DESCRIPTION AND IDENTIFICATION OF SOME TRISPECIES HARD PINE HYBRIDS

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ABSTRACT

Three trispecies hybrid pines are described: (1) *Pinus* × *vidakovićii* = *Pinus sylvestris* L. × (*P. densiflora* Siebold et Zucc. × *P. nigra* J. F. Arnold); (2) *Pinus* × *kriebelii* = (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc.; (3) *Pinus* × *hagmanii* = (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco. Those hybrids were produced by controlled hybridization, the first in 1962, the second and the third in 1985.

Nineteen morphological and anatomical characteristics of needles and shoots were analyzed. Discriminant analysis was used to investigate the possibility of identification and differentiation of the trispecies hybrids among themselves and from the parental species.

Based on analyzed characteristics, the *P.* ×*vidakovićii* trispecies hybrid could be identified in 100 % of cases. It could also be differentiated from the parental species (*P. sylvestris*, *P. densiflora*, *P. nigra*) and from the F_1 hybrid *P. densiflora* × *P. nigra*. The latter was used as a pollen donor when the trispecies hybrid was produced. The *P.* × *kriebelii* hybrid was also successfully differentiated from the parental species (*P. nigra*, *P. sylvestris*, *P. densiflora*), but when compared with the F_1 hybrid *P. nigra* × *P. sylvestris*, which was used as the mother plant for the trispecies hybrid, misidentification occurred in 12 % of cases. *P.* × *hagmanii* could be identified in 100 % of cases when compared with *P. sylvestris* and *P. thunbergiana*, but in 5 % of cases the trispecies was misidentified when compared with *P. nigra* and in 10 % of cases when compared with the F_1 mother plant hybrid *P. nigra* × *P. sylvestris*.

Identification of each trispecies hybrid was 100 % accurate when *P.* × *vidakovićii* and *P.* × *hagmanii* were compared with the other two trispecies hybrids. When trispecies hybrid *P.* × *kriebelii* was compared with the other two trispecies hybrids, in 2.4 % of cases they were misidentified as *P.* × *vidakovićii* and as *P.* × *hagmanii*.

Key words: Trispecies hybrids, *Pinus* × *vidakovićii* = *Pinus sylvestris* L. × (*P. densiflora* Siebold et Zucc. × *P. nigra* J. F. Arnold), *Pinus* × *kriebelii* = (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc., *Pinus* × *hagmanii* = (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco, morphology, discriminant analysis

INTRODUCTION

Trispecies pine hybrids, described and identified in this paper, were produced by controlled hybridization at the Department of Forest Genetics and Dendrology at the Faculty of Forestry, University of Zagreb. They were produced as a part of different projects that dealt with European black pine (*Pinus nigra* J. F. Arnold) and Scots pine (*P. sylvestris* L.) hybrids production, and the problem of their incompatibility. The span of these projects included two other species; Japanese red pine (*P. densiflora* Siebold et Zucc.) and Korean black pine (*P. thunbergiana* Franco). Those four species were used to create different hybrid families by various crossing combinations, including production of F_1 hybrids, back-crossed and reciprocal hybrids, F_2 hybrids, and trispecies hybrids. Produced seedlings were planted in 15 experimental areas in the Arboretum Lisičine and Đurđevački peski between 1979 and 1991. Some of the numerous papers published in the last 40 years as a result of the mentioned work of hard pines improvement projects, are: VIDAKOVIĆ (1958, 1974, 1977, 1986), VIDAKOVIĆ & BORZAN (1973, 1991), PETRIČEVIĆ *et al.* (1977), BORZAN & PAPEŠ (1978), BORZAN *et al.* (1995), BORZAN & IDŽOJTIĆ (1996). Detailed description of conducted controlled hybridization with number of trees used in those experiments, number of pollinated female flowers, obtained cones and fertile seeds was given in annual and final reports of the projects: VIDAKOVIĆ *et al.* (1973, 1977, 1985, 1991).

Two survived plants of the trispecies hybrid *Pinus sylvestris* × (*P. densiflora* × *P. nigra*) were planted in 1962 in the nursery of the Department of Forest Genetics and Dendrology. Production of that hybrid was mentioned by VIDAKOVIĆ (1963). VIDAKOVIĆ (1974) described some of morphological traits and pointed out their similarity to Scots pine, intermediacy of bud color and size between Scots and European black pine, and that their growth was good. Hybrids (*Pinus nigra* × *P. sylvestris*) × *P. densiflora* and (*Pinus nigra* × *P. sylvestris*) × *P. thunbergiana* were produced in 1985 and planted on the experimental plot at the Arboretum Lisičine. Their morphology and progress has not yet been published.

Abbreviations for species and hybrid names in the text have been used as first two letters of the species name or of the hybrid combination (i.e. *P. nigra* = *ni*, *P. nigra* × *P. sylvestris* = *nisy*, *P. sylvestris* × (*P. densiflora* × *P. nigra*) = *sy* × *deni*, etc.).

MATERIALS AND METHODS

Analysis of the trispecies hybrid *Pinus sylvestris* × (*P. densiflora* × *P. nigra*) was performed using samples from the two trees that survived. Samples of the hybrid (*Pinus nigra* × *P. sylvestris*) × *P. densiflora* were taken from 21 different trees, while for the hybrid (*Pinus nigra* × *P. sylvestris*) × *P. thunbergiana* samples were taken from 8 trees. Species samples and F_1 hybrid samples were taken from different genotypes (trees) as follows: *ni* = 41 tree, *sy* = 29 trees, *de* = 40 trees, *th* = 9 trees, *nisy* = 32 trees, *deni* = 10 trees. Parental trees used for controlled hybridization were also included in this analysis as a part of the regarding group. Two one-year-old shoots with fully developed needles were collected from each sample tree at the end of October, in 1996.

Length of twenty-five cones of each analyzed hybrid was measured for calculating average cone length.

