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MORPHOMETRIC ANALYSIS ON CHROMOSOMES OF TROPICAL *Pinus* SPECIES

ANÁLISE MORFOMÉTRICA EM CROMOSSOMOS DE ESPÉCIES TROPICAIS DE *Pinus*

Lília Rosário Ribeiro[1] Lisete Chamma Davide[2] Cássia Ângela Pedrozo[3]
Sandro Barbosa[4] Giovana Augusta Torres[5]

RESUMO

Foram realizados estudos citogenéticos em *Pinus oocarpa* Schiede ex Schltdl., *Pinus patula* Schltdl. & Cham. e nas procedências Jócon Yoro, Las Camelias e San Rafael del Norte do *Pinus tecunumanii* Eguiluz & J. P. Perry, visando subsidiar a definição da categoria taxonômica do *Pinus tecunumanii*. A caracterização dos cromossomos mitóticos, utilizando coloração com Giemsa e com o fluorocromo 4', 6-diamidino-2-fenilindoldiidroclorídrico (DAPI), confirmou o padrão cariotípico descrito para a maioria das espécies do gênero, isto é, onze pares de cromossomos metacêntricos, muito semelhantes quanto ao comprimento e morfologia, e um par submetacêntrico, para todos os taxa estudados. A coloração com o fluorocromo forneceu definição clara das constrições secundárias, permitindo a diferenciação das espécies e procedências pelo número e posição das mesmas. As procedências de *Pinus tecunumanii* também se diferenciaram dos outros dois materiais em relação ao comprimento total do complemento haplóide. Os resultados obtidos suportam o "status" específico para o *Pinus tecunumanii*, bem como fornecem evidências de que além das mutações de ponto, alterações estruturais contribuíram para a diferenciação intra e interespecífica no gênero *Pinus*.

Palavras-chave: cariótipo; constrição secundária; *Pinus*; citotaxonomia.

ABSTRACT

Karyotypic analysis of *Pinus oocarpa* Schiede ex Schltdl., *Pinus patula* Schltdl. & Cham. and of provenances Jócon Yoro, Las Camelias and San Rafael del Norte of *Pinus tecunumanii* Eguiluz & J. P. Perry were accomplished to provide information for definition of P*inus tecunumanii* taxonomic status. Characterization of mitotic chromosomes stained with Giemsa and with 4', 6-diamidino-2-phenylindoldihydrochlorid (DAPI) fluorochrome confirmed the karyotypic pattern reported for most of the *Pinus* species, which present eleven pairs of metacentric chromosomes, very similar in regard to length and morphology, and a submetacentric pair for all the taxa studied. Sharp definition of secondary constrictions revealed by DAPI staining allowed distinction of species and provenances by the number and position of this cytological marker. *Pinus tecunumanii* was also different from the other two species in regard to total length of haploid set. The results support the species status for *Pinus tecunumanii* as well as present evidence that in addition to point mutations, structural alterations contributed toward intra and interspecies differentiation into the genus *Pinus*.

Keywords: karyotype; secondary constriction; *Pinus*; cytotaxonomy.

INTRODUCTION

Some species of the genus *Pinus* outstand among the tropical conifers introduced in Brazil due to their productivity and adaptation to a wide range of soils and environmental conditions. The species *Pinus oocarpa, Pinus patula* and *Pinus tecunumanii* have been widely used at Southeastern and Southern regions of the country for reforestation, resin and wood extraction and paper production (SUASSUNA, 2001).

Considering the three taxa above, only *Pinus tecunumanii* still does not have a well defined species

1. Bióloga, Mestre, Professora Adjunta do Centro Universitário de Formiga, Av. Doutor Arnaldo de Senna, 328, Bairro Água Vermelha, CEP 35570-000, Formiga (MG). lilia@uniformg.edu.br
2. Bióloga, Drª., Professora Titular do Departamento de Biologia, Universidade Federal de Lavras, Caixa Postal 3037, CEP 37200-000, Lavras (MG). lcdavide@ufla.br
3. Engenheira Agrônoma, MSc., Doutoranda no Programa de Genética e Melhoramento, Universidade Federal de Viçosa, CEP 36570-000, Viçosa (MG). cassiapedrozo@yahoo.com.br
4. Biólogo, Dr., Professor do Departamento de Ciências Biológicas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 714, CEP 37130-000, Alfenas (MG). barbosa@superig.com.br
5. Engenheira Agrônoma, Drª., Professora Adjunta do Departamento de Biologia, Universidade Federal de Lavras, Caixa Postal 3037, CEP 37200-000, Lavras (MG). gatorres@ufla.br

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status, although it is listed as a species of the subsection Oocarpae, along with the other two (FRANKIS *et al.*, 1999). Some of the previous works consider that taxon as a species while others consider it as a subspecies of *Pinus patula* (STYLES, 1985; LEÃO and DAVIDE, 1993; FURMAN *et al.*, 1997).

