  --GGCGTTAT   1195  OSC 56749
1150    CCTCCGAAAT  TCATTGGCGG  -AcTGTTTCA  TGTTCCATTg  --GGcGTTAT   1196  NSW 7574
 946    CCTTTGAAAT  TTGCTGGCGG  -ATCGCTCCA  TGTTCCATTT  --GGCGTTAT    992  OSC 56756
 946    CCTTTGAAAT  TTGCTGGCGG  -ATCGCTCCA  TGTTCCATTT  --GGCGTTAT    992  OSC 56761
 946    CCTTTGAAAT  TTGCTGGCGG  -ATCGCTCCA  TGTTCCATTT  --GGCGTTAT    992  OSC 56826
 952    TCTTTAAAAT  TTACAGGCGG  GCGACTTCAA  -GT-CCTTTC  ATAGCGTTGT    999  OSC 56829
 949    TCTTTAAAAT  TTACAGGCGG  GCGACTTCAA  -GT-CCTTTC  ATAGCGTTGT    996  NSW 6354
 980    TCTTTAAAAT  TTACAGGCGG  GCGACTTCAA  -GT-CCTTTC  ATAGCGTTGT   1027  OSC 56745
 974    CTTTTGAAAT  CAAATGGCGG  AAAGC-TCCA  TGT-GCCCC-  --GGCGTAGT   1018  OSC 56831
 942    G-----AAAT  TCAATGGCGG  AAAGCC-CCA  TGT-GCCCC-  --GGCGTAGT    981  Scutellinia
 988    CCTTTGAAAT  TTACAGGCGG  -ATTGCGTCG  TGTTCCTTTT  --GGCGTTAT   1034  AHS 8843
 987    CCTTTGAAAT  TTACAGGCGG  -ATTGCGTCG  TGTTCCTTTT  --GGCGTT-T   1032  OSC 56830
 959    TCTTTAAAAT  TTACAGGCGG  GCGACTTCAA  --TTCCTTTC  ATAGCGTTGT   1006  EGS 2179
 960    TCTTTAAAAT  TTACAGGCGG  GCGACTTCAA  -GT-CCTTTT  TCAGCGTTGT   1007  AHS 30502
 960    TCTTTAAAAT  TTACAGGCgG  GCGACTTCAA  -GT-CCTTTT  TTAGCGTTGT   1007  AHS 21147
 952    CCTTTGAAAT  TTACAGGCGG  -ACTCTTCCA  TGTTCCATTT  --GGCGTTAT    998  ML 941947
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	1251	1261	1271	1281	1291			
1029	AAGANAACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1071	OSC	56811
1024	AGGATAACTC	TTTCG-TT-G	GACTCGGTCG	TC-TGTCctG	CCcTA	1065	OSC	56823
1025	AGGATAACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1067	OSC	56753
1023	AGGATAACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1065	OSC	56799
1023	AGGATAACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1065	NSW	7536
1000	AGGTTANCTC	TCTCGCTT-G	GATN-CGTGA	GCCTGTCCTG	CCCCA	1042	OSC	56734
998	AGGTTAACTC	TCTCGCTT-G	GATA-CGTGA	AC-TGTCCTG	CCCCA	1039	OSC	56801
999	A-GTTAACTC	TCTCGCTT-G	GATA-CGTGA	ACCTGTCCTG	CCCCA	1040	OSC	56759
1041	AAGTTTTCTC	TTTCGCTT-G	GATTACATGG	TCATCTC--G	CCACA	1082	OSC	56754
1014	AAGTTTTCTC	TTTCGCTT-G	GATTACATGG	TCATCTC--G	CCACA	1055	OSC	56747
1036	AAGTTTGCTC	TTTCGCTC-G	GATTACATGG	CCATCTC--G	CCGCA	1077	OSC	56758
1041	AAGTTTTCTC	TTTCGCTT-G	GATTACATGG	TCATCTC--G	CCACA	1082	OSC	56777
1040	AAGTTTTCTC	TTTCGCTT-G	GATTACATGG	TCATCTC--G	CCACA	1081	OSC	56798
1035	AAGTTTGCTC	TTTCGCTC-G	GATTACATGG	CCATCTC--G	CCGCA	1076	OSC	56813
1035	AAGTTTTCTC	TTTCGCTT-G	GATTACATGG	TCATCTC---	CCACA	1075	OSC	56770
1038	AAGTTTGCTC	TTTCGCTC-G	GATTACATGG	CCATCTC--G	CCGnn	1079	OSC	56782
1195	AGGATAACTC	TTTCGCTT-G	GACTTATGAG	GC-TGTCCAG	CCC-A	1236	OSC	56784
1193	AGGATAACTC	TTTCGCTT-G	GACTTATGAG	GC-TGTCCAG	CCC-A	1234	OSC	56825
1189	AGGAAAACTC	TT-CCCTT-G	GANTTATGAA	GC-TTTCCA-	CCCnn	1229	OSC	56824
1189	AGGANAACTC	TTNC-CTT-G	GAATTATNNA	GN-TNTCCTN	CC--A	1228	OSC	56809
1193	ANGAtAACaC	TTTCCCTT-G	GAATTAATAA	AGCTGTCCTG	CCCAA	1236	OSC	56760
1196	AGGATAACTC	TTTCGCTT-G	GACTTATGAG	GC-TGTCNTG	CCC-A	1237	OSC	56749
1197	AGGATAATNT	TTGGGNN--G	GACTATTGAG	GN-TGTNGNG	CCC-A	1237	NSW	7574
993	AGGATAACTC	TTTCGCTT-G	GACTCATGAG	GC-TGTCCTG	CCCAA	1035	OSC	56756
993	AGGATAACTC	TTTCGCTT-G	GACTCATGAG	GC-TGTCGTG	CCCAA	1035	OSC	56761
993	AGGATAACTC	TTTCGCTT-G	GACTCATGAg	GC-TGTCCTG	CCCAA	1035	OSC	56826
1000	AAGTTAACTC	TTTCGCTTAG	GACTTGGTGG	AT-TGTCCAG	CCTCG	1043	OSC	56829
997	AAGTTAACTC	TTTCGCTTAG	GACTTGGTGG	CT-TGTCCAG	CCTCG	1040	NSW	6354
1028	AAGTTAGCTC	TTTCGCTTAG	GACTTGGTGG	CT-TGTCCAG	CCTCG	1071	OSC	56745
1019	AAGTTTTC--	TTTCGCTT-G	GAACATGAGG	TG--ATCCTG	CCCCn	1058	OSC	56831
982	AAGTTTTC--	TTTCGCTT-G	GAACGTGAGG	TG--ATCCTG	CCGCA	1021	*Scutellinia*	
1035	AGGATAACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1077	AHS	*8843*
1033	AGGATNACTC	TTTCGCTT-G	GACTCGGTCG	TC-TGTCCTG	CCCTA	1075	OSC	*56830*
1007	AAGTTAACTC	TTTCGCTTAa	GACTTGGTGG	CT-TGTCCAg	CCTCG	1050	EGS	*2179*
1008	AAGTTAACTC	TTTCGCT-AG	GACTTTGTGG	CT-TGTCCAG	CCTTG	1050	AHS	*30502*
1008	AAGTTAACTC	TTTCGCT-AG	GACTTTGTGG	TT-TGTCCAG	CCTTG	1050	AHS	*21147*
999	AAGATTACTC	TTTCNCTT-G	GACTCATGGG	TTCTGTCCTN	CCnnn	1042	ML	*941947*