Nineteen different features were analyzed from the needle and shoot samples: 1. *NL* = needle length (cm), and 2. *FSL* = fascicle sheath length (cm); 3. *TL* = length, and 4. *TW* = width in the middle of tracheid from a one year old sample in μm. The shoots were macerated by boiling 1–2 minutes in 10 % HNO_3 (GERLACH 1969), tracheids isolated and fixed on slides; 5. *NVSR* = number of ventral, and 6. *NDSR* = dorsal stomatal rows; 7. *NS/cm* = number of stomata per 1 cm of one ventral stomata row. Each needle-cut was 1cm long, 0.5 cm above and below the middle of the needle. Mid-row of the needle was used if possible, unless the stomatal row was broken. In that case, the closest, non interrupted stomatal row was used; 8. *NNS/cm* = number of needle serrations per cm along one margin in the middle of the needle; 9. *NA* = needle cross-section area in mm^2 at the mid-length of the needle. Each cross-section was used to measure features described under numbers 9–14. Permanent samples of the needle cross-sections were made according to GERLACH (1969). 10. *NH* = height, and 11. *ND* = diameter of the needle cross-section; 12. *SRA* = stellar region area; 13. *SRH* = height, and 14. *SRD* = diameter of the stellar region of the cross-section; 15. *NHLmax* = maximum no. of layers of hypodermal cells. Since the number of hypodermal layers varies, only the maximum number of layers in each cross-section was recorded; 16. *NMRC* = number of medial, and 17. *NERC* = external resin canals; 18. *NSCmax* = maximum, and 19. *NSCmin* = minimum number of sheath cells surrounding a single resin canal in each cross-section.

The needles were photocopied, scanned, and different features were measured by the computer. The length and the width of the tracheids, the cross-sections and the stellar region were measured using Zeiss Axioscope connected with a video camera. The video camera was connected to a computer, which analyzed the data by using *Optimas 6.5* software. Stomatal rows were counted under a binocular magnifying lens (64×). Data were processed by discriminant analysis using a statistical package StatSoft. Inc (2001).

RESULTS

Descriptive Statistics

Table 1 gives a description of the nineteen studied morphological and anatomical traits of needles and shoots for four hard pine species and their hybrids. For each trait, *F*- and *t*-tests were made. Differences between the groups are commented in the text. For each trait four analyses were made, according to species – hybrid relationship: first analysis is the comparison of samples *sy*, *de*, *ni*, *deni*, *sy* × *deni*; second analysis of *ni*, *sy*, *de*, *nisy*, *nisy* × *de*; third analysis of *ni*, *sy*, *th*, *nisy*, *nisy* × *th*, and fourth analysis of trispecies hybrids *sy* × *deni*, *nisy* × *de*, *nisy* × *th*.

1. Needle length, *NL* (Table 1)

First analysis: needles of *sy* × *deni* trees are on average significantly shorter than needles of the three parent species, as well as needles of the hybrid *deni*.

Second analysis: needle length of the hybrid *nisy* × *de* is not significantly different from the *de*, but significantly shorter than those of *ni*, and significantly longer than those of *sy*.

Third analysis: *NL* of the trispecies hybrid *nisy* × *th*

Table 1. Statistical parameters of nineteen analysed morphological and anatomical traits for pure species (*ni, sy, de, th*), F_1 hybrids (*deni, nisy*) and trispecies hybrids (*sy× deni, nisy × de, nisy × th*). $\bar{x}$ = arithmetic mean, CV = coefficient of variability (%), *n* = sample size.

Group	Statist. Param.	*NL* cm	*FSL* cm	*TL* mm	*TW* µm	*NVSR*	*NDSR*	*NS/cm*	*NNS/cm*	*NA* mm²
ni	$\bar{x}$	12.2	1.0	1.055	24.1	8	12	101	32	1.0031
n = 82	CV %	23.8	20.0	10.8	12.5	15.9	16.7	9.2	20.8	19.8
sy	$\bar{x}$	9.6	0.8	1.030	25.1	13	15	115	37	0.9956
n = 58	CV %	15.6	13.3	10.8	14.3	21.5	18.7	8.7	20.3	22.1
de	$\bar{x}$	12.0	1.0	1.232	21.9	7	10	119	54	0.5935
n = 80	CV %	15.8	17.3	12.5	12.0	16.8	18.6	8.0	14.6	15.2
th	$\bar{x}$	12.7	1.1	1.430	26.5	7	13	92	60	1.0056
n = 36	CV %	21.2	14.6	10.7	9.8	14.7	12.8	9.2	13.0	20.0
deni	$\bar{x}$	11.5	0.9	1.087	20.3	8	13	103	40	0.8142
n = 40	CV %	18.3	16.4	13.4	12.3	15.8	14.6	8.5	13.8	16.8
nisy	$\bar{x}$	10.9	0.9	1.074	23.1	10	14	106	29	1.0489
n = 64	CV %	22.0	22.5	14.2	13.0	20.4	21.6	10.1	23.0	19.1
sy × *deni*	$\bar{x}$	7.8	0.6	0.945	18.8	11	13	116	48	0.8175
n = 36	CV %	20.5	21.3	14.6	15.3	19.0	28.0	9.4	14.9	22.6
nisy × *de*	$\bar{x}$	11.9	0.9	1.004	24.2	8	11	101	35	0.7554
n = 42	CV %	17.6	16.1	19.1	17.6	20.7	18.6	8.1	15.8	26.5
nisy × *th*	$\bar{x}$	10.5	0.8	1.152	25.5	9	14	108	30	0.9242
n = 40	CV %	20.0	16.3	22.3	14.9	19.8	17.3	8.3	22.2	14.3