Several authors adopted cytogenetical approach to answer questions on *Pinus* taxonomy (SAX and SAX, 1933; SAYLOR, 1961 and 1964, HIZUME *et al.*, 1983 and 1989; DAVIDE and ARAÚJO, 1993; SILVA-MANN *et al.*, 2002; HIZUME, *et al.*, 2002). They showed that the karyotypes are quite uniform within the *Pinus* genus, presenting twelve chromosomes very similar in size and morphology. Only chromosomes I and XII and, in some species, chromosome XI, may be distinguished using measurement and statistical analysis. In spite of that symmetry, when details of the complements were taken into account, the distinction of the species was performed successfully in species different from the ones evaluated in this work (SAYLOR, 1961 and 1964). Secondary constriction is one of the markers that may aid in identifying single chromosomes in the genus *Pinus* (SAYLOR, 1961; PEDERICK, 1967; DAVIDE and ARAÚJO, 1993). Nevertheless, some works report that secondary constrictions are not reliable enough to be used to distinguish chromosomes of similar sizes (NATARAJAN *et al.*, 1961; DAVIDE and ARAÚJO, 1993). Consistent identification of this cytological marker can provide important further information for the distinction of *Pinus* chromosomes.

The aim of this work was to search for chromosomes features that can be useful to elucidate the taxonomic status of *Pinus tecunumanii* Eguiluz & J. P. Perry when compared to *Pinus oocarpa* ex Schltdl. and *Pinus patula* Schltdl. & Cham.

MATERIAL AND METHODS

The species analyzed, along with their origin, were: *Pinus oocarpa*, from Central America and found in altitudes from 700 to 2000m; *Pinus patula*, from South Africa and found in altitudes ranging from 1500 to 3000m; three provenances of *Pinus tecunumanii*, Jócon Yoro, from Honduras and found in altitudes ranging from 775 to 1000m; Las Camelias, from Nicaragua and found in altitudes from 950 to 1060m and San Rafael del Norte from Nicaragua and found in altitudes from 1080 to 1330m. Seeds from all of them were obtained from Brazilian plantations.

Cytogenetic analysis was carried out on root tip meristematic tissue. The roots were pre-treated with 0.05% colchicine, at 4^{o}C, for 24 hours, and fixed in Carnoy solution. The roots were submitted to enzymatic treatment with cellulase (102UmL^{-1}) and pectinase (24UmL^{-1}) solution. Meristems were isolated and squashed in 45% acetic acid. The coverglasses were removed using liquid nitrogen. Slides were air-dried and stained with Giemsa 2% or with 4', 6-diamidino-2-phenylindoldihydrochlorid (DAPI) 1µgµL^{-1} (HIZUME *et al.*, 1989).

For each taxon, 10 Giemsa–stained metaphase images were selected for digitalization. The measures were performed by using Jandel Sigma Scan r Pro v.2.0 software. The following data were obtained: large arm's length (LA); short arm's length (SA); total length of chromosome i: TLi = LA+ SA; arm ratio: SA/LA (SAYLOR, 1961); total length of haploid set: TLHS = ΣTLi; relative length of chromosome i: RLi = TLi/TLHS x 100. Secondary constrictions were measured from the telomere.

Chromosome pairing was based on short arm length, since it is supposed to be less affected by squashing technique, while morphologic classification was based on arm ratio. Species karyotypes were compared based on position of long arm length in the decreasing normal sequence (SAYLOR, 1961, 1964, 1972).

Slides stained with DAPI fluorochrome were observed under epifluorescence microscope (Olympus BX60) using a 330-385nm filter. Selected images were digitalized by a CCD camera (Optronics) attached to the microscope.

Variance analysis was performed to detect variation among the genotypes for relative length (RL) and for total length of haploid set (TLHS). Grouping of genotypes, regarding to their means for RL and TLHS, was verified using Scott-Knott's test (SCOTT and KNOTT, 1974). All the analyses were performed on Sisvar software, version 4.0 (FERREIRA, 2000).

RESULTS AND DISCUSSION

Karyotypic pattern of the five genotypes analysed was the same of those ones previously described

in the *Pinus* genus, presenting twelve chromosomes in their haploid complement, eleven of them metacentric and highly similar to each other and one submetacentric (Figure 1, Table 1 and Table 2).

