7 RESULTS

7.1 DNA EXTRACTION

Extracted DNA samples are presented in the next 1% Agarose gels (Fig. 15)

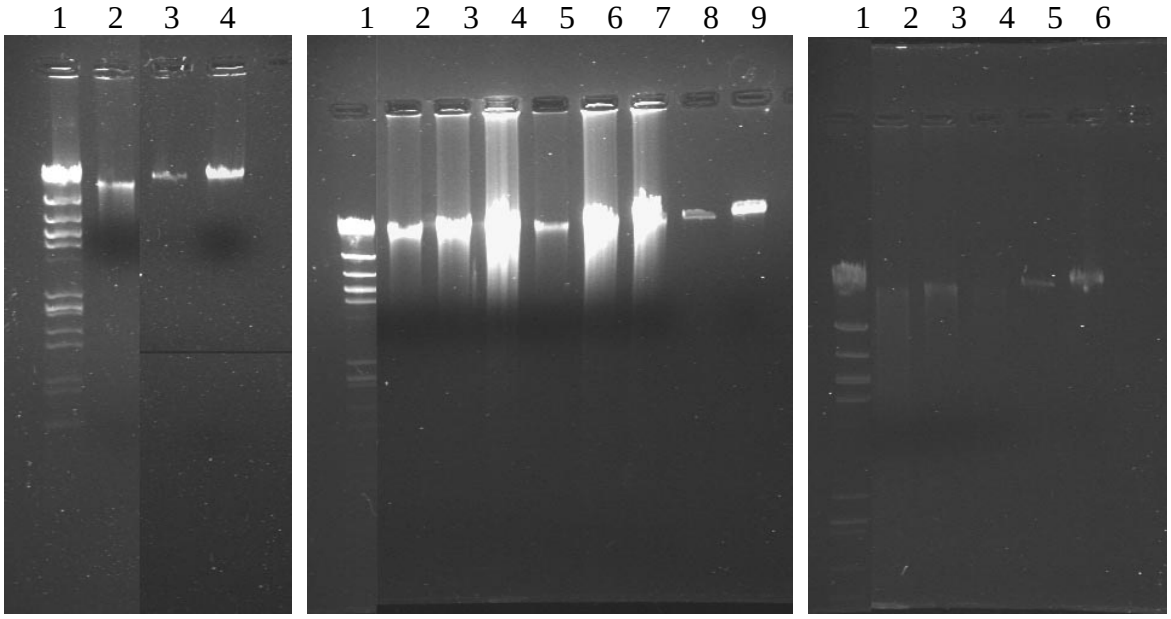

Fig. 15. 1% Agarose gels of extracted DNA. First gel (left). lane1:ladder, lane2:CP-9, lane3:10ng/µl, lane4:50 ng/µl. Second gel (middle). lane1:ladder, lane2:CP-8, lane3:CP-13, lane4:CP-95, lane5:CP-163, lane6:CP-172, lane7:CP-188, lane8:10ng/µl, lane9:50 ng/µl. Third gel (right). lane1:ladder, lane2:CP-5, lane3:CP-7, lane4:CP-189, lane5:10ng/µl, lane6:50 ng/µl.

DNA concentrations were obtained using the computer program BIORAD Gel Doc 2000™

(Table 5):

Strain	Concentration (ng/µl)	Strain	Concentration (ng/µl)
CP-5	16.6	CP-95	188.7
CP-7	12.4	CP-163	35.3
CP-8	62.6	CP-172	191.3
CP-9	62.1	CP-188	116.2
CP-13	112	CP-189	1.6

Table 5. Extracted DNA concentrations for the different strains

7.2 PCR AMPLIFICATION OF ITS REGION

Amplified ITS regions (PCR) are presented in the next 1% Agarose gel (Fig. 16).

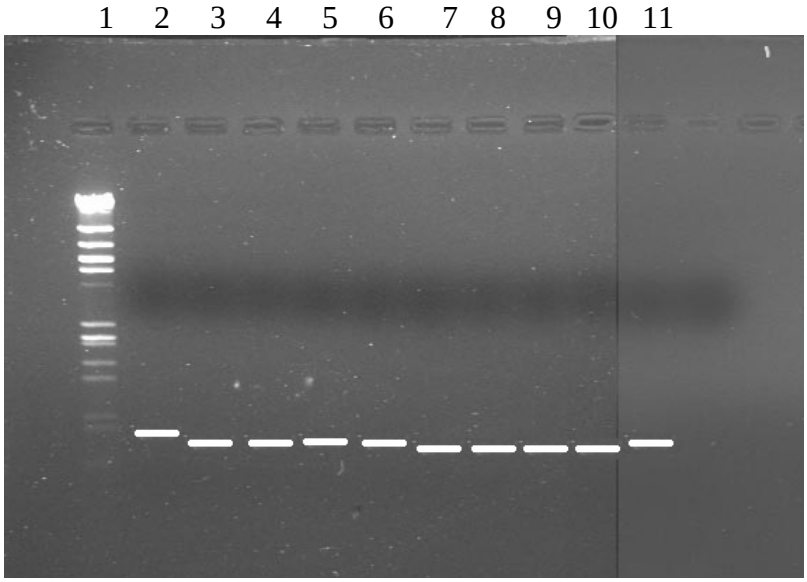

Fig. 16. 1% Agarose gel of amplified ITS regions. PCR's directed by genomic DNA templates were primed with ITS1 and ITS4 complementary to conserved sequences. Lane1:ladder. Amplified products are shown from lane 2 to lane 11 in the next order: CP-5, CP-7, CP-8, CP-9, CP-13, CP-95, CP-163, CP-172, CP-188, CP-189. Concentrations were not calculated. Schematic version of the gel.

Purified ITS regions (PCR samples) are presented in the next 1% Agarose gel (Fig. 17)

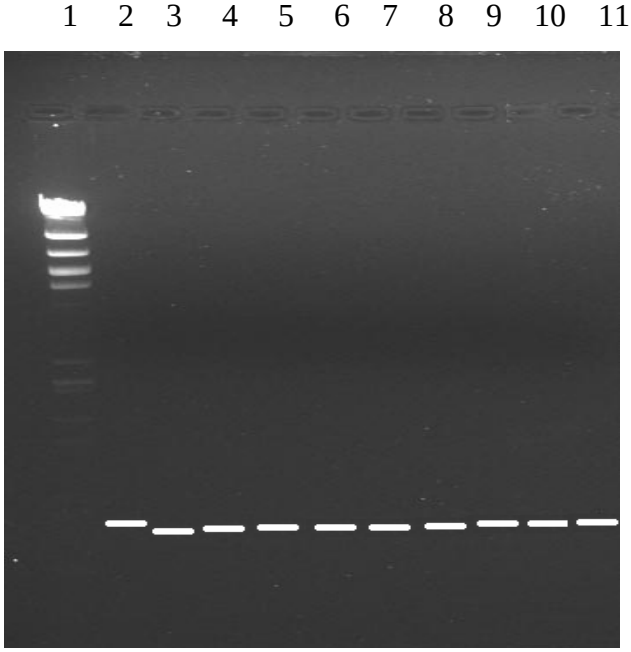

Fig. 17. 1% Agarose gel of purified ITS regions. The bands are barely visible since staining with EtBr was minimal. Lane:ladder. Amplified products are shown from lane 2 to lane 11 in the next order: CP-5, CP-7, CP-8, CP-9, CP-13, CP-95, CP-163, CP-172, CP-188, CP-189. Concentrations were not calculated. Schematic version of the gel.

7.3 ANALYSIS OF CLASSICAL AND MOLECULAR DATA

The next phylogenetic tree shows the relationships between the strains used throughout this research project (Fig. 18).