Group	Statist. Param.	*NH* mm	*ND* mm	*SRA* mm²	*SRH* mm	*SRD* mm	*NHL* max	*NMRC*	*NERC*	*NSC* max	*NSC* min
ni	$\bar{x}$	0.959	1.445	0.2724	0.427	0.796	3.4	6.0		13.4	8.3
n = 82	CV %	13.3	10.3	21.2	10.7	12.7	20.6	45.0		15.9	16.7
sy	$\bar{x}$	0.818	1.647	0.3083	0.371	1.041	1.3		11.5	18.7	7.7
n = 58	CV %	12.9	13.0	24.1	11.9	14.9	35.7		20.5	25.2	26.3
de	$\bar{x}$	0.733	1.135	0.1479	0.319	0.591	1.3		6.5	11.6	7.3
n = 80	CV %	11.0	7.8	22.7	13.2	10.6	35.6		22.3	18.6	23.8
th	$\bar{x}$	1.058	1.379	0.2501	0.452	0.704	3.1	4.6		11.3	7.7
n = 36	CV %	14.0	10.0	23.5	13.3	12.7	18.0	45.6		17.3	18.7
deni	$\bar{x}$	0.897	1.307	0.2250	0.384	0.742	2.5	4.1	2.9	14.1	8.7
n = 40	CV %	10.3	8.0	17.7	11.1	9.5	20.2	53.4	51.0	12.0	13.9
nisy	$\bar{x}$	0.917	1.573	0.3057	0.417	0.931	2.3	6.0	4.5	14.1	7.7
n =64	CV %	12.7	10.8	19.3	10.0	13.0	21.6	56.5	71.4	22.1	17.9
sy× *deni*	$\bar{x}$	0.767	1.467	0.2141	0.324	0.844	1.6		9.5	11.6	5.4
n = 36	CV %	10.2	13.9	32.2	14.7	19.1	32.4		33.8	13.5	28.6
nisy × *de*	$\bar{x}$	0.811	1.346	0.2249	0.361	0.789	1.9		5.6	12.4	7.6
n = 42	CV %	14.1	12.9	36.2	15.9	18.4	21.0		38.7	18.7	19.1
nisy×*th*		0.909	1.417	0.2466	0.398	0.787	2.3	4.3	3.1	12.4	7.3
n = *40*		10.0	8.5	15.1	7.1	10.7	19.9	40.3	70.4	15.6	10.0

is not significantly different from the hybrid *nisy*, but significantly shorter than needles of *ni* and *th*, and significantly longer than *sy*.

Fourth analysis: trispecies hybrids differ significantly in this trait.

2. Fascicle sheath length, *FSL* (Table 1)

First analysis: *FSL* of the hybrid sy × *deni* is significantly shorter than those of three pure species, as well as from the hybrid *deni*.

Second analysis: the hybrid *nisy* × *de* does not differ significantly in *FSL* from *de* and from hybrid *nisy*, but it's fascicles are significantly shorter than by *ni*, and longer than by *sy*.

Third analysis: trispecies hybrid *nisy* × *th* has identical *FSL* as *sy*, but significantly longer than *ni*, *th*, and *nisy* hybrid respectively.

Fourth analysis: trispecies hybrids differ significantly in this trait.

3. One-year shoot tracheid length, *TL* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly shortest tracheids.

Second analysis: trispecies hybrid *nisy* × *de* has significantly shorter tracheids than the three pure species as well as the F_1 hybrid *nisy*.

Third analysis: trispecies hybrid *nisy* × *th* has significantly longer tracheids than *sy*, *ni*, and hybrid *nisy* respectively, but significantly shorter than *th*.

Fourth analysis: variances for hybrids *sy* × *deni* and *nisy* × *th* differ significantly, while the difference between *sy* × *deni* and *nisy* × *de* is on the border of significance. Hybrid *nisy* × *de* has significantly shorter *TL* than *nisy* × *th* hybrid.

4. One-year shoot tracheid width, *TW* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly narrowest tracheids.

Second analysis: trispecies hybrid *nisy* × *de* has significantly wider tracheids than *ni*, *de* and hybrid *nisy*, but narrower and not significantly different than *sy*.

Third analysis: *TW* value in trispecies hybrid *nisy* × *th* is significantly larger than in *ni*, and in hybrid *nisy*, significantly narrower than in *th*, but not significantly larger than *sy*.

Fourth analysis: the hybrid *sy* × *deni* has significantly smallest value, while the difference between hybrids *nisy* × *de* and *nisy* × *th* is not significant.

5. Number of ventral stomatal rows, *NVSR* (Table 1)

First analysis: *NVSR* in trispecies hybrids *sy* × *deni* is significantly larger than in *ni*, *de*, and in hybrid *deni*, but significantly smaller than in *sy*.

Second analysis: trispecies hybrid *nisy* × *de* and *ni* have the same *NVSR*, but they differ significantly in variances. Average *NVSR* in the trispecies hybrid is significantly larger than in *de*, significantly smaller than in *sy*, but not significantly smaller than in F_1 hybrid *nisy*.

Third analysis: *NVSR* in the hybrid *nisy* × *th* is significantly larger than in *ni* and *th*, but significantly smaller than in *sy* and in F_1 hybrid *nisy*.

Fourth analysis: only hybrids *nisy* × *de* and *nisy* × *th* do not differ significantly.

6. Number of dorsal stomatal rows, *NDSR* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* and F_1 hybrid *deni* differ significantly in variances. The average *NDSR* in *sy* × *deni* is significantly larger than in *ni* and *de*, but significantly smaller than in *sy*.

Second analysis: *NDSR* in the hybrid *nisy* × *de* is significantly larger than in *de* but significantly smaller than in *ni*, *sy* and in F_1 hybrid *nisy*.

Third analysis: the hybrid *nisy* × *th* has the same *NDSR* as F_1 hybrid *nisy*, significantly larger than *ni* and *th*, but not significantly smaller than *sy*.

Fourth analysis: all trispecies hybrids are significantly different in this trait.

7. Number of stomata along one row, *NS/cm* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly larger average *NS/cm* than *ni* and F_1 hybrid *deni*, but not significantly smaller than *de*, and most similar to *sy*.

Second analysis: trispecies hybrid *nisy* × *de* has the same average *NS/cm* as *ni*, significantly smaller than *sy*, *de* and hybrid *nisy*.

Third analysis: trispecies hybrid *nisy* × *th* has the average *NS/cm* significantly larger than *th* and *ni*, significantly smaller than *sy*, but most similar to the hybrid *nisy*.

Fourth analysis: all hybrids are significantly different in this trait.

8. Number of serrations along one needle margin, *NNS/cm* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has a significantly larger average *NNS/cm* than *ni*, *sy* and hybrid *deni*, but significantly smaller than *de*.

Second analysis: trispecies hybrid *nisy* × *de* has a significantly larger average *NNS/cm* than *ni* and F_1 hybrid *nisy*, but significantly smaller than *sy* and *de*.

Third analysis: average *NNS/cm* of trispecies hybrid *nisy* × *th* is not significantly different from F_1 hybrid *nisy* but significantly smaller than in *ni*, *sy* and *th*.