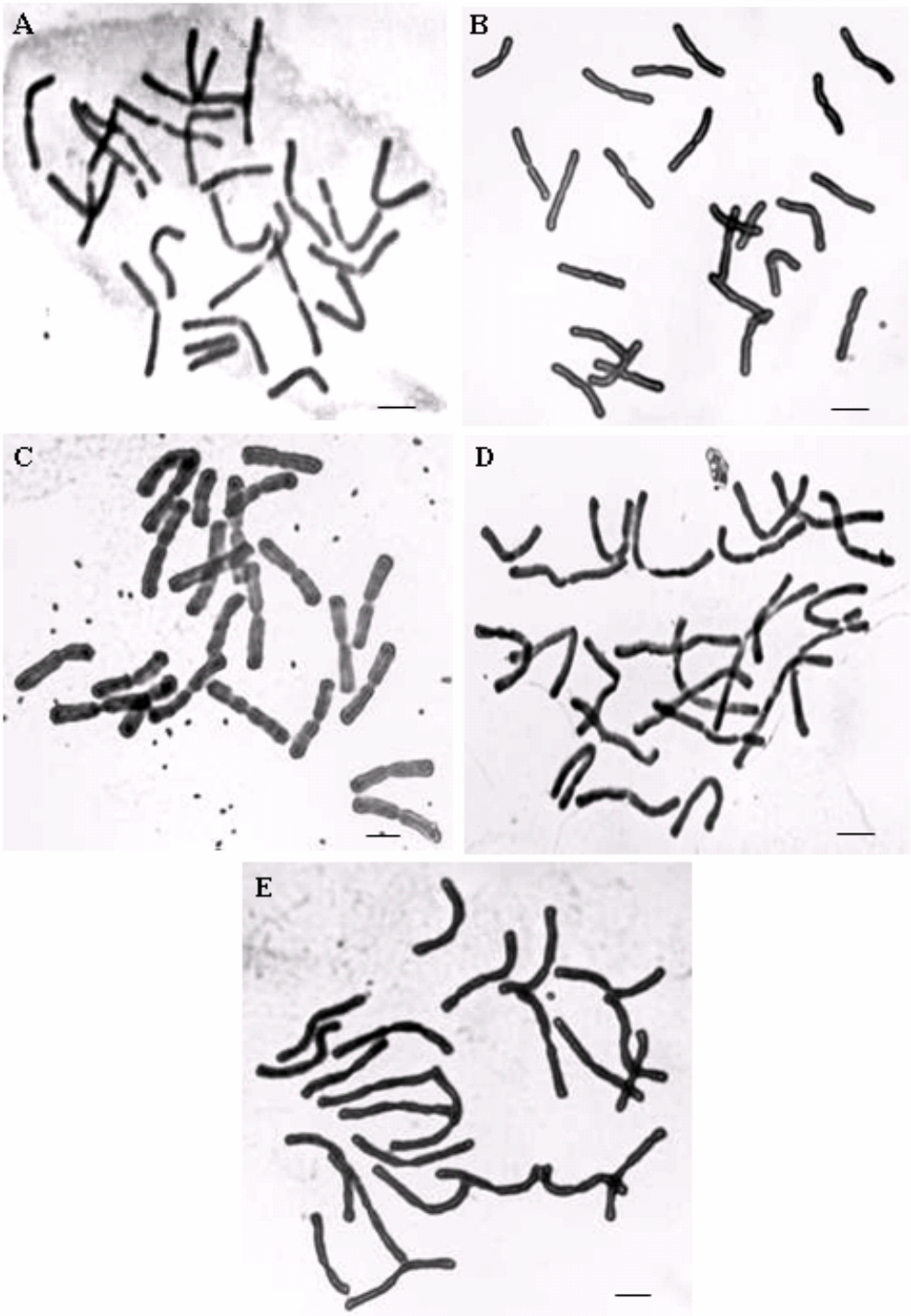

FIGURE 1: Mitotic metaphases stained with Giemsa. (A) *Pinus oocarpa*; (B) *Pinus patula*; *Pinus tecunumanii*: (C) Las Camélias; (D) Jócon Yoro; (E) San Rafael del Norte. (Bar = 5µm).

FIGURA 1: Metáfases mitóticas coradas com Giemsa. (A) *Pinus oocarpa*; (B) *Pinus patula*; *Pinus tecunumanii*: (C) Las Camélias; (D) Jócon Yoro; (E) San Rafael del Norte. (Barra = 5µm).

The alteration in the decreasing order of the long arm length was observed neither in *Pinus oocarpa* nor in the Provenance San Rafael del Norte. In *Pinus patula* and in provenances Las Camelias and Jócon Yoro, chromosomes VII, IV and VI, respectively, interrupted this order. Saylor (1972) and Davide and Araújo (1993) found three and four chromosomes, respectively, breaking that decreasing order and even so there was no coincidence between the pairs in both works, when comparing these data for the same species.

