

Ploidy levels and evolution in Boletales*

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Cytofluorometric analyses of nuclear DNA contents have been carried out with species of Boletales which have never or only little been studied in this respect. Differences being whole multiples of a basic value are interpreted as different ploidy levels. Thirty-eight species originating from Europe, North America, and from Malaysia have been studied. The data are discussed in relation to data from 123 additional species previously examined, and they are set in a general evolutionary context.

Keywords: cytofluorometry, DNA, ploidy, evolution, Boletales.

Polyploidy in higher plants is closely related to evolution. Obtaining knowledge about polyploidy and its possible role in evolution of fungi is hampered by the fact that fungal chromosomes are very small and are not readily seen during mitosis and meiosis. Fungal ploidy levels can rarely be analyzed successfully by counting chromosomes. In addition, such analysis requires complicated methods. Therefore fungal chromosome numbers are known with certainty in only a few cases.

An indirect method to obtain ploidy levels is to analyse the nuclear DNA content after DAPI (4',6-diamino-2-phenylindole) staining and evaluate the data of different species comparatively (Bresinsky & al., 1987a; 1987b). This method has its limits (Wittmann-Meixner & al., 1989b; Arnold, 1993) and the results allow inference of ploidy level only if the analyzed taxa (or their nuclear DNA-values) are comparable in the following aspects:

- (1) Base composition (GC-contents), because DAPI binds to AT-rich segments of DNA;
- (2) the percentage of repetitive DNA;
- (3) the percentage of insertions and deletions in the DNA;
- (4) the current phase of the cell cycle;
- (5) permeability to DAPI;
- (6) optical characteristics of the cell structures; the background should show little fluorescence.

* This paper is dedicated to Professor M. Moser on the occasion of his seventieth birthday.

Within a surveyable range of taxonomic relationships of fungi these conditions seem to be fulfilled. GC-content and amount of repetitive DNA usually show little variation within genera and families of fungi (for references see Wittmann-Meixner, 1989). Variation of DNA content due to phase of cell cycle can be kept under control by observation of comparable stages. The measured DNA contents provide reliable, reproducible and constant taxonomic characters of fungal species. Small differences in the measured nuclear DNA contents (within the extent of standard deviation) of two compared samples do not necessarily mean different number of chromosomes and may be dependent on the above mentioned parameters or to inaccuracy of measurement. However, large differences in the nuclear DNA contents expressed as multiples of a basic value are to be interpreted as different ploidy levels.

Previous analyses of Boletales (Meixner & Bresinsky, 1988; Wittmann-Meixner & Bresinsky, 1989a; Wittmann-Meixner, 1989), Ascomycetes (Weber, 1992), and the subgenus *Telamonia* within *Cortinarius* (Arnold, 1993) indicated the DNA-values of different species within closely related groups (e.g. genera) frequently represent whole multiples of a basic value. Taxa shown to be polyploid by this method mostly live under the same environmental conditions as is known for higher plants. As in higher plants, polyploidy may indicate previously unknown differentiation into several species or infraspecific taxa (Weber, 1992; Arnold, 1993).

This study relates to Boletales *s.l.*, as indicated by chemical and molecular biological characteristics (Bresinsky & Besl, 1979; Bruns & al., 1989) and includes members of following families: Coniophoraceae, Paxillaceae, Omphalotaceae, Boletaceae, Strobilomycetaceae, Gomphidiaceae, Rhizopogonaceae and further gastroid families.

Previous studies (Wittmann-Meixner, 1989) could not demonstrate heteroploidy (infraspecific polyploidy) in Boletales (278 strains for a totale of 128 investigated species). Nuclear DNA contents remained constant within one single species. This holds true within several strains of *Paxillus involutus* originating from different hosts and therefore probably belonging to separate, incompatible groups (Fries, 1985): identical nuclear DNA values were observed in spite of beginning speciation. One sample of *P. involutus* from Chile, probably imported from Europe, was significantly different from European strains, although the difference was smaller than a whole ploidy level. On the other hand, speciation could be correlated with differences in nuclear DNA contents in *Boletus edulis s.l.* and *Leccinum scaber s.l.* All ploidy levels from 1x to 10x were found in Boletales; mostly they were even-numbered levels (2x, 4x, 6x). Up to four different levels were determined within one genus. Closely related pairs of species

were compared: species occurring in glacially disturbed areas, as well as species with advanced characters in general belonged to a higher ploidy level (e.g. *Suillus sibiricus* versus *S. americanus*; *Paxillus panuoides* versus *P. curtisii*). In areas with great glacial disturbance the percentage of polyploid species was high; in refuge areas (e.g. the deciduous forest belt of North America) monoploid species were more common.

The goal of the present study is to analyse additional species of Boletales *s.l.* from Central Europe and North America and some species from the tropical regions of Malaysia. Tropical species of Boletales have not yet been analysed. Thirteen Malayan species could be examined that had been collected during an excursion of the University of Regensburg. Such a small number of species is of course not representative of the total tropical diversity, yet it was of interest to determine, even if only approximately, the ratio of polyploid species within tropical Boletales because this portion is known to be often quite large in higher plants due to paleopolyploidy. Six Californian species were also examined. All but one had never been studied cytologically. The material from Europe contained some rare species not yet analysed; also included are subspecies that belong to taxa apparently in continuous evolutionary progress and species that have been studied in North American samples only (e.g. *Tylopilus felleus*).

Material and methods

Material examined. *Austroboletus dictyotus* (Bredijn) Wolfe: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Boletellus emodensis* (Berk.) Sing.: MALAYSIA, Cameron Highlands, Gunung Berembang, 25. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Boletellus dissiliens* (Corner) Pegler et Young: MALAYSIA, Pulan Penang, Forest Reserve near Telok Bahang, 9. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. *Boletus aereus* Bull.: Fr.: GERMANY, Karlstadt near Würzburg, 18. 10. 1989, leg. & det. W. Steglich & A. Reisinger. *Boletus appendiculatus* Schaeff.: ROMANIA, frozen material obtained from mushroom seller, 1989. – *Boletus aureomycelinus* Pat. & Bak.: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. *Boletus craspedius* Mass.: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Boletus depilatus* Redeuilh: GERMANY, Herrsching, Ammersee, Sept. 1989, mushroom foray. *Boletus edulis* Bull.: Fr. var. *quercicola* Vasilk.: UKRAINIA, Kiev region, 17. 8. 1989, leg. & det. S.P. Wasser & A. Bresinsky. – *Boletus edulis* Bull.: Fr. var. *betulicola* Vasilk.: UKRAINIA, Kiev region, 15. 7. 1989, leg. & det. S. P. Wasser & A. Bresinsky. *Boletus graveolens* Corner: MALAYSIA, Pulan Penang, Penang Hill, 19. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Boletus gyrodontoides* Corner: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl. – *Boletus rhodopurpureus* Smotl.: GERMANY, Kaltenbrunn, Garmisch, 10. 10. 1991, leg. M. Beisenherz, det. A. Bresinsky (ss.

Bresinsky & al., 1982, cf. *Boletus satanoides*). *Boletus sylvestris* Petsch: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Boletus tristiculus* Mass.: MALAYSIA, Pulan Penang, Muka head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Chamonixia caespitosa* Roll.: GERMANY, Oberpfalz, Elm near Kaimling (MTB 6339/4), 28. 9. 1991, leg. & det. H. Zitzmann. – *Chroogomphus helveticus* (Sing.) Mos. ssp. *tatrensis* (Pil.) Kuthan & Sing.: No. 119, GERMANY, Wettersteingebirge, Garmisch, Schachen, 15. 8. 1989, leg. & det. A. Bresinsky. – No 118, GERMANY, Pillnach, 4. 10. 1991, leg. & det. M. Kronfeldner. – *Chroogomphus vinicolor* (Peck) O. K. Miller: USA, California, Pt. Reyes, 15. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Gomphidium subroseus* Kauffm.: USA, California, Pt. Reyes, 22. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Heimiella retispora* (Pat. & Bak.) Boedijn: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Leccinum griseum* (Qué.) Sing.: No. 114, GERMANY, Paintner Forst, Rothenbügl, Lkr. Kelheim, 7.1992, leg. & det. A. Bresinsky. No 115 (91-3b), GERMANY, Kaltenbrunn, Garmisch-Mittenwald, 15. 8. 1991, leg. & det. A. Bresinsky. – *Leccinum piceinum* Pilát & Dermek: GERMANY, National Park Bayerischer Wald, 7. 1989, leg. & det. N. Luschka. – *Leccinum subcinnamomeum* Pilát & Dermek: GERMANY, Neubäu-Bodenwöhr, 9. 9. 1989, leg. & det. A. Bresinsky. – *Phylloporus bellus* (Mass.) Corner: MALAYSIA, Pulan Penang, Forest Reserve near Telok Bahang, 9. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Phylloporus rhodoxanthus* (Schw.) Bres. ssp. *rhodoxanthus*: USA, California, Pt. Reyes, 15. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Phylloporus* cf. *rufescens* Corner: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Pulveroboletus cramesinus* (Secr.) Mos.: GERMANY, Eifel, Daun, 8. 10. 1989, leg. & det. N. Arnold. – *Pulveroboletus icterinus* (Pat. & Bak.) Watling: MALAYSIA, Pulan Penang, Muka Head Field Station, 8. 4. 1991, leg. N. Arnold & H. Besl, det. H. Besl. – *Suillus caeruleus* Smith & Thiers: No. 4, USA, California, Pt. Reyes, 15. 3. 1991, leg. M. Fischer, det. Th. Bruns. No. 10, USA, California, Pt. Reyes, 22. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Suillus flavidus* (Fr.) Sing.: GERMANY, Oberpfalz, Neubäu, 1. 10. 1989, leg. & det. A. Bresinsky. *Suillus placidus* (Bon.) Sing.: GERMANY, Garmisch, Schachen, sub *Pinus cembra*, 15. 8. 1991, leg. & det. A. Bresinsky. *Suillus plorans* (Roll.) Sing.: GERMANY, Garmisch, Schachen, 15. 8. 1991, leg. & det. A. Bresinsky. – *Suillus pseudobrevipes* Smith & Thiers: USA, California, Auburn, 6. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Suillus pungens* Thiers & Smith: USA, California, Pt. Reyes, 15. 3. 1991, leg. M. Fischer, det. Th. Bruns. – *Suillus sibiricus* Sing.: GERMANY, Garmisch, Schachen, 15. 8. 1991, leg. & det. A. Bresinsky. *Tylopilus felleus* (Bull. Fr.) Karst.: GERMANY, Regensburg, Scheuchenberg, 17.8.1991, leg. & det. A. Bresinsky. *Xerocomus fragilipes* (C. Martin) Pouz.: GERMANY, Lichtenfels, Kümmersreuth, 27. 10. 1989, leg. H. Engel. – *Xerocomus truncatus* Sing., Snell & Dick: No. 104, GERMANY, Regensburg, Scheuchenberg, 31. 7. 1991, leg. A. Bresinsky, det. J. Linzenkirchner. – No. 105, GERMANY, Regensburg, Pielenhofen, 10. 8. 1989, leg. & det. H. Besl.

Specimens are deposited in the herbarium of the University of Regensburg (REG). Material fixed in carnoy was used to measure the relative nuclear DNA contents. Small pieces of hymenophore were laid onto slides and firmly pressed. The slides were then dried on a bench at 60 °C for 30 min and afterwards fixed in ethanol:acetic acid (3:1) for 15 min. The samples were washed in buffer for 15 min, stained with DAPI (0.5 µg/ml dissolved in McIlvaine buffer pH 7.0) at 37 °C for 15 min and then washed for 5–7 min. The DNA contents were derived by measuring the fluorescence intensity of the samples with a

ZEISS Microscope Universal supplemented with an epifluorescence illuminator III RS, a ZEISS Microscope Photometer 03, a U 87701 set of filters (excitation filter BP 365/11, beam splitter FT 395, suppression filter LP 397), and an additional short pass filter KP 500. The fluorescence intensity of the different samples is given in relative units (= relative nuclear DNA content). The variation coefficient is equivalent to the standard deviation as a percentage divided by the mean value. Strain no. 3515 of *Suillus placidus* served as internal standard, to allow a comparison with previous studies (Wittmann-Meixner, 1989).

Results

The DNA values obtained are listed according to the origin of the material (Europe: Tab. 1; North America: Tab. 2; Malaysia: Tab. 3).

Investigation of European material (Tab. 1) confirms earlier results, as in the case of *Suillus sibiricus* (4x). *S. plorans* is also 4x. Both species are mycorrhizal fungi associated with *Pinus cembra* and their habitats are mostly located in glacially disturbed areas. According to the modified rule of Tischler (1934), this fits well with a higher ploidy level. *Suillus placidus* on the other hand was determined to belong to the 2x-level in all North American and European strains. Usually *S. placidus* is a mycorrhizal fungus associated with *Pinus strobus*, but in the Alps it also exists associated with *P. cembra*. For a long time *S. placidus* was thought to be exclusively synanthropic in Europe and to have been imported from North America together with *Pinus strobus*. Based on Favre's work (1960), *S. placidus* occurs in the Alps with native *Pinus cembra* and might therefore not only be a synanthrope in Europe. In case of an earlier adaptation to *P. cembra* and an immigration history correlated with this host, a differentiation at least into two taxa with specific characters should be expected. In addition, a higher ploidy level for the taxon connected with *Pinus cembra* could be expected, in a similar way as for *S. sibiricus* and *S. plorans*. The strain of *S. placidus* associated with *Pinus cembra* possessed a DNA value of 2x and was in no way different from those associated to the other host. *Boletus impolitus* (2x) and the recently separated species *B. depilatus* (4x) are apparently related to each other closely. According to Heinemann & Rammeloo (1992), *B. depilatus* produces mostly 2, *B. impolitus* 4 spores per basidia. It is thus possible that in *B. depilatus* the half number of spores per basidia as well as simultaneous polyploidization are responsible for the difference in DNA content. *Boletus rhodopurpureus*, collected in the Alps of Bavaria and possibly not yet identified correctly (Besl & al., 1982), is just like *B. depilatus* a

Tab. 1. Ploidy levels of Boletaceae, Gomphidiaceae, and *Chamonixia* collected in Europe. In parentheses: the strain number.

Taxon	No. of nuclei investigated	rel. DNA content (\pm standard dev.)	ploidy level
<i>Boletus appendiculatus</i>	50	141.9 \pm 15.4	2x
<i>Boletus depilatus</i>	100	277.3 \pm 11	4x
<i>Boletus edulis</i> var. <i>querceticola</i>	50	144.3 \pm 8.4	2x
<i>Boletus edulis</i> var. <i>betulicola</i>	75	150.5 \pm 10.6	2x
<i>Boletus aereus</i>	100	149.6 \pm 11.0	2x
<i>Boletus rhodopurpureus</i>	50	276.9 \pm 14.1	4x
<i>Pulveroboletus cramesinus</i>	75	147.5 \pm 7.6	2x
<i>Xerocomus truncatus</i> (104)	25	140.3 \pm 10.3	2x
(105)	75	132.0 \pm 4.8	2x
<i>Xerocomus fragilipes</i>	50	133.8 \pm 12.0	2x
<i>Suillus flavidus</i>	50	126.9 \pm 7.0	2x
<i>Suillus placidus</i>	75	125.9 \pm 7.8	2x
<i>Suillus plorans</i>	50	254.3 \pm 7.0	4x
<i>Suillus sibiricus</i>	50	250.2 \pm 7.8	4x
<i>Tylopilus felleus</i>	50	152.2 \pm 6.2	2x
<i>Leccinum griseum</i> (114)	75	111.2 \pm 10.4	2xa
(115)	50	112.9 \pm 8.8	2xa
<i>Leccinum piceinum</i>	75	153.3 \pm 5.3	2x
<i>Leccinum subcinnamomeum</i>	75	192.1 \pm 8.4	3x
<i>Chroogomphus helveticus</i> ssp. <i>tatrensis</i> (118)	50	889.7 \pm 5.6	10x
(119)	50	929.5 \pm 6.9	10x
<i>Chamonixia caespitosa</i>	100	366.8 \pm 9.6	4-6x