Fourth analysis: hybrids *sy* × *deni* and *nisy* × *th* differ significantly in variances, while hybrids *sy* × *deni* and *nisy* × *de*, as well as hybrids *nisy* × *de* and *nisy* × *th* differ significantly in the average.

9. Needle cross-section area, *NA* (Table 1)

First analysis: the hybrid *sy* × *deni* and F_1 hybrid *deni* have similar *NA*, but trispecies hybrid has significantly larger *NA* than *de*, and significantly smaller than *ni* and *sy*.

Second analysis: average *NA* in trispecies hybrid *nisy* × *de* is significantly larger than in *de* but significantly smaller than in the other groups (*ni*, *sy*, *nisy*).

Third analysis: trispecies hybrid *nisy* × *th* has the smallest *NA*. Significant are differences in variances between the trispecies hybrid and the other groups.

Fourth analysis: differences in *NA* between hybrids *sy* × *deni* and *nisy* × *de* are not significant. Hybrids *sy* × *deni* and *nisy* × *th*, as well as hybrids *nisy* × *de* and *nisy* × *th* differ significantly in variances.

10. Needle cross-section height, *NH* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly larger *NH* than *de*, but significantly smaller than the other groups.

Second analysis: trispecies hybrid *nisy* × *de* has the average *NH* most similar to *sy*, significantly larger than *de*, but significantly smaller than *ni* and hybrid *nisy*.

Third analysis: trispecies hybrid *nisy* × *th* is in *NH* not significantly different from F_1 hybrid *nisy*. *NH* in the trispecies hybrid is significantly smaller than in *ni* and *th*, but significantly larger than in *sy*.

Fourth analysis: all groups differ significantly in this trait.

11. Needle cross-section diameter, *ND* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly wider needles than *ni*, *de*, and F_1 hybrid *deni*, but significantly narrower than *sy*.

Second analysis: needles of trispecies hybrid *nisy* × *de* are significantly wider than needles of *de*, but significantly narrower than needles of the other groups (*ni*, *sy*, *nisy*).

Third analysis: only differences between trispecies hybrid and the *sy* and *nisy* groups are significant.

Fourth analysis: differences between all hybrids are significant.

12. Stellar region cross-section area, *SRA* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly larger *SRA* than *de* but significantly smaller than all other groups.

Second analysis: trispecies hybrid *nisy* × *de* has significantly larger average *SRA* than *de* but significantly smaller than all other groups.

Third analysis: trispecies hybrid *nisy* × *th* has significantly lowest average *SRA* value.

Fourth analysis: difference between hybrids *sy* × *deni* and *nisy* × *de* is not significant. Hybrids *sy* × *deni* and *nisy* × *th* as well as hybrids *nisy* × *de* and *nisy* × *th* differ significantly in variances.

13. Stellar region cross-section height, *SRH* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has not significantly larger *SRH* than *de*, but significantly smaller from the other groups.

Second analysis: only the difference in *SRH* between trispecies hybrid *nisy* × *de* and *sy* is not significant.

Third analysis: trispecies hybrid *nisy* × *th* has significantly larger average *SRH* than *sy*, but significantly smaller than the other groups.

Fourth analysis: all investigated trispecies hybrids differ in *SRH* significantly.

14. Stellar region cross-section diameter, *SRD* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly smaller average *SRD* than *sy*, but significantly larger than the other groups.

Second analysis: trispecies hybrid *nisy* × *de* has significantly larger average *SRD* than *de*, but significantly smaller than the other groups.

Third analysis: average *SRD* of hybrid *nisy* × *th* is not significantly different from *ni*, nor from *de*, but significantly smaller than *sy* and hybrid *nisy*.

Fourth analysis: only difference in *SRD* between *sy* × *deni* and *nisy* × *de* is not significant. Other hybrids differ significantly in variances.

15. The largest number of hypodermal cell layers in needle cross-section, *NHLmax* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* has significantly larger average *NHLmax* than *sy* and *de*, but significantly smaller than *ni* and hybrid *deni*.

Second analysis: trispecies hybrid *nisy* × *de* has significantly larger average *NHLmax* than *sy* and *de*, but significantly smaller than *ni* and hybrid *nisy*.

Third analysis: average *NHLmax* in trispecies hybrid *nisy* × *th* and in hybrid *nisy* are equal, significantly larger than in *sy*, but significantly smaller than in *ni* and *th*.

Fourth analysis: hybrids differ significantly in the *NHLmax* value.

16. Number of medial resin canals in needle cross-section, *NMRC*

The number of medial resin canals in *sy*, *de*, and in trispecies hybrids *sy* × *deni* and *nisy* × *de* is not distributed normally, but most likely according to Poisson's distribution. This is due to the fact that resin canals are external in these groups, only a few being medial. The mode and the median have been calculated for these groups as the central tendency value, while interquartiles have been calculated as the variability value. For the other groups, Table 1 gives size of the samples, arithmetical means and coefficients of variability (CV %).

First analysis: there is a significant difference between *ni* and hybrid *deni*. Trispecies hybrid *sy* × *deni*, and two species (*sy*, *de*) have external resin canals. The mode and the median for these groups are zero. The interquartile is one for *sy* and for the *sy* × *deni* hybrid, zero for *de*, implying that the variability of this trait is higher in *sy* and the trispecies hybrid. The largest number of medial resin canals on one cross-section is four for *sy*, and three for *de* as well as for *sy* × *deni*.

Second analysis: *ni* and the hybrid *nisy* differ significantly in variances. The mode and the median for *sy*, *de* and the hybrid *nisy* × *de* are zero. The interquartile for *sy* is one, for *de* zero, and for *nisy* × *de* two. The largest number of medial resin canals is four for *sy*, three for *de*, and five for the trispecies hybrid.

Third analysis: *sy* has external resin canals. Hybrid *nisy* × *th* differs significantly in *NMRC* from *ni*, as well as from the *nisy* hybrid, but the difference between *nisy* × *th* and *th* is not significant.

Fourth analysis: trispecies hybrids *sy* × *deni* and *nisy* × *de* have external resin canals. Hybrid *nisy* × *th* has on average 4.3 medial resin canals.

17. Number of external resin canals in the needle cross-section, *NERC*

The number of external resin canals in *ni* and *th* are most likely distributed according to Poisson's distribution. These two species have medial resin canals, only a few are external. The mode and median have been calculated as the central tendency value, and interquartiles as the variability value. For the other groups, Table 1 gives size of the samples, arithmetical means and coefficients of variability (CV %).

First analysis: the largest *NERC* is in *sy*. The trispecies hybrid *sy* × *deni* differs significantly from *sy*, *de* and *deni* by variances. Medial resin canals are in *ni*.

Second analysis: trispecies hybrid *nisy* × *de* has significantly different *NERC* from other groups.

Third analysis: medial resin canals are in *ni* and *th*. *NERC* average values are in: *sy* 11.5, hybrid *nisy* 4.5, and in *nisy* × *th* 3.1. Hybrids have high CV %. Trispecies hybrid *nisy* × *th* differs significantly from hybrid *nisy* in variance, and from *sy* in the arithmetical mean.

Fourth analysis: hybrid *sy* × *deni* has the largest *NERC*, while hybrid *nisy* × *th* has the smallest. Differences between the trispecies hybrids are significant.

18. The largest number of sheath cells surrounding one resin canal, in the needle cross-section, *NSCmax* (Table 1)

First analysis: trispecies hybrid *sy* × *deni* and *de* have the same average *NSCmax* value, but smaller than the other groups. Hybrid *sy* × *deni* differs significantly from the pure species in variance, and from hybrid *deni* in the arithmetical mean.

Second analysis: the average *NSCmax* in trispecies hybrid *nisy* × *de* is not significantly larger than in *de*, but significantly smaller than in the other groups.

Third analysis: the average *NSCmax* in hybrid *nisy* × *th* is not significantly larger than in *th*, but significantly smaller than in *ni*, *sy* and *nisy*.

Fourth analysis: there is a significant difference in *NSCmax* only between hybrids *sy* × *deni* and *nisy* × *de*.

19. The smallest number of sheath cells surrounding one resin canal, in the needle cross-section, *NSCmin* (Table 1)

First analysis: hybrid *sy* × *deni* has significantly smallest average *NSCmin* value.

Second analysis: trispecies hybrid *nisy* × *de* has not significantly larger *NSCmin* than *de*, significantly smaller than *ni* and *sy*, but significantly smaller than *nisy*.

Third analysis: hybrid *nisy* × *th* has significantly smallest average *NSCmin*.

Fourth analysis: trispecies hybrids are significantly different in *NSCmin* from each other.

Discriminant Analysis

Four separate analyses were done. Morphological and anatomical traits were used as independent variables. First analysis was done for: *sy*, *de*, *ni*, *deni* and *sy* × *deni*; second analysis for: *ni*, *sy*, *de*, *nisy* and *nisy* × *de*; third analysis for: *ni*, *sy*, *th*, *nisy* and *nisy* × *th*; fourth analysis for: *sy* × *deni*, *nisy* × *de* and *nisy* × *th*. Tolerance was 0.01.

First Analysis

The possibility of differentiating between trispecies hybrid *P.* × *vidakovićii* (*sy* × *deni*) and the pure species (*sy*, *de*, *ni*) as well as hybrid *deni* was analysed. Nineteen morphological and anatomical traits were included in the analysis.

According to the summary statistics, it can be stated that discrimination between the groups is significant. The following values were obtained: Wilk's $\lambda = 0.0027$; $F = 52.7$ (d. f. 72, 1079); $p < 0.01$. Eighteen variables were included into the model, namely all variables except *ND*, which was not significant.

Based on the eighteen analyzed traits the trispecies hybrid samples *sy* × *deni* were correct classified in 100 % (Table 2, Figure 1). This means that, based on the eighteen morphological and anatomical needle and shoot traits, the trispecies hybrid sample can be fully discriminated from the pure species (*sy*, *de* and *ni*) as well as from hybrid *deni*. Five traits by which the trispecies hybrid is best discriminated from the other groups are shown in Table 3.

Second Analysis

The possibility of differentiating between trispecies hybrid *P.* × *kriebelii* (*nisy* × *de*) and *ni*, *sy*, *de*, as well as F_1 hybrid *nisy* was analyzed. Nineteen morphological and anatomical traits were included into the analysis as variables. Discrimination between the groups was significant (Wilk's $\lambda = 0.0027$; $F = 52.7$ (d. f. 72, 1079); $p < 0.01$).

On the basis of the nineteen morphological and anatomical needle and shoot traits, samples of trispecies hybrid *nisy* × *de* (Table 4, Figure 2) can be fully discriminated from the pure species (*ni*, *sy* and *de*). Discrimination between *nisy* × *de* and F_1 hybrid *nisy* is not significant. Samples of the trispecies hybrid can be misclassified as samples of hybrid *nisy* in 12 % cases. Five traits by which the trispecies hybrid is best discriminated from the other groups are shown in Table 5.

Third Analysis

The possibility of differentiating between trispecies hybrid *P.* × *hagmanii* (*nisy* × *th*) and the pure species (*ni*, *sy* and *th*) as well as F_1 hybrid *nisy* was analysed. Nineteen morphological and anatomical traits were included into the analysis. Discrimination between the groups was significant, and Wilk's λ was 0.0081; F =

Table 2. Classification matrix for *sy* × *deni*, *sy*, *de*, *ni* and *deni*. According to classification functions, all measured data were classified into groups (by rows in the table) to which they are most likely to belong.

Group	Classified as				
	sy	*de*	*ni*	*deni*	*sy* × *deni*
			%		
sy	98.3	0	0	0	1.7
de	0	100	0	0	0
ni	0	0	100	0	0
deni	0	0	7.5	92.5	0
sy × *deni*	0	0	0	0	100

Table 3. Five traits that trispecies hybrid *sy* × *deni* most easily differentiate from pure species (*sy*, *de*, *ni*) and from F_1 hybrid *deni*.

Groups	1st trait	2nd trait	3rd trait	4th trait	5th trait
sydeni – *sy*	*SRA*	*NA*	*SRH*	*TW*	*NSCmax*
sydeni – *de*	*SRD*	*SRA*	*NA*	*ND*	*SRH*
sydeni – *ni*	*NERC*	*NA*	*ND*	*SRH*	*NSCmin*
sydeni – *deni*	*SRA*	*SRD*	*ND*	*NERC*	*SRH*

Table 4. Classification matrix for *nisy* × *de*, *ni*, *sy*, *de* and *nisy*. According to classification functions, all measured data were classified into groups (by rows in the table) to which they are most likely to belong.