Another characteristic evaluated by Saylor (1972) was the long arm length of chromosome I. In the species of the Subsection Oocarpae, the author found that only *Pinus greggi* does not have chromosome I with the largest long arm. In this work, as pointed out by Davide and Araújo (1993) for species of that subsection, all taxa presented chromosome I with the largest long arm (Tables 1 and 2).

TABLE 1: Mean values, in micrometers, of the chromosome features of *Pinus oocarpa* and *Pinus patula*.

TABELA 1: Médias, em micrômetros, para as características cromossômicas de *Pinus oocarpa* and *Pinus patula*.

C	*Pinus oocarpa*						*Pinus patula*					
	LA	SA	TLi	RL	AR	CM	LA	SA	TLi	RL	AR	CM
I	8.25	7.81	16.07	9.92	0.95	M	**7.74**	**7.10**	14.84	9.71	0.92	M
II	**7.86**	7.20	15.06	9.30	0.92	M	7.40	6.72	14.12	9.24	0.91	M
III	**7.70**	6.83	14.53	8.97	0.89	M	**7.18**	6.52	13.70	8.96	0.91	M
IV	7.48	6.75	14.23	8.78	0.90	M	7.06	6.38	13.45	8.80	0.90	M
V	7.43	6.62	14.05	8.67	0.89	M	6.92	6.32	13.24	8.67	0.91	M
VI	7.21	6.61	13.83	8.53	0.92	M	**6.85**	6.24	13.09	8.57	0.91	M
VII	7.20	**6.40**	13.60	8.39	0.89	M	6.87	6.07	12.94	8.47	0.88	M
VIII	**6.99**	6.42	13.41	8.28	0.92	M	6.62	**6.12**	12.73	8.33	0.92	M
IX	6.88	6.23	13.11	8.09	0.91	M	**6.54**	5.94	12.48	8.17	0.91	M
X	6.67	5.99	12.65	7.81	0.90	M	**6.27**	5.67	11.94	7.81	0.90	M
XI	6.23	5.60	11.82	7.30	0.90	M	5.95	5.23	11.19	7.32	0.88	M
XII	5.89	3.79	9.68	5.97	0.64	SM	5.48	3.61	9.09	5.95	0.66	SM

In that: C = chromosome; LA = long arm; SA = short arm; TLi = total length of chromosome i; AR = arm ratio; RL = relative length; CM = chromosome morphology considering the centromere position; M = metacentric; SM = submetacentric; TLHS = total length of haploid set. Bold values indicate presence of secondary constriction.

TABLE 2: Mean values, in micrometers, of the chromosome features of the provenances of *Pinus tecunumanii*.

TABELA 2: Médias, em micrômetros, para as características cromossômicas das procedências de *Pinus tecunumanii*.

C	Las Camelias						Jócon Yoro						San Rafael del Norte					
	LA	SA	TLi	RL	AR	CM	LA	SA	TLi	RL	AR	CM	LA	SA	TLi	RL	AR	CM
I	**10.16**	9.25	19.41	9.91	0.91	M	**11.85**	10.55	22.39	10.17	0.89	M	**10.48**	9.47	19.95	9.80	0.90	M
II	**9.53**	8.76	18.29	9.34	0.92	M	**10.89**	9.85	20.74	9.42	0.90	M	**10.01**	9.09	19.10	9.38	0.91	M
III	9.24	8.67	17.91	9.15	0.94	M	10.79	9.46	20.25	9.20	0.88	M	9.78	8.74	18.52	9.10	0.89	M
IV	9.31	**8.28**	17.59	8.98	0.89	M	10.28	**9.30**	19.58	8.89	0.90	M	9.64	**8.62**	18.26	8.97	0.89	M
V	**9.00**	8.02	17.01	8.69	0.89	M	**10.13**	9.00	19.13	8.69	0.89	M	**9.26**	8.47	17.73	8.71	0.92	M
VI	8.70	7.98	16.67	8.51	0.92	M	10.22	8.72	18.94	8.60	0.85	M	9.19	8.21	17.40	8.55	0.89	M
VII	8.59	**7.85**	16.44	8.39	0.91	M	9.76	**8.71**	18.47	8.39	0.89	M	8.99	**8.09**	17.07	8.39	0.90	M
VIII	8.40	7.68	16.08	8.21	0.91	M	9.26	8.49	17.75	8.06	0.92	M	8.77	7.85	16.62	8.17	0.90	M
IX	**8.17**	7.51	15.68	8.01	0.92	M	9.06	**8.22**	17.27	7.84	0.91	M	**8.65**	7.70	16.36	8.04	0.89	M
X	8.09	7.11	15.19	7.76	0.88	M	8.87	7.89	16.76	7.61	0.89	M	8.27	7.34	15.61	7.67	0.89	M
XI	7.60	6.70	14.30	7.30	0.88	M	8.31	7.44	15.76	7.16	0.90	M	7.73	6.66	14.38	7.07	0.86	M
XII	6.80	4.44	11.24	5.74	0.65	SM	7.72	5.32	13.03	5.92	0.69	SM	7.28	5.25	12.52	6.15	0.72	SM

In that: C = chromosome; LA = Long arm; SA = short arm; TLi = total length of chromosome i; AR = arm ratio; RL = relative length; CM = chromosome morphology considering the centromere position; M = metacentric; SM = submetacentric; TLHS = total length of haploid set. Bold values indicate presence of secondary constriction.

Variance analysis for TLHS revealed significant difference among the three species. Scott-Knott test formed one group with the provenances of *Pinus tecunumanii* and another one with *Pinus oocarpa* and *Pinus patula* (Table 3). Similarity between *Pinus oocarpa* and *Pinus patula* TLHS as well as the fact that genomes of *Pinus oocarpa* and *Pinus patula* are smaller than those ones of *Pinus tecunumani*i was also showed by Davide and Araújo (1993).

In terms of relative length, chromosome IX of Jócon Yoro is significantly shorter than the same chromosome in other two *Pinus tecunumanii* provenances, as well as in *Pinus patula* and *Pinus oocarpa* (Table 3). The occurrence of deletions may be considered, even though no difference concerning TLHS was observed among the *Pinus tecunumanni* provenances, since the loss of chromatin of only one of the chromosomes would be diluted in the complement as a whole. Although the data lead mainly to the deletion hypothesis, other types of structural alterations, such as inversion and translocation, must be considered.

TABLE 3: Mean values of chromosome IX relative length (RL) and total length of haploid set (TLHS), both in micrometers, for *Pinus oocarpa*, *Pinus patula* and for different provenances of *Pinus tecunumanii*.

TABELA 3: Médias para comprimento relativo (RL) do cromossomo IX e para comprimento total do complemento haplóide, em micrômetros, de *Pinus oocarpa*, *Pinus patula* e de três procedências de *Pinus tecunumanii*.

Taxa	TLHS*	RL*
Pinus tecunumanii Eguiluz & J. P. Perry - Jócon Yoro	220.07a	7.84 b
Pinus tecunumanii Eguiluz & J. P. Perry -San Rafael del Norte	203.52a	8.04 a
Pinus tecunumanii Eguiluz & J. P. Perry - Las Camelias	195.81a	8.01 a
Pinus oocarpa Schiede ex Schltdl.	162.05b	8.09 a
Pinus patula Schltdl. & Cham.	152.81b	8.17 a
CV%	22.33	2.00

In that: *Means followed by the same letter do not differ significantly by the Scott-Knott test (P<0.05).

In several works using staining with Giemsa solution, secondary constrictions were not considered as reliable data for chromosome characterization (SAYLOR, 1961, 1964, 1972; NATARAJAN *et al.*, 1961; PEDERICK, 1967; DAVIDE and ARAÚJO, 1993). In this work, as well, the secondary constrictions were not revealed clearly when chromosomes were stained by Giemsa. However, when the chromosomes were staining with DAPI, a very sharp definition of secondary constrictions was achieved. Each species presented a characteristic number of secondary constrictions: *Pinus oocarpa* with four, *Pinus patula* with seven and the provenances of *Pinus tecunumanii* with six (Figures 2 and 3).

Secondary constriction on chromosome IX of the *Pinus tecunumanii* provenances was the only one localized in different arms, being on the short arm in Jócon Yoro and on the long one in either Las Camelias or San Rafael del Norte (Figure 2 and 3). That variation in the secondary constriction position may be correlated with the geographical distribution of those provenances, since Las Camelias and San Rafael del Norte come from high altitude sites (950 to 1330 m), while Jócon Yoro comes from lower altitude sites (775 to 1000m). Sedelnikova and Muratova (2002) showed variation in location of secondary constriction among populations of *Pinus sibirica* Du Tour and assumed it was resulted from different levels of environmental stress. Chromosome races resulting from rearrangements and fixed according to geographical conditions are described in some plant species by Levin (2002). For *Pinus tecunumanii* the existence of high- and low-elevation ecotypes has already been considered based on morphology and monoterpens (DVORAK, 1989) and on RADP marker data (FURMAN *et al*., 1997).

The data obtained in this work support the statement that the karyotypes into the genus *Pinus* are similar but not identical, what allow us to detect differences that can be correlated to evolutionary events and that can be used as important tools for taxonomic and genetic purposes (SAYLOR, 1961, 1964 and 1972). The karyotype features, such as variation in the decreasing order of long arm length, secondary constrictions and total length of the haploid set provided information enough to distinguish the three taxa investigated. The distinction of *Pinus tecunumanii* from the other two *Pinus* species provides further subsides to confer upon the species taxonomic category, corroborating the latest classifications (FRANKIS *et al.*, 1999).

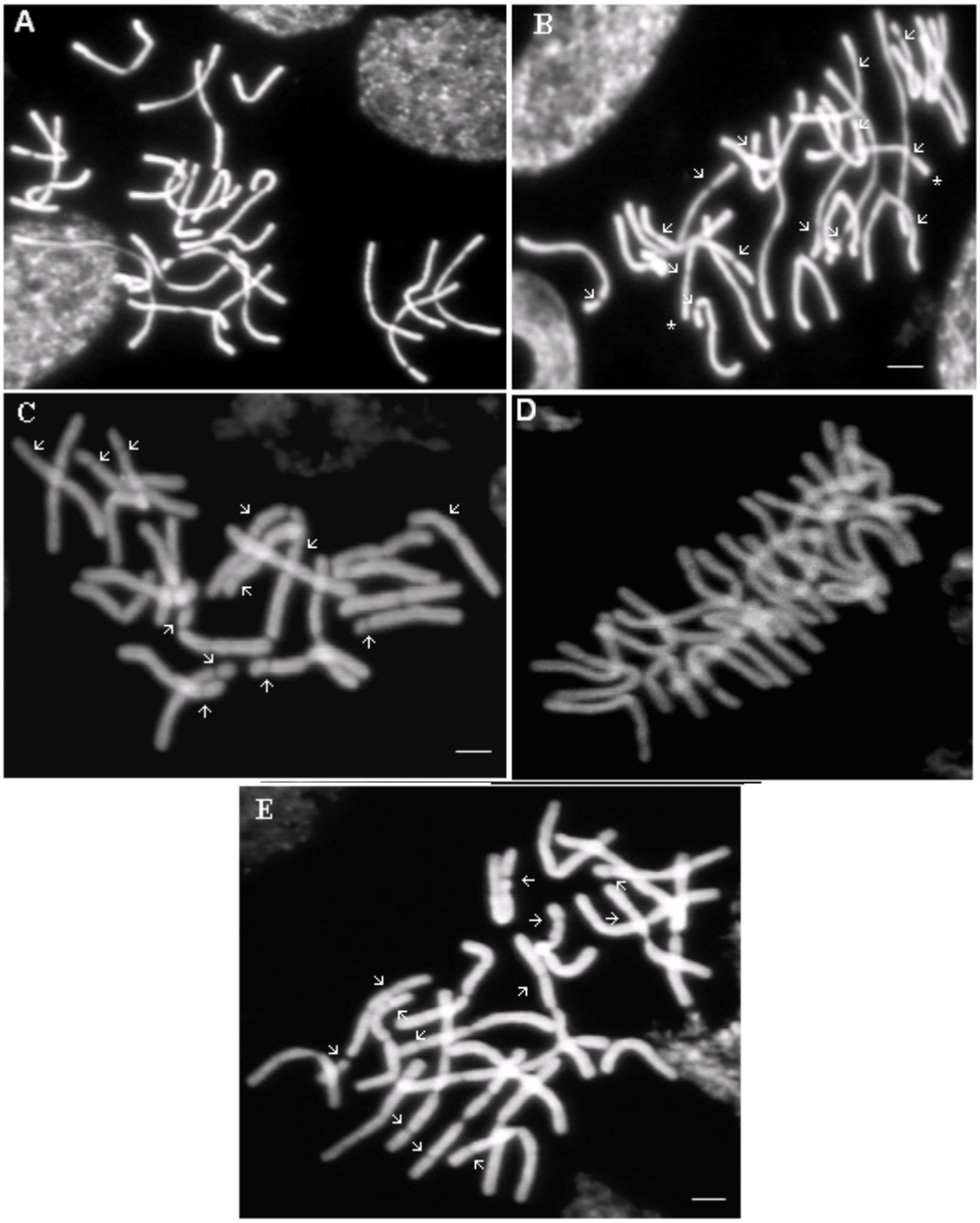

FIGURE 2: Mitotic metaphases stained with DAPI fluorochrome. (A) *Pinus oocarpa*; (B) *Pinus patula*; *Pinus tecunumanii*: (C) Las Camelias; (D) Jócon Yoro; (E) San Rafael del Norte. Arrows = secondary constrictions; * = chromosomes with constrictions in both chromosome arms. (Bar = 5µm).

FIGURA 2: Metáfases mitóticas coradas com fluorocromo DAPI. (A) *Pinus oocarpa*; (B) *Pinus patula*; *Pinus tecunumanii*: (C) Las Camélias; (D) Jócon Yoro; (E) San Rafael del Norte. Setas = constrições secundárias; * = cromossomos com constrições nos dois braços cromossômicos. (Barra = 5µm).

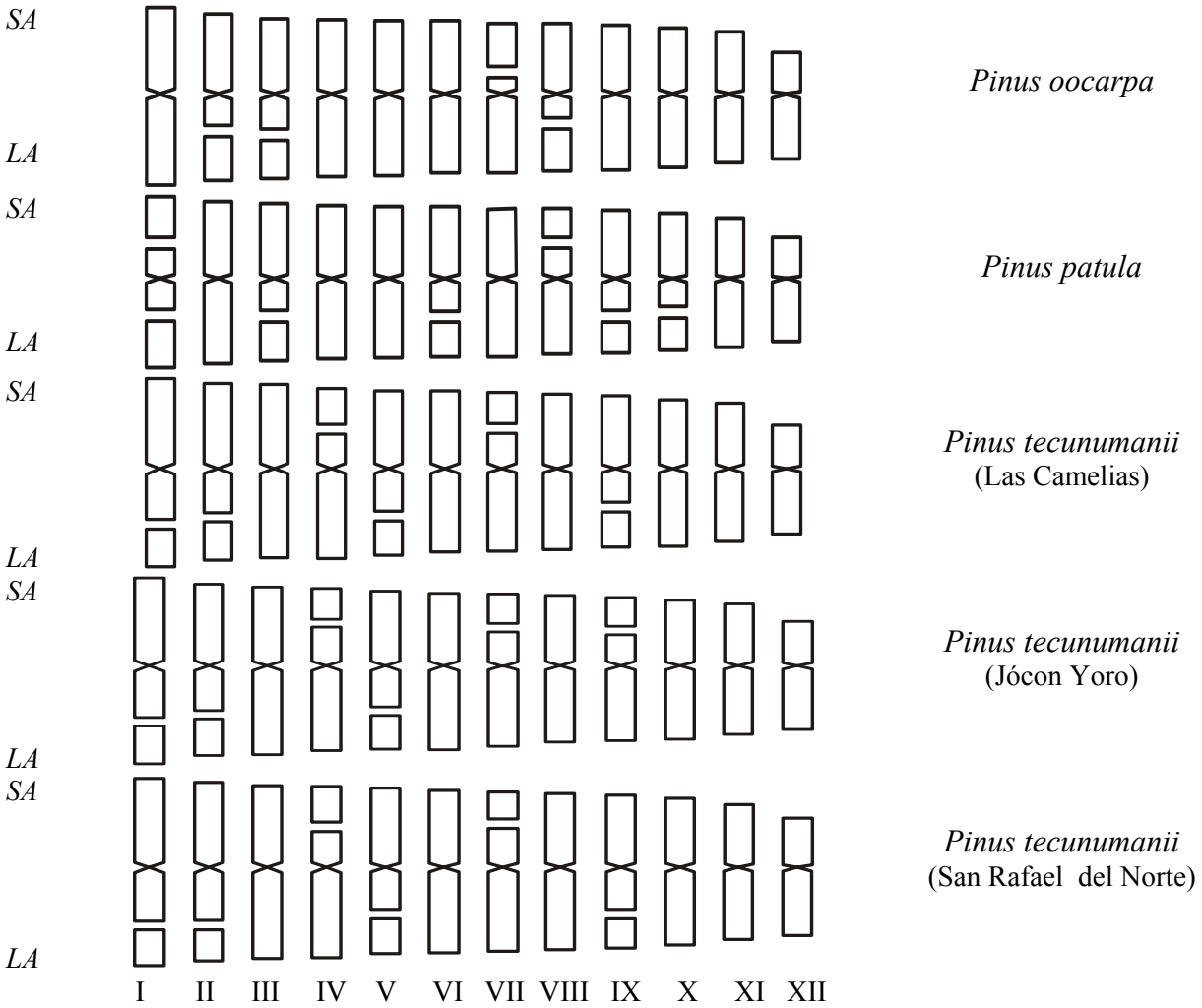

FIGURE 3: Idiograms of tropical *Pinus* species. SA = short arm; LA = long arm. The gaps correspond to the location of secondary constrictions.

FIGURA 3: Idiogramas de três espécies tropicais de *Pinus*. SA= braço curto; LA= braço longo. As interrupções correspondem à localização das constrições secundárias.

CONCLUSIONS

Karyotypic features support the classification of *Pinus tecunumanni* as a species distinct from *Pinus patula* and from *Pinus oocarpa.*

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BIBLIOGRAPHICAL REFERENCES

DAVIDE, L. C.; ARAÚJO, A. J. DE. Características cromossômicas para a identificação de fenótipos diferentes em povoamentos de *Pinus oocarpa* Schiede. **Revista Árvore**, Viçosa, v. 17, n. 3, p.327-338, 1993.