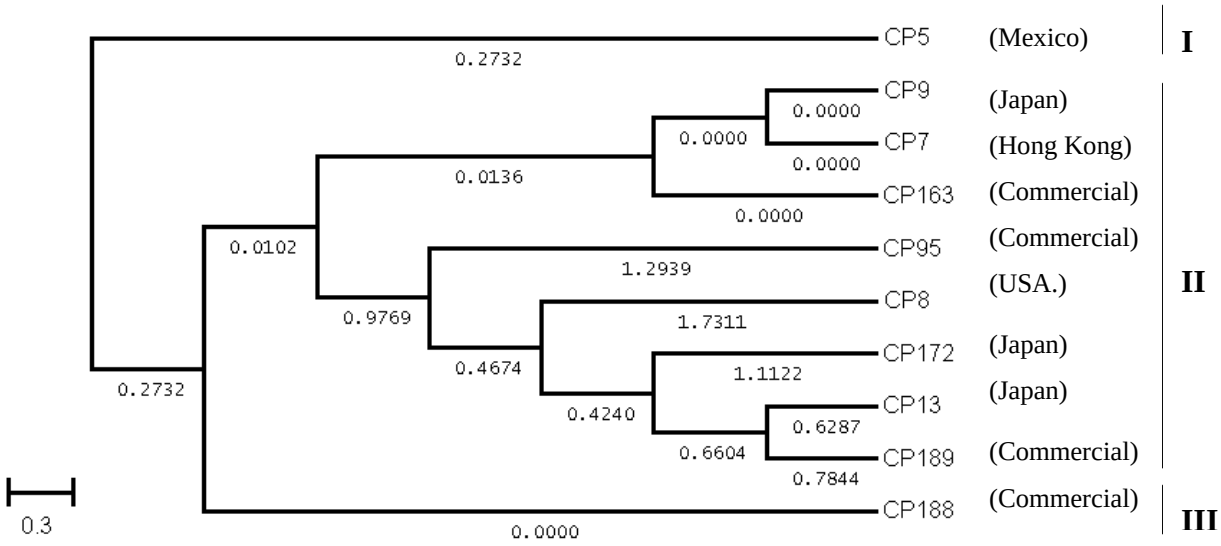

Fig. 18. Dendrogram frrom the cluster analysis and genetic distance Tajima-Nei among 9 *Lentinula edodes* strains and one *Lentinula boryana* strain (CP-5) for polymorphisms generated by the use of ITS molecular markers. Origin of strains is showed in parentheses. Number of terminal nodes in tree: 10. Outgroup rooted. Mode: Best tree.

Distance Matrix

	CP-13	**CP-9**	**CP-8**	**CP-7**	**CP-189**	**CP-188**	**CP-172**	**CP-163**	**CP-95**
CP-9	3.4586								
CP-8	3.4586	3.1989							
CP-7	3.4586	0.0000	3.1989						
CP-189	1.4130	3.4586	3.2028	3.4586					
CP-188	3.4586	0.0159	3.4586	0.0159	3.0011				
CP-172	1.4996	3.4586	3.4586	3.4586	3.4586	2.9693			
CP-163	3.4586	0.0000	3.1989	0.0000	3.4586	0.0159	3.4586		
CP-95	3.4586	2.1109	3.4586	2.1109	3.4586	2.1662	3.4586	2.1109	
CP-5	3.4586	0.5737	3.4586	0.5737	3.4586	0.5420	1.8084	0.5737	3.4586

As can be seen in the tree, there are three distinct groups. One comprises *L. boryana* (outgroup), and the other two contain strains from different origins. The coefficients of genetic similarity (Tajima-Nei's distance) ranged from 0.000 (CP-9, CP-7, CP-163) to

3.4586 (CP-13 and CP-189, all other strains). The closest related pair of strains were CP-9 and CP-7 from Japan and Hong Kong, respectively. Strain CP-5, *L. boryana* from Mexico, however, was most distantly related to the remaining nine strains.

Table 6 shows the average production and biological efficiency of several *Lentinula edodes* strains (after two harvests) in 2.5 kg units of production (humid weigth). These differential yields are correlated with the three groups in the dendrogram and are going to be crucial in advocating for a determinate genetic improvement programme. Following the table a figure can be found with morphological differences among several strains (Fig. 19).