Group	Classified as				
	ni	*sy*	*de*	*nisy*	*nisy* × *de*
	%				
ni	98.3	0	0	1.2	0
sy	0	98.3	0	0	1.7
de	0	0	100	0	0
nisy	0	0	0	89.1	6.2
nisy × *de*	0	0	0	12.0	88.0

Table 5. Five traits that trispecies hybrid *nisy* × *de* most easily differentiate from pure species (*ni*, *sy*, *de*) and from F_1 hybrid *nisy*.

Groups	1st trait	2nd trait	3rd trait	4th trait	5th trait
nisyde – ni	*NA*	*NERC*	*SRD*	*NHLmax*	*NL*
nisyde – sy	*SRA*	*SRH*	*NA*	*NERC*	*NDSR*
nisyde – de	*SRA*	*SRD*	*SRH*	*NNS/cm*	*TL*
nisyde – nisy	*NMRC*	*NNS/cm*	*NDSR*	*NS/cm*	*NERC*

Table 6. Classification matrix for *nisy* × *th*, *ni*, *sy*, *th* and *nisy*. According to classification functions, all measured data were classified into groups (by rows in the table) to which they are most likely to belong.

Group	Classified as				
	ni	*sy*	*th*	*nisy*	*nisy* × *th*
	%				
ni	96.3	0	0	0	3.7
sy	0	98.3	0	1.7	0
th	0	0	100	0	0
nisy	4.7	0	0	92.2	3.1
nisy × *th*	5	0	0	10	85.0

Table 7. Five traits that trispecies hybrid *nisy* × *th* most easily differentiate from pure species (*ni*, *sy*, *th*) and from F_1 hybrid *nisy*.

Groups	1st trait	2nd trait	3rd trait	4th trait	5th trait
nisyth – th	*NERC*	*NHLmax*	*NH*	*NDSR*	*NL*
nisyth – sy	*SRA*	*SRH*	*NA*	*SRD*	*NH*
nisyth – th	*NNS/cm*	*ND*	*SRD*	*SRA*	*NS/cm*
nisyth – nisy	*NA*	*SRA*	*ND*	*NMRC*	*NERC*

36.1 (d. f. 68, 1018); $p < 0.01$. Seventeen variables were included into the model, viz. all variables except *ND* and *SRH*, which were not significant.

According to the seventeen morphological and anatomical needle and shoot traits analysed, samples of trispecies hybrid *nisy* × *th* can be fully discriminated

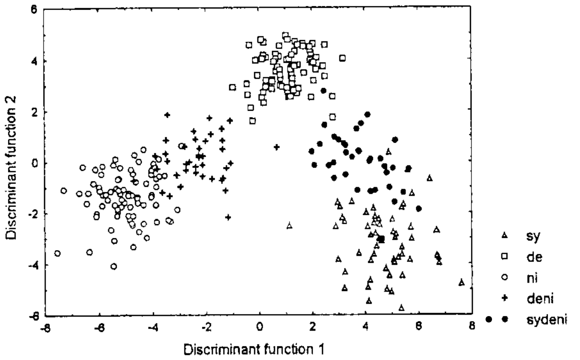

Figure 1. Scatterplot of the canonical scores for *sy* × *deni*, *sy*, *de*, *ni* and *deni*. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

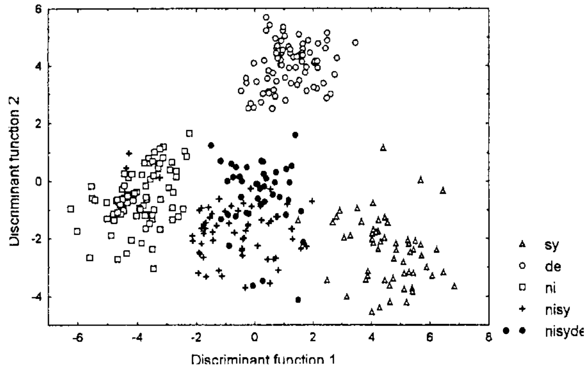

Figure 2. Scatterplot of the canonical scores for *nisy* × *de*, *ni*, *sy*, *de* and *nisy*. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

from *sy* and *th* (Table 6, Figure 3). Misclassification of the trispecies hybrid samples as samples of *ni* is possible in 5 % of cases, while 10 % of trispecies hybrid samples could be misclassified as *nisy* hybrid samples. Five traits by which the trispecies hybrid is best discriminated from the other groups are shown in Table 7.

Fourth Analysis

The possibility of differentiating between trispecies hybrids *P.* × *vidakovićii* (*sy* × *deni*), *Pinus* ×*kriebelii* (*nisy* × *de*) and *P.* × *hagmanii* (*nisy* × *th*) was analysed. Nineteen morphological and anatomical traits were included as variables. Discrimination between the hybrids is significant (Wilk's $\lambda = 0.0034$; $F = 25.7$ (d. f. 34, 198); $p < 0.01$). Seventeen variables were included into the model (variables *TW* and *NH* were not significant).

Based on the analyses of seventeen morphological and anatomical needle and shoot traits, the classifica tion accuracy of samples of trispecies hybrids *sy* × *deni* and *nisy* × *th* is 100 % (Table 8, Figure 4). Misclassification of samples of trispecies hybrid *nisy* × *de* as samples of hybrid *sy* × *deni* is possible in 2.4 % of cases and in the same percent as hybrid *nisy* × *th*. Five traits by which trispecies hybrids are best discriminated are shown in Table 9.