4x species within the genus. Other closely related species to *B. rhodopurpureus* such as *B. satanas* and *B. rhodoxanthus* are 2x. *Boletus aereus* (2x) is probably a more primitive taxon within the complex of *B. edulis*. This correlates well to its more southern habitats on the northern hemisphere, i.e. to its relictic status. Only the North American species *B. separans* belongs to a lower evolutionary level within the group of *B. edulis*. In addition to a ploidy level of 1x, it also accumulates thelephoric acid in the cortex of its stipe (Bresinsky & Besl, 1979) which has to be regarded as a more primitive character. This acid is not accumulated otherwise in Boletales. *B. edulis* var. *querceticola* and *B. edulis* var. *betulicola*, too, were determined to be 2x. Some probably more advanced taxa such as *B. edulis* var. *edulis*, *B. aestivalis*, and *B. pinophilus* have significantly lower DNA values than those belonging to the 2x group. In this study these values are called 2xa; the addition „a“ stands for a deviation by a lower value from the whole multiple of the DNA-value named „x“

Different nuclear DNA contents reflect speciation also within the complex of *Leccinum scabrum* and *L. rufum*. According to an earlier study by Wittmann-Meixner (1989), their DNA values vary between 2x and 2xa. Compared to *L. scabrum* (2x), the 2xa-species *L.*

Tab. 2. – Ploidy levels of Boletaceae and Gomphidiaceae collected in North America. In parentheses: the strain number.

Taxon	No. of nuclei investigated	rel. DNA content (\pm standard dev.)	ploidy level
<i>Suillus pungens</i>	50	69.4 \pm 4.5	1x
<i>Suillus pseudobrevipes</i>	75	133.4 \pm 5.8	2x
<i>Suillus caerulescens</i> (10)	50	142.2 \pm 8.7	2x
(4)	25	129.8 \pm 9.9	2x
<i>Phylloporus rhodoxanthus</i> ssp. <i>rhodoxanthus</i>	25	99.3 \pm 3.9	2xa
<i>Gomphidius subroseus</i>	75	181.0 \pm 7.8	2x
<i>Chroogomphus vinicolor</i>	75	731.7 \pm 9.8	8x

melaneum, *L. variicolor*, and *L. griseum* can be seen again as derived taxa. In addition, the form of the cortical layer of the pileus (being a cellular epithelium) of *L. griseum* may confirm the above statement; yet its association with various deciduous trees including *Carpinus* somehow contradicts this assumption.

L. subcinnamomeum is 3x and thus triploid. It is the first species of this genus found to have such a high ploidy level. Therefore *L. subcinnamomeum* is quite distinct from all other *Leccinum* species including *L. scabrum*, even though the former was considered to be only an infraspecific taxon of the latter. *L. piceinum* on the other hand displays the same DNA value as *L. aurantiacum* and related taxa.

If the assumption is correct that *Chamonixia caespitosa* is phylogenetically derived from ancestors similar to *Gyroporus cyanescens*, the data collected about this gastroid fungus are not surprising. The nuclear DNA contents of *C. caespitosa* (4x–6x) equals twice the value of its supposed ancestor-like species (2x–3x). The derived status of *C. caespitosa* is thus reflected by its DNA value.

The overall highest DNA values in Boletales were found in *Chroogomphus* in the family Gomphidiaceae. They were up to 10 times higher than the lowest values in Boletaceae and 5 times higher than the lowest values in *Gomphidius*. Therefore *Chroogomphus* species such as *C. rutilus* and *C. helveticus* ssp. *tatrensis* (which has not been analyzed before) may be considered to be decaploid or at least pentaploid.

For material from North America (Tab. 2), a ploidy level of 8x was determined in *Chroogomphus vinicolor*. This ploidy level had never been found in Gomphidiaceae. *G. subroseus* displayed identical DNA values as *G. glutinosus*, the latter having been analyzed in earlier studies.

The rest of the material originating from North America contains three different *Suillus*-species, one of which, *S. pungens*, belongs to

Tab. 3. – Ploidy levels of Malayan Boletaceae.

Taxon	No. of nuclei investigated	rel. DNA content (\pm standard dev.)	ploidy level
<i>Phylloporus bellus</i>	50	129.0 \pm 7.3	2x
<i>Phylloporus</i> cf. <i>rufescens</i>	75	129.4 \pm 9.3	2x
<i>Pulveroboletus icterinus</i>	25	97.2 \pm 10.0	1x
<i>Boletus craspedius</i>	75	87.7 \pm 10.7	1x
<i>Boletus graveolens</i>	75	83.8 \pm 11.7	1x
<i>Boletus sylvestris</i>	75	121.3 \pm 7.6	2xa
<i>Boletus gyrodontoides</i>	75	124.6 \pm 7.4	2xa
<i>Boletus aureomycelinus</i>	75	124.3 \pm 10.2	2xa
<i>Boletus tristiculus</i> (3)	50	141.6 \pm 10.2	2x
(10)	50	146.7 \pm 7.7	2x
<i>Boletellus emodensis</i>	25	159.6 \pm 18.0	2x
<i>Boletellus dissiliens</i>	75	279.5 \pm 8.6	4x
<i>Heimiella retispora</i>	75	224.8 \pm 10.0	4x

the 1x-level. According to previous studies, other species belonging to this ploidy level are exclusively of North-American origin. Examples are *S. pictus*, *S. acidus*, *S. americanus*, *S. cothurnatus*, and *S. glandulosus*. *S. pungens* is considered to be a close relative of *S. granulatus* (Smith & Thiers, 1964). It is probably a primitive relictic taxon, associated with *Pinus radiata* in California. In addition to other characteristics, its distinctness from *S. granulatus* and *S. placidus* is now confirmed by its low nuclear DNA value (1x). The host itself, *Pinus radiata*, needs to be understood as a relictic species. According to fossil records, its original habitat must have been the whole coastal region of California (Axelrod, 1967); nowadays it can only be found naturally in three separated small areas. An additional host seems to be *P. attenuata*, another three-needled pine. Yet this species, too, is hardly further spread than the previous one.

S. granulatus is 2x. With its hosts being *P. sylvestris*, *P. strobus*, etc. it is much more common than *S. pungens*. *S. albidipes* and *S. brevipes* are closely related to *S. granulatus* and belong also to the 2x-level. Their habitats and distribution patterns mediate between those of *S. pungens* and *S. granulatus* (Smith & Thiers, 1964; Thiers, 1975). *S. placidus*, which in a young state may be mistaken for *S. pungens* (Thiers, 1975), is in contrast to the latter a 2x species and is commonly associated with five-needled pine trees growing in eastern North America.

The data collected for *Phylloporus rhodoxanthus* ssp. *rhodoxanthus* are exactly the same as those derived from samples of other origin. Unfortunately the European taxon *P. rhodoxanthus* ssp. *europaeus* (= *P. pelletieri*) could not yet be analyzed in a comparable study. Fresh samples or material fixed in carnoy would be welcome.

Finally, the two Malayan species of *Phylloporus* studied belong to the 2x-level (Tab. 3). Thus the above mentioned American non-tropical taxon appears to be derived by loss of DNA (single chromosomes ?). The genus occurs apparently mainly in tropical regions (Corner, 1972). *Pulveroboletus icterinus* from Malaysia has the same low DNA value (1x) as the earlier analyzed species *Pulveroboletus ravenelii* from North America. Two of the six different *Boletus* species belong to the 1x-level. The remaining species as well as various European taxa may be assigned to the 2xa category. The relation of 1x 2x (including 2xa in a broad sense) is about 1 2 for all *Boletus* species. The percentage of monoploid species is therefore quite high.

As in the case of *Phylloporus* and *Strobilomyces*, which was discussed earlier in this paper, the center of distribution of *Austroboletus*, *Boletellus*, and *Heimiella* is also mainly in tropical regions. Species of these genera expand into the northern and southern hemispheres (Singer, 1986; Corner, 1972). The few previously studied species of *Austroboletus* and *Boletellus* from North America can all be assigned to the 2x-level. Strikingly, often a 4x-level occurs in Malayan species of these genera (including *Heimiella retispora* 3 out of 4 times). Although the evolutionary age of these species is hard to estimate, nevertheless the phenomenon of paleopolyploidy observed in tropical vascular plants comes into mind.

In the material from Malaysia the percentage of polyploid species as well as the percentages of species with other ploidy levels are close to the values found for the 128 previously studied species collected in localities spread over the northern and southern hemispheres (Wittmann-Meixner, 1989). In the following list percentage values of polyploidy taken from earlier studies are given in brackets behind the values obtained from material of Malaysia: 1x: 23 % (23.4 %); 2x/2xa: 54 % (53.6 %); >2x: 23 % (22.7 %).

Discussion

Because of the small number of samples, the standard distribution of the frequency with which different ploidy levels occur in Malayan species cannot be calculated with certainty. Corner (1972) mentions 140 different Malayan species of Boletaceae and Strobilomycetaceae. Thus our 13 taxa are only a minute part of the known diversity.

The Indomalayan region might even represent an evolutionary center for some genera. Mycorrhiza-forming species are frequently associated with Dipterocarpaceae in this region (Lee, 1990), but *Pinus* and various Fagaceae may also serve at least as potential hosts. In

Tab. 4. – Phylogenetic progression within the Boletales.

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- a: saprophytic → symbiotic
 - b: on wood producing brown rot → forming mycorrhiza
 - c: producing brown rot → (→ producing white rot)
 - d: conifer host → deciduous trees (→ conifers)
 - e: broad specialization, different hosts → narrow specialization
 - f: fruiting body: corticoid → pileate → gastroid
 - g: fruiting body: long-living → short-living
 - h: hymenophore: smooth → with wrinkles → with lamellae/tubes
tubes: radially elongated/compound → narrow/simple
 - j: gymnocarpous → hemiangiocarpous → angiocarpous
 - k: velum: not present → present (→ not present)
 - l: columella of angiocarp fruiting bodies: present → not present
 - m: active discharge of spores → passive release of spores
 - n: anemochorous → endozoochorous (by vertebrates)
 - o: small ellipsoid spores → big spindle-like spores
 - p: smooth spores → ornamented spores → smooth spores
 - q: hymenophoral trama: bilateral → regular
 - r: cortex of pileus with elongated hyphae → with spherical cells
 - s: set of metabolites complete → not complete
 - t: metabolites are little oxidized → highly oxidized
 - u: hyphae oligonucleate → dikaryotic
 - v: dikaryotic hyphae: with clamps → without clamps
 - w: monoploid → diploid → polyploid
-

addition, the biogeographical significance of the Indomalayan region is related to its position on the globe, acting as land bridge between the northern and southern hemispheres. It may be expected that the frequency distribution of ploidy levels of Boletales is different for tropical species than it is for taxa from refuge areas of the northern hemisphere or from glacially disturbed areas. The difference to the latter should be true in spite of the phenomenon of tropical paleopolyploidy. Examples for glacially disturbed areas are Central Europe (1x = 5.9%; 2x = 82.3%; 4–10x = 11.8%) and the South American Andes (1x = 0%; 2x = 45.5%; 4–10x = 54.5%) (Wittmann-Meixner, 1989); the North American deciduous forest is an example for a refuge area (1x = 47.7%; 2x = 50%; 4–10x = 2.3%). The few Malayan species so far studied seem to confirm a frequency distribution that is quite different from the others.

In addition to categorising floras of the world according to their percentages of monoploid and polyploid species, it is interesting to find out whether different levels of phylogenetic progression are also different in their ploidy rates. For Boletales in a broader sense, including Coniophoraceae and gastroid families, the progressions presented in Tab. 4 may be assumed. Regressive developments or returns to the original evolutionary level must, however, be considered.

The selective pressure responsible for such progression during the phylogeny of the Boletales is caused by the optimal use of an

enormous variety of ecological niches, especially provided by symbiosis, and the best possible distribution of genetic potential through spores.

Some of the trends of progression are correlated directly, e.g. the development of fruiting bodies (gymnocarpous versus angiocarpous) and the lack or presence of a velum. However, a direct correlation of special (e.g. advanced) characters with a given (e.g. high) ploidy level cannot be expected. Except for the above mentioned paleopolyploidy found in tropical regions, polyploidy is probably characteristic for recent speciation. The most likely way of interpretation will be to look at complexes of closely related species with evolutionary processes in mind. Polyploidy will also repeatedly confirm taxonomically described speciation as in *Boletus* and *Leccinum* discussed here.

Speciation connected with polyploidy may include groups with advanced characters as well as those with conservative, original ones. Yet it may be possible that groups with advanced characters show no recent speciation. They may therefore exhibit only very small polyploidy rates. Thus the correlation between polyploidy and advanced characters must not necessarily be high.

Several karyological aspects in Boletales discussed by Wittmann-Meixner (1989) should be reiterated here. In *Paxillus* species, the greater the volume of spores and the more the spore form had progressed from ellipsoid to spindle-like the higher was the DNA content (up to 6x). Species with big spindle-like spores, however, come from glacially disturbed areas of Chile, where a high polyploidy rate can be observed anyway. Belonging to the 1x-level, *P. curtisii* has an extremely simple type of fruiting body and small ellipsoid spores.

The species of one genus with a complete set of pigments are considered to be more primitive as compared to the ones without pigments. *Leccinum* species which share a complete set of pulvinic acid derivatives with other genera of Boletaceae are associated with a low ploidy level. The 1x *Suillus* species accumulate grevillins instead of biogenetically related pulvinic acids. A lack of pulvinic acids in fruiting bodies should be expected for all studied species of the 1x level. This observation should also apply to *S. cothurnatus*, *S. glandulosus*, and *S. pungens* (even though they have not yet been analyzed sufficiently from a chemical point of view), because their fruiting bodies do not stain blue when injured (due to lack of pulvinic acids), and because they are closely related to species containing only grevillins. Taxa belonging either to the 2x level or to the 4x level produce both types of pigments (grevillins and pulvinic acids), sometimes within one species (usually only one or the other). For example, *S. sibiricus* (4x) stores grevillins whereas *S. plorans* (also 4x) accumulates derivatives of pulvinic acid. Several species of the families Gomphidiaceae and Rhizopogonaceae (the latter should be

seen as derived; Bruns & al., 1989) showed extremely high DNA values, but had no grevillins. Whether grevillins should be considered a relatively primitive character in regard to pulvinic acids cannot be decided here, especially because species accumulating grevillins in their fruiting bodies secrete derivatives of pulvinic acid in mycelial cultures.

Suilloideae and the closely related Gomphidiaceae and Rhizopogonaceae are exclusively associated with conifers, which are phylogenetically older than angiosperms. Especially the more primitive species of Suilloideae, e.g. 1x species, may have kept more primitive characteristics such as the synthesis of grevillins. A 1x level species of the subfamilies of Boletaceae with clamp connections in its fruiting bodies has not yet been found. Species of these subfamilies (Gyroporoideae, Gyrodontoideae) or groups (Suilloideae p.p.) belong mostly to the 2x level; a higher ploidy level, however, was determined in *Gyroporus castaneus* (4x–6x) only. Even in this case, there is no clear correlation between primitive characteristics and a low ploidy level.

The lowest measured DNA value of each genus is always considered to be 1x. It increases more or less with evolutionary level: Coniophoraceae: 1x = 45–58; Paxillaceae: 1x = 43–49; Omphalotaceae 1x = 64–70; Suilloideae: 1x = 54–77; Xerocomoideae: 1x = 72; Boletoidae: 1x = 68–89. For some families no 1x level taxon has yet been found. From the known values of the 2x levels, the following values are to be expected for the 1x levels: Strobilomycetaceae: 1x = ? approx. 85; Gyroporoideae 1x = ? approx. 95; Gomphidiaceae 1x = ? approx. 95; Rhizopogonaceae: 1x = ? approx. 100.

More investigations on diversification, relationship, and progression in Boletales are needed. Besides increased application of cytological and molecular biological methods, it would be essential to study the still unexplored diversity of endangered and perishing tropical species, such as the flora of the Indomalayan region.

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