Strain	**Production (g)**	**Biological efficiency (%)**
CP-188 (Group III)	995.5	134.10
CP-172 (Group II)	868.12	116.92
CP-163 (Group II)	829.37	111.70
CP-7 (Group II)	712.38	95.94
CP- 13 (Group II)	524	70.57
CP-95 (Group II)	370.12	49.85
CP-189 (Group II)	307.12	41.36
CP-5 (Group I)	0	0

Table 6. Production and biological efficiency of several *Lentinula edodes* strains [20]

Fig. 19. Morphological differences in fruiting bodies of several *L. edodes* strains [20]

7.4 COMPARISON WITH GENETIC SEQUENCES FROM INTERNATIONAL DATABASES

The next dendrogram shows the relationships between CP, NCBI and EBI strains (Fig. 20)

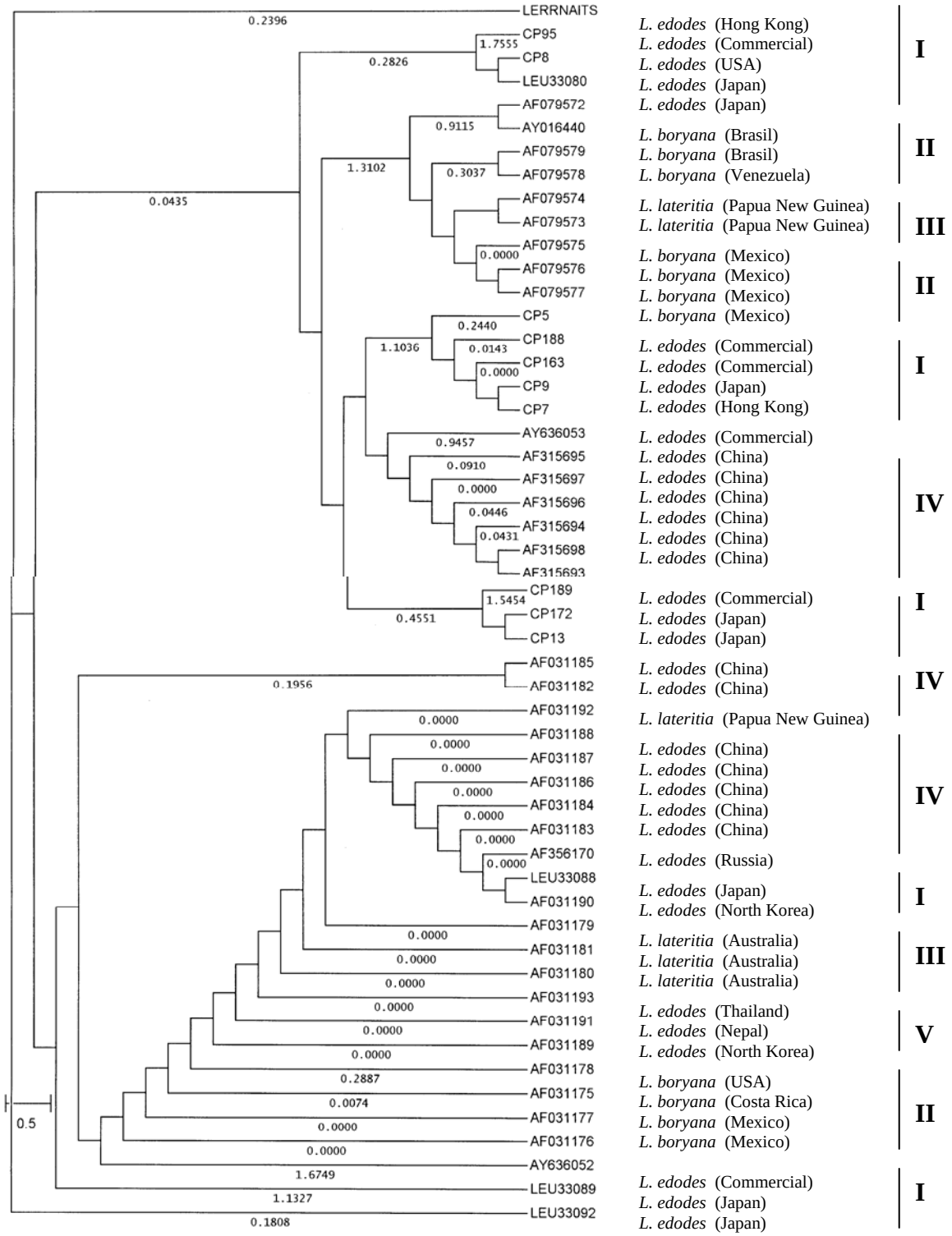

Fig. 20. Dendrogram from the cluster analysis and genetic distance Tajima-Nei among 52 *Lentinula* strains for polymorphisms generated by the use of ITS molecular markers. Origin of strains is showed in parentheses. Number of terminal nodes in tree: 52. Unrooted. Mode: Best tree.