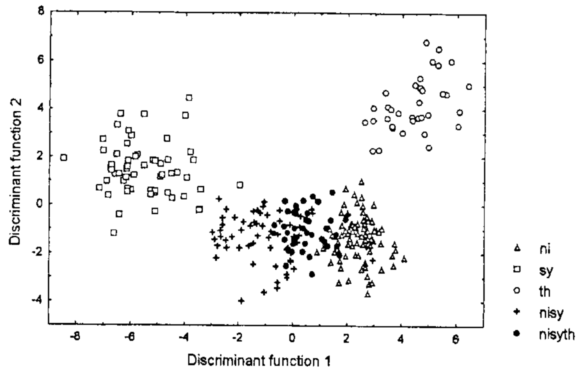

Figure 3. Scatterplot of the canonical scores for *nisy* × *th*, *ni*, *sy*, *th* and *nisy*. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

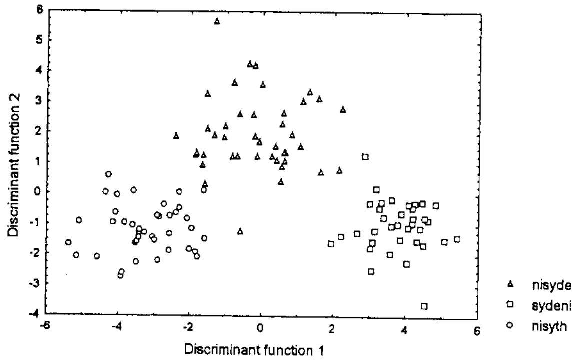

Figure 4. Scatterplot of the canonical scores for *sy* × *deni*, *nisy* × *de* and *nisy* × *th*. Particular values for the first discriminant function are plotted on axis x, and for the second discriminant function on axis y.

The Description of Trispecies Hybrids

The tree dimensions, the length of cones, the average morphological characteristics of the needles and the shoot tracheids of the tree trispecies hybrids, can be described as follows:

Pinus × *vidakovićii* Borzan & Idžojtić, hibr. nov. (= *Pinus sylvestris* L. × (*P. densiflora* Siebold et Zucc. × *P. nigra* J. F. Arnold)

The mean diameter of the two 38 year old trees at breast height (DBH) is 34 cm. Their height is 24 m.

The needles are on average 7.8 cm long, with a 0.6

Table 8. Classification matrix for *sy × deni*, *nisy × de* and *nisy × th*. According to classification functions, all measured data were classified into groups (by rows in the table) to which they are most likely to belong.

Groups	Classified as		
	sy × deni	*nisy × de*	*nisy × th*
		%	
sy × deni	100	0	0
nisy × de	2.4	95.2	2.4
nisy × th	0	0	100

Table 9. Five traits that trispecies hybrids *sy × deni*, *nisy × de* and *nisy × th* most easily differentiate from each other.

Groups	1st trait	2nd trait	3rd trait	4th trait	5th trait
sydeni -- nisyde	*NNS/cm*	*NVSR*	*NS/cm*	*NL*	*NHLmax*
sydeni – nisyth	*SRA*	*SRD*	*NERC*	*NNS/cm*	*NSCmin*
nisyde – nisyth	*SRD*	*NA*	*SRH*	*NNS/cm*	*NDSR*

cm fascicle sheath length. At the midlength of the needle there are 11 ventral and 13 dorsal stomatal rows.

The number of ventral stomata per 1 cm of a row is 116. There are 48 serrations per 1 cm of the needle. The needle cross-section area is 0.8176 mm^2, its height is 0.767 mm, and its width is 1.467 mm. The cross-section of the stelar region area in the middle of the needle length is 0.2141 mm^2, with a height of 0.324 mm and a width of 0.844 mm. The maximum number of hypodermal cell layers is on average 1.6. There are on average 0.4 medial, and 9.5 external resin canals. Each resin canal is surrounded by a layer of epithelial cells, surrounded by one or more layers of sheath cells. The sheath cells from the cross-section were counted around those canals which were surrounded by the maximum and the minimum number of cells. On average, the maximum number of the cells is 11.6, and the minimum is 5.4.

The tracheids of one year old shoots are 0.945 mm long and 18.9 μm wide.

The ripe cones are 4.0–6.5 cm long (5.3 in average).

***Pinus* × *vidakovići* Borzan & Idžojtić, hibr. nov. (= *Pinus sylvestris* L. × (*P. densiflora* Siebold et Zucc. × *P. nigra* J. F. Arnold)**

Diametrum duarum arborum, aetate 38 annorum, circa 34 cm, altitudo circa 24 m.

Longitudo media aciculae 7.8 cm, vaginae 0.6 cm. Aciculae in partem mediam ad summum 24 striis stomatalibus provisae, quarum in parte interna 11, in parte externa 13. In segmento partis mediae aciculae, longitudinis 1 cm, stria mediana stomatalis partis internae stomatibus 116 instructa. Segmentum ejusdem partis aciculae, iuxta unam marginem dentibus 48 provisa. Superficies sectionis transversalis in medio aciculae 0.8176 mm^2, altitudo 0.767 mm, latitudo 1.467 mm. Medium aciculae in sectione transversale, cylindro centrale, cujus superficies 0.2141 mm^2, altitudo 0.324 mm et latitudo 0.844 mm, provisum. Numerus maximus stratorum hypodermalium in sectione transversale plus minusve 1.6. Canales resiniferi plus minusve 9.9, quorum mediani 0.4, axiales 9.5. Canales resiniferi strato cellularum epithelialium circumdati, externe uno vel pluribus stratis sclerenchymaticis cincti. Numerus maximus cellularum sclerenchymatarum in sectione transversale canalem resiniferum circa 11.6, minimus circa 5.4.

Longitudo tracheidarum sobolum annualium circa 0.945 mm, latitudo 18.9 μm.

Strobi 4.0–6.5 cm longi (mediocriter 5.3 cm).

***Pinus* × *kriebelii* Borzan & Idžojtić, hibr. nov. (= (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc.)**

The mean DBH of 11 year old trees is 6.5 cm. Their mean height is 3.5 m.

The needles are on average 11.9 cm long, with a 0.8 cm fascicle sheath length. At the midlength of the needle there are 8 ventral, and 11 dorsal stomata rows. The number of ventral stomata per 1 cm of a row is 101. There are 35 serrations per 1 cm of the needle. The needle cross-section area is 0.7554 mm^2, its height is

0.811 mm, and its width is 1.346 mm. The cross-section of the stelar region area in the middle of the needle length is 0.2249 mm^2, with a height of 0.361 mm and a width of 0.789 mm. The maximum number of hypodermal cell layers is 1.9 on average. There are on average 1.0 medial, and 5.6 external resin canals. The maximum number of the sheath cells surrounding the resin canals is 12.4 and the minimum is 7.6.

The mean tracheid length is 1.004 mm, and their width is 24.3 µm.

The ripe cones are 4.6–7.4 cm long (5.8 in average).

***Pinus* × *kriebelii* Borzan & Idžojtić, hibr. nov. (= (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc.)**

Diametrum arborum, aetate 11 annorum, circa 6.5 cm, altitudo circa 3.5 m.

Longitudo media aciculae 11.9 cm, vaginae 0.8 cm. Aciculae in parte media ad summum 19 striis stomatalibus notatae, quarum in parte interna 8, in parte externa 11. Stria mediana stomatalis partis internae aciculae, in segmento partis mediae aciculae, longitudinis 1 cm, stomatibus 101 instructa. Segmentum ejusdem partis aciculae, iuxta unam marginem dentibus 35 provisum. Superficies mediocris sectionis transversalis in medio aciculae 0.7554 mm^2, altitudo 0.811 mm, latitudo 1.346 mm. Superficies cylindri centralis in sectione transversale 0.2249 mm^2, altitudo 0.361 mm, latitudo 0.789 mm. Numerus maximus stratorum cellularum hypodermalium in sectione transversale plus minusve 1.9. Canales resiniferi axiales circa 5.6, mediani 1.0. Numerus maximus cellularum sclerenchymatarum circa canalem resiniferum 12.4, numerus minimus 7.6.

Longitudo media tracheidarum sobolum annualium circa 1.004 mm, latitudo circa 24.3 µm.

Strobi 4.6–7.4 cm longi (mediocriter 5.8 cm).

***Pinus* × *hagmanii* Borzan & Idžojtić, hibr. nov. (= (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco)**

The mean DBH of 11 year old trees is 8.1 cm. Their mean height is 4.1 m.

The needles are on average 10.5 cm long, with a 0.8 cm fascicle sheath length. At the midlength of the needle there are 9 ventral, and 14 dorsal stomatal rows. The number of ventral stomata per 1 cm of a row is 108. There are 30 serrations per 1 cm of the needle. The needle cross-section area is 0.9242 mm^2, its height is 0.910 mm, and its width is 1.417 mm. The cross-section of the stelar region area in the middle of the needle length is 0.2466 mm^2, with a height of 0.398 mm and a width of 0.787 mm. The maximum number of hypodermal cell layers is on average 2.3. There are on average 4.3 medial, and 3.1 external resin canals. The maximum number of the sheath cells surrounding the resin canals is 12.4 and the minimum is 7.4.

The mean tracheid length is 1.152 mm, and their width is 25.5 µm.

The ripe cones are 4.1–6.5 cm long (5.1 in average).

***Pinus* × *hagmanii* Borzan & Idžojtić, hibr. nov. (= (*Pinus nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco)**

Diametrum arborum, aetate 11 annorum, circa 8.1 cm, altitudo circa 4.1 m.

Longitudo media aciculae 10.5 cm, vaginae 0.8 cm. Aciculae in parte media ad summum 23 striis stomatalibus notatae, quarum in parte interna 9, in parte externa 14. Stria media stomatalis partis internae aciculae, in segmento partis mediae aciculae, longitudinis 1 cm, stomatibus 108 instructa. Segmentum ejusdem partis aciculae, iuxta unam marginem dentibus 30 provisa. Superficies mediocris sectionis transversalis aciculae 0.9242 mm^2, altitudo 0.910 mm, latitudo 1.417 mm. Medium aciculae in sectione transversale, cylindro centrale, cujus superficies 0.2466 mm^2, altitudo 0.398 mm et latitudo 0.787 mm, provisum. Numerus maximus stratorum cellularum hypodermalium in sectione transversale plus minusve 2.3. Canales resiniferi mediane dispositi plus minusve 4.3, axialiter dispositi 3.1. Numerus maximus cellularum sclerenchymatarum circa canalem resiniferum 12.4, minimus 7.4.

Longitudo media tracheidarum sobolum annualium circa 1.152 mm, latitudo 25.5 µm.

Strobi 4.1–6.5 cm longi (mediocriter 5.1 cm).

DISCUSSION AND CONCLUSIONS

Considering the ability to accurately identify and distinguish the three analyzed trispecies hybrids and the statistically significant ability to differentiate them from related species and hybrid combinations, it is proper to give them nothospecific names as opposed to naming them by using the hybrid formula.

The hybrids are named after three eminent and meritorious researchers who worked on pine improvement: Mirko Vidaković, Howard B. Kriebel and Max. Hagman. The trispecies hybrid *Pinus* × *vidakovićii* has a hybrid formula: *Pinus sylvestris* L. × (*P. densiflora* Siebold et Zucc. × *P. nigra* J. F. Arnold). The trispecies hybrid *Pinus* × *kriebelii* has a hybrid formula: (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. densiflora* Siebold et Zucc. The trispecies hybrid *Pinus* × *hagmanii* has a trispecies formula: (*P. nigra* J. F. Arnold × *P. sylvestris* L.) × *P. thunbergiana* Franco. In the formulas, female parent species are mentioned first.

Results of the analysis and comparison of morphological traits of the parental species: *P. sylvestris*, *P. nigra*, *P. densiflora* and *P. thunbergiana*, (of the trespecies hybrids analysed in this paper) by IDŽOJTIĆ (1998) have shown that pure species can be completely discriminated too.

The need for increase the number of analysed morphological traits in identifying different taxa, esspecialy hybrids, and to discriminate them from parental or other species or hybrids has been shown in paper by BORZAN & IDŽOJTIĆ (1996). They have increased the number of analysed morphological traits from three to five enabling more accurate discrimination of *Pinus nigra*, *P. sylvestris* and *P. densiflora* species from their F_1 and F_2 hybrids. The accuracy of determination was increased for F_1 and F_2 hybrids from 53 to 66 percent and from 88 to 98 percent, respectively. That was the reason for searching the appropriate number of morphological traits for accurate discrimination of hybrids.

Tables 3, 5, 7 and 9 give the five traits by which the analysed groups are best discriminated. These are the variables that contribute most to discrimination. However, use of these traits alone would make the correctness of discrimination lower than that presented in the results of this research, where nineteen different traits were involved in the analysis.

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