DVORAK, W.S.; BALOCCHI, C.E.; RAYMOND, R.H. Performance and stability of provenances and families of *P. tecunumanii* in the tropics and subtropics. **Proceedings**… Pattaya, Thailand, IUFRO, p. 412 - 414, 1989. " Breeding Tropical Trees: Population and Genetic Improvement Strategies in Clonal and Seedling Forestry Breeding tropical

trees"

FERREIRA, D. F. Análises estatísticas por meio do Sisvar para Windows versão 4.0. In: 45º REUNIÃO ANUAL DA REGIÃO BRASILEIRA DA SOCIEDADE INTERNACIONAL DE BIOMETRIA, 45. 2000, **Anais**... São Carlos, SP: Sociedade Brasileira de Biometria/UFSCar, 2000, p.255-258.

FRANKIS, M. *et al.* **Classification of the genus *Pinus*.** 1999. Captured in 13 nov. 2001. Online. Available on the Internet: http://www.lovett-pinetun.org.

FURMAN, B. J.; GRATTAPAGLIA, D.; DVORAK, W. S.; O'MALLEY, D. M. Analysis of genetic relationships of Central American and Mexican pines using RAPD markers that distinguish species. **Molecular Ecology**, v. 6, p. 321–331, 1997.

HIZUME, M.; OHGIKU, A.; TANAKA, A. Chromosome banding in the genus *Pinus*. II. Interspecific variation of fluorescent banding patterns in *P. densiflora* e *P. thunbergii*. **Botanycal Magazine of Tokyo,** Tokyo, v. 102, p. 25 - 36, 1989.

HIZUME, M.; OHGIKU, A.; TANAKA, A. Chromosome banding in the genus *Pinus*. I. Identification of chromosome in *P. nigra* by fluorescent banding method. **Botanycal Magazine of Tokyo,** Tokyo, v. 96, p.273 - 276, 1983.

HIZUME, M.; SHIBATA, F.; MATSUSAKI, Y.; GARAJOVA, Z. Chromosome identification and comparative karyotipic analyses of four *Pinus* species. **Theoretical and Applied Genetics**, v.105, p.491-497, 2002.

LEÃO, F. F.; DAVIDE, L. C. Posição taxonômica do Pinus de Tecun Umán: análise das características de acículas. **IPEF,** Piracicaba, v. 46, p. 96-106, 1993

LEVIN, D.A. **The role of chromosomal change in plant evolution.** New York, USA: Oxford University Press, 2002. 230 p.

NATARAJAN, A. T.; OHBA, K.; SIMAK, M. Karyotypes analysis in *Pinus sylvestris*. **Hereditas**, Lund - Sweden, v. 47, p. 379 - 382, 1961.

PEDERICK, L. A. The structure and identification of the chromosomes of *Pinus radiata* D. Don. **Silvae Genetica**, v. 16, n. 2, p. 69 - 77, 1967.

SAX, K.; SAX, H. J. Chromosome number and morphology in the conifers **Journal of the Arnold Arboretum**, Cambridge, v. 14, p. 357 - 375, 1933.

SAYLOR, L. C. Karyotype analysis of *Pinus* - group Lariciones. **Silvae Genetica**, Frankfurt, v. 13, p. 165 - 170, 1964.

SAYLOR, L. C. Karyotype analysis of *Pinus* - subgenus *Pinus*. **Silvae Genetica**, Frankfurt, v. 21, p.155-163, 1972.

SAYLOR, L. C. Karyotype analysis of selected species of *Pinus*. **Silvae Genetica** , Frankfurt, v. 10, p. 77 - 84, 1961.

SCOTT, A. J.; KNOTT, M. A cluster analysis method for grouping means in the analysis of variance. **Biometrics,** Raleigh, v. 30, p. 507-512, 1974.

SEDELNIKOVA, T.S.; MURATOVA, E.N. Specific karyological features of Siberian stone pine (*Pinus sibirica* Du Tour) in western Siberian bogs. **Russian Journal of Ecology.** V.33, n.5, p. 303-308, 2002.

SILVA-MANN, R. DAVIDE, L.C.; REIS, M.A. Fluorescent banding in tropical *Pinus* chromosomes. **Scientia Forestalis,** Piracicaba, n. 61, p. 59-63. 2002.

STYLES, B. T. The identify of Schwerdtferger's Central American pine. **Forest Genetic Resources Information** , Rome, n. 13, p. 47 - 51, 1985.

SUASSUNA, J. **A cultura do *Pinus***: uma perspectiva e uma preocupação. Captured in 11 nov. 2001. Online. Available on the Internet: http://www.fundaj.gov.br.