The Distance Matrix for the dendrogram can be found in the APPENDIX 11.8. Almost all isolates from similar origins clustered together. Data divided the isolates in five geographically distinct population groups (Fig. 21). The coefficients of genetic similarity (Tajima-Nei's distance) ranged from 0.000 (mostly among China strains) to 4.8712 (mainly Japan strains with all other groups). The closest related strains were China strains and *L. boryana* strains from America. Most species from China are considerd to be synonymous since their similarity coefficients range from 0.000 to 0.0910. All *L. boryana* species from Mexico appear to be very closely related. The same happens with *L. boryana* species from around America, which differ only slightly. *L. boryana* and *L. lateritia* seem to be highly similar as well.

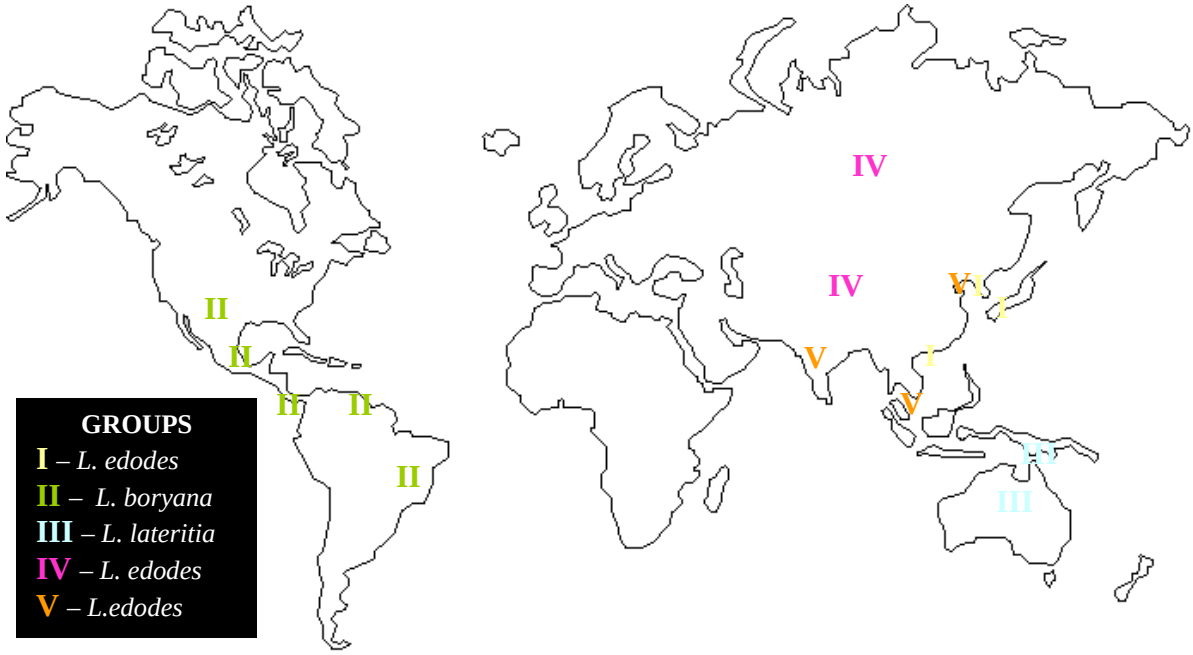

Fig. 21. Approximate distribution of *L.edodes, L. boryana* and *L. lateritia* as studied in the dendrogram obtained in this study with strains from international databases. rDNA lineages are shown in five distinct groups: I – Japan, Hong Kong, North Korea; II – Brasil, Venezuela, Mexico, Costa Rica, USA; III – Papua New Guinea, Australia; IV – China, Russia; V – Thailand, Nepal, North Korea.