UNIVERSITY OF PÉCS

Biology Doctoral School

Botany Programme

Evaluation of leaf anatomical and molecular phylogenetic methods on critical *Rubiaceae* taxa

Ph.D. Thesis

Szilvia Stranczinger

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1. INTRODUCTION

Nowadays phylogenetic analyses based on DNA are gaining ground in the systematization of plant families besides the traditional morphological investigations. The Rubiaceae is in the focus of these researches as one of the largest flowering plant families, which is on the top of the evolution of the dicotyledons.

From the modern taxonomic methods, following the cladistic analysis based on morphology, more and more phylogenetic systems were created based on molecular data. Despite examining various DNA regions, there are results in almost all studies that cannot be explained with the current description of the given taxa.

During the investigation of the species-rich *Rondeletia* genus belonging to the Rondeletieae tribe it turned out that this species group interpreted in the broad sense should be separated into several genera, already described in the 19th century.

The majority of the species has been transferred systematically and their phylogenetic description has been modified. The phylogenetic study of DNA sequence data supported the segregation of the *Rondeletia* genus into smaller genera.

In order to separate the species and sections of the *Exostema* genus several morphological and molecular based attempts have been made. Despite of the combined morphological and phylogenetic studies there is no unified view for the taxonomy of the group.

The systematic position and inner division of the *Randia* genus has not been clarified to date. In the beginning there were attempts to clarify the taxonomy of the genus on the basis of morphological characteristics and later with phylogenetic investigations.

In the present study the systematic study of the above mentioned taxa has been carried out partly on leaf venation micromorphological basis (*Exostema* and *Rondeletia* genus), and partly on phylogenetic basis (*Rondeletia*, *Randia* and *Hintonia* taxa).

2. AIMS

In earlier flora descriptions plants were discussed mainly on the basis of external morphological features. In more recent taxonomic works taxonomists aim at characterising species and higher systematic categories on the basis of more and more considerations. In the systematization of angiosperms floristic characters are used most frequently. Vegetative features have been considered as dubious, since in many cases seemingly similar characteristics may appear in non-related plant species. Nevetherless, in the best systems both reproductive and vegetative characters are taken into consideration.

From the non-reproductive organs leaf studies are the most wide-spread, because the leaves can be compared in a wide taxonomic range, and usually they are present on the plant for a long time. Therefore, in the systematization of the Rubiaceae family the taxonomic value of the so far not used leaf venation micromorphological features was studied.

Nowadays DNA based phylogenetic studies comprise a new trend in systematic investigations. Several plant groups with debatable position could be revised with the help of sequence analysis of various DNA regions. The goal of our research is to join this international trend by elaborating a molecular phylogenetic system which can help to clarify the phylogenetic position of genera and species belonging to certain tribes of the Rubiaceae family.

The aim of our phylogenetic studies was to contribute to the solution of three questions: 1. separation of 3 genera of the Rondeletia complex in the Rondeletieae tribe; 2. verification of a species level difference in the *Hintonia* genus of the Cinchoneae tribe; 3. the more exact description of the inner division of the *Randia* genus in the Gardenieae tribe.

The clarification of the above questions forms part of the goals of the international project Flora Mesoamerica.

3. MATERIAL AND METHODS

3.1. Leaf venation examination

In order to examine the leaf venation of species belonging to the *Exostema* and *Rondeletia* genera it was necessary to develop a new leaf clearing method. The procedure that proved to be suitable is a combination of several well-known methods, using the herbarium leaf material of the species. Samples were taken from 5 leaves in each species. Leaf pieces were boiled in distilled water for a few minutes, then in 10% alcoholic KOH for further 4-5 minutes. After that 5% H_2O_2 was poured on the leaves until boiling again. Then leaves were kept in 10% alcoholic KOH for 24 hours. After removing this solution samples were rinsed 2-3 times with distilled water. Then 10% KOH was applied once more, boiling the leaves in it for 3-4 minutes. Then 96% alcohol was added to the KOH and samples were boiled until colourless. Finally, leaves were rinsed in distilled water 2-3 times. Samples were suitable for microscopic investigation after 1-2-day drying.

Leaf venation of the cleared leaves was studied with a LEICA WILD M 420 stereo microscope at a 10× magnification. 20 micrographs were taken in 3 positions of the cleared leaves (at the basal, middle and apical part of the leaf), sized 2×2 mm or 1×4 mm. Mean and standard variation values were calculated and graphs were prepared with the Windows Excel program. In the case of the Rondeletia species samples were investigated with a NIKON ECLIPSE 80i microscope. Morphological parameters were measured with the software Image Tool 1.27.

The studied parameters of leaf venation were the following: areole number, veinlet termination number, veinlet types, veinlet divergence, areole morphology, veinlet morphology, areole size, density of leaf venation.

3.2. Phylogenetic analysis

Total genomic DNA was extracted from leaf tissue. Samples were obtained from herbarium material (FCME). Amplification of the target loci (rps16 chlorplast intron and ITS nuclear DNA) was made on a PTC-200 GradientCycler (MJ Research, INC.). The whole ITS region was amplified with ITS4 and ITS5 primers, the ITS1 was multiplied with ITS2 and ITS5 primers. The *rps*16 intron was amplified with rpsF and rpsR2 primers. The PCR reactions were performed in a total reaction volume of 50 µl. The PCR reaction resulted approximately 800 bp long *rps*16 intron, ca. 700 bp ITS region and 300 bp ITS1 fragment. After fragment isolation sequencing reactions were made on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) using BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer’s instructions at the Agricultural Biotechnology Center, Gödöllő. Sequencing was carried out with the same primers used for amplification.

The sequence analyses were divided into three main groups: 1. separating the genera of the Rondeletia complex, 2. getting acquainted with the inside division of the *Randia* genus, 3. verification of species level differentiation of *Hintonia latiflora* and *H. standleyana*. In all three examinations self-analyzed sequences were completed with sequences from the NCBI GenBank.

For rooting the phylogenetic tree an outgroop was used, whose members are systematically closely connected with members of the ingroup, but are evolutionally further from the ingroup than its members from one another. The sequences were first aligned by the ClustalW v1.7 computer program. The nucleotide sequences were added and aligned by

GeneDoc v2.3 computer program using the corresponding DNA sequences as template. After the optimization of the alignments the evolving gaps were coded as missing during the final phylogenetic analyses. The cladistic analyses were completed with PAUP* 4.0 software on Windows® XP® using maximum parsimony. This process provides a phylogenetic tree which allows the least character state changes (evolution steps) to explain genealogical relations. For testing the accuracy of the phylogenetic tree, we ran bootstrap analyses, which are suitable to estimate standard error. As a general rule it is can be declared that if the bootstrap value is over 85%, than the topology of the concrete ramification is adequate, between 75-84% it is moderate, and between 50-74% the bootstrap value is low and not competent.

Tree statistics included the consistency index (CI) and retention index (RI). Both indexes show the degree of homoplasy in the current phylogenetic tree.

4. RESULTS

4.1. Leaf venation characters of the studied taxa

Characterisation of *Exostema* and *Rondeletia* taxa was done on the basis of quantitative and qualitative analysis of areoles and veinlets. Following this results were evaluated in the relation of species, sections and the two genera. Examining the apical parts of the leaves, areole numbers vary to a great extent both at genus and section level. Highest values were measured in the case of *E. myrtifolium* (45.45) and *E. purpureum* (45.2) that belong to different sections and live in different habitats. The smallest number of areoles is characteristic of the *Rondeletia* genus, within the genus, however of *R. ekmanii* (8.7) and *R. brigandiana* (11.6) belonging to different sections and living in various habitats. From the veinlet termination values measured in this position of the leaves *E. velutinum* (108.4) and *E. lineatum* (105.8) are conspicuous. The veinlet termination numbers of the leaf apex separate the two genera, being lower in the studied *Rondeletia* species. The mean values are 29.98 and 77.24 in *Rondeletia* and *Exostema* species, respectively. Considering the terminal veinlets of the leaf apex there are significant differences between species.

Studying the areole number of the middle part of the leaf the lowest values were found in *E. parviflorum* (12.1) and *R. brigandiana* (11.6 db), while the most areoles in this leaf position were detected in *E. selleanum* (31.2) and *E. velutinum* (34.8), belonging to the same section.

Also in the middle part of the leaf, with the exception of *R. ekmanii*, lower veinlet termination number characterises *Rondeletia* species (35.06), while in *Exostema* species the mean number of veinlet terminations is 73.53. Highest veinlet termination number was found in the leaves of *E. acuminatum* (104.5), while in the case of *R. stereocarpa* this value was 6.7.

In the basal part of the leaf both the highest and the lowest areole numbers were measured in the *Exostema* genus: 40.2 and 6.65 in *E. myrtifolium* and *E. ellipticum*, respectively. The areole numbers of the studied *Rondeletia* species showed lower standard deviation in the basal part of the leaf (6.3) than in the case of *Exostema* species (10.04). In the basal part of the leaf , similarly to the middle part, with the exception of *R. ekmanii*, *Rondeletia* species could be characterised with lower veinlet termination numbers. The mean values per unit area were 73.15 and 31.63 in *Exostema* and *Rondeletia* species, respectively.

In different parts of the leaves differences were found between the studied species, considering both vein islet and veinlet termination number. The standard deviation of results was lower in each case in the studied species of the *Rondeletia* genus than in the *Exostema* genus. The mean number of the terminal veinlets in *Rondeletia* species was lower than in *Exostema* species, regardless of the position in which it was determined. Veinlet termination number is more suitable for differentiating leaves. *Rondeletia* species are characterised by lower veinlet termination numbers. If the means of areole and veinlet termination numbers measured in the various parts of the leaf in the studied species are considered, it can be stated that these values are very similar within the investigated genera. Considering areole numbers there is no difference between the genera, however, as mentioned earlier, the mean number of terminal veinlets is characteristic for the genus.

The number of veinlet terminations is higher in each studied *Exostema* species than that of areoles, irrespective of the sampling position. The difference between these two values is smaller in the case of *Rondeletia* species, and in the case of *R. stereocarpa* and *R. longibracteata* the areole number is higher than the veinlet termination number. No correlation was found between the number of areoles and veinlet terminations and the measurement positions, the values were variable in the different parts of the leaf in most studied species. In some cases the values are constant, in other cases they are increasing or decreasing towards the apical, basal or middle part of the leaf.

The two genera can be well separated by examining the vein islet and veinlet termination categories. The lower veinlet termination numbers of the *Rondeletia* genus are the result of the dominance of areoles with 2, 3 and 4 terminal veinlets, and no areoles with many (10, 12) veinlet terminations. However, these vein islet categories (areoles with a great number of terminal veinlets) are characteristic of the studied *Exostema* species.

The percentage of areoles with veinlet terminations is relatively constant in some cases, regardless of the measurement positions (e.g. *E. purpureum*, *E. selleanum*, *R. peduncularis*, *R. ekmanii*), in other cases, however, there is great variation in the different parts of the leaf (e.g. *R. clarendonensis*, *E. spinosum*). No relationship was found between these percentages and measurement positions and the various taxonomic categories. In the case of taxa from deciduous forests, high mountains and rainforests these proportions were higher than in the species from limestone and serpentine shrubs.

No significant differences were found between the leaf venation densities of the studied *Rondeletia* species. In the course of leaf venation studies it was stated that venation density decreases from the leaf apex to the basal part. Leaf venation density did not reach the value of 100 cm/cm^2, which is considered to be the sign of xeromorphy, in any of the species.

The investigated *Rondeletia* genus can be characterised with quadrangular or pentagonal areoles, and simple veinlet terminations without branches, linear or curved. The studied species of the *Exostema* genus can be characterised by triangular, quadrangular and rounded vein islets, and once or twice branched veinlet terminations. Concerning the morphological characteristics there were differences not only between the two studied genera, but also between the sections and species. Unified features were found within the *Exostema* genus in terms of areole and veinlet termination morphology:

Morphology of vein islets

The studied representatives of the *Floribundae* section can be characterised by the frequent occurrence of irregular areoles and by the absence of triangular vein islets in the basal part of the leaf. Species of the *Oliganthae* section are uniformly characterised by triangular areoles that are frequent in the apical part of the leaf and by scattered pentagonal areoles. The common characteristics of the studied species in the *Exostema* section are the great number of quadrangular vein islets in the whole leaf and the small number of rounded areoles in the middle part of the leaf. Within the section there is variation in the distribution of pentagonal vein islets. In the case of *E. myrtifolium* this type of areole can be found in great number in the basal part of the leaf, while in other species of the section it is present only in small numbers in the whole leaf.

Morphology of vein terminations

The studied species of the *Floribundae* section can be characterised by the great number of once branched veinlet terminations in the apical part of the leaf, as well as by three times branched veinlet terminations in the 2nd and 3rd position. The same species can be characterised also by the small number of areoles without veinlets. The leaves of species in the *Oliganthae* section are characterised by the small number of areoles without veinlets in the basal and apical part, as well as of areoles with curved veinlets. Three times branched veinlets can be found in the whole leaf, but in smaller numbers. The species of the *Exostema* section can be characterised by the scattered occurrence of linear and curved, as well as once and twice branched veinlets.

In the case of *Rondeletia* species the size of the most frequent vein islets was also measured. Areole size varied in several cases within the same leaf, therefore no systematic significance was attached to this value. Areole sizes varied to a great extent within the studied sections, as well, hence no relationship was found in the results either at genus or section level. As expected, the reason for a higher areole number was the smaller areole size. In the case of species where the number of irregular vein islets was high, their size was also bigger than that of quadrangular or pentagonal areoles.

The examined representatives of the *Exostema* and *Rondeletia* genera are distributed in the neotropics. Their leaf morphology indicates the effect of the tropical climate. During their spread the impact of the base-rock became more pronounced, and the climatic factors changed by the features of the terrain also influenced leaf venation, and consequently resulted in differences between leaf venation in the species. The effect of the habitat on the quantitative and qualitative features of leaf venation can be demonstrated in some cases (density of leaf venation, veinlet termination number, percentage of areoles with veinlet terminations). However, despite the above facts, the influence of the habitat on the micromorphological characteristics of leaf venation could not be verified. The studied features may vary in species living in the same habitat but belonging to different taxonomic categories or even within a single leaf.

4.2. Phylogenetic analysis of the studied taxa

4.2.1. Examination of the ITS region of species of the Rondeletia complex

The amplification of the whole ITS (internal transcribed spacer) region (ITS1-5, 8S rDNS-ITS2) was succesful only in 10 species from the Rondeletia complex, because of the older herbarium material, where DNA is damaged, fragmented. It turned out from the investigation of the alignment of the10 entire ITS sequences that, as expected, the decisive majority of the sequence differences between the species was limited to the two intergenic parts (ITS1 and ITS2) and from these mainly to the ITS1. In the next step our goal was to amplify only this DNA region (ITS1) with ITS2 and ITS5 primers. So we could include 16 species — as members of the ingroup — in the phylogenetic analysis. The total G/C content of the entire ITS region was >60% in most species.

We did not find any sequence difference between the 2 samples of *Rondeletia deamii* collected from different locations (Mexico, Nicaragua). Differences were found only in 2 positions (95 and 217) between *Arachnothryx leucophylla* and *Arachnothryx leucophylla* var. *virgata* nom. prov. The sequences were found quite variable in length (*Arachnothryx monteverdensis* 642 bp, *Rondeletia deamii* from Nicaragua 558 bp). The optimization of the first alignment was neccesary.

Besides the five *Arachnothryx*, two *Rogiera* and two *Rondeletia* taxa four *Randia* species — as members of the outgroup — were involved in the phylogenetica analysis of the whole ITS region. Although the *Randia* genus is a member of the Rubiaceae family, it belongs to the Ixoroideae subfamily contrary to the Rondeletia complex, which is a member of Cinchonoideae. So the selected species are systematically closely connected with the members of the ingroup, but are further from the ingroup than from each other. The final matrix comprised 569 positions, where 434 (76.3%) were constant; 23 (4.1%) variable but uninformative, and 112 (19.6%) informative. The members of Rondeletia complex form a strongly supported (94% bootstrap) group. Although *Arachnothryx*, *Rogiera* and *Rondeletia* species were separated within this clade, they had only low bootstrap support. The Arachnothryx clade had 69%, while the *Rondeletia* and *Rogiera* together had 59% bootstrap. In the latter clade two *Rogiera* and two *Rondeletia* taxa had high bootstrap support (97% and 100%). In spite of the low bootstraps and the limited number of the studied sequences, the *Rondeletia* genus seems polyphyletic.

The internal transcribed spacer 1 (ITS1) sequences of 16 taxa were studied in order to recognize the more exact phylogenetic relationships inside the Rondeletia complex. Three species — representing genera outside the Rondeletia complex (one African and two neotropical) — were chosen from GenBank as outgroup taxa. The final matrix comprised 306 positions, where 168 were constant; 50 (16%) variable but uninformative, and 88 (29%) informative. The strict consensus tree shows three major groups within the studied taxa of the Rondeletia complex. The *Arachnothryx* clade had high bootstrap support (86%) and included two strongly supported clades (88 and 92% bootstrap). The studied *Rogiera* and *Rondeletia* species formed a highly supported clade (98% bootstrap), within this clade the *Rondeletia* genus had 100% bootstrap support. The two studied *Rogiera* species form a moderately supported (75%) clade. By analyzing the whole ITS and ITS1 region, *Arachnothryx*, *Rondeletia* s. str. and *Rogiera* genera were well separated within the Rondeletieae tribe.

4.2.2. Phylogenetic analysis of the *Randia* genus

The ITS region and *rps*16 intron (chloroplast DNA) — which have not been used for the examination of the genus before — were chosen to clarify the phylogenetic relationships of the Central American *Randia* taxa. The outgroup comprised the sequences of 5 taxa representing the Rondeletieae tribe. The *rps*16 intron was found quite variable in length within the studied species: the aligned portion ranged from 683 (*Arachnothryx guerrerensis*) to 850 (*Randia thurberi*) bp. In spite of this aligning the sequences was easy due to their great similarity. The final matrix comprised 734 characters, where 622 were constant; 28 (4%) variable but uninformative and 84 (11%) informative. In the course of investigating the *rps*16 intron two well supported (87% and 99% bootstrap) subclades were separated within the *Randia* clade.

The entire ITS region, containing ITS1, ITS2 and 5.8S rDNA sequences was approximately 571 bp long in the studied taxa. The ingroup comprised 16 taxa, from which the sequences of 7 were determined by us for the first time, the other sequences, including the 3 sequences of the outgroup taxa — were chosen from the GenBank. The final matrix comprised 571 positions, where 397 were constant; 55 (10%) variable, but uninformative and 119 (21%) informative.

The strict consensus tree shows 3 major clades. A group of mainly lowland, South American *Randia* species (*Randia altiscandens*, *R. wigginsii*, R. *carlosiana*, *R. longifolia*, *R. pubistyla*) is moderately supported (75%). Three species from Mexico (*R. guerrerensis*, *R. capitata*, *R. induta*) form a strongly supported (97%) clade. The third group consisting of Central American and Mexican *Randia* species is less supported (58%). The latter two groups are well supported together (95%).

Both of our analyses (*rps*16 intron and ITS region) show that contemporary circumscription of the possible groups within *Randia* s. l. is uncertain. A literature survey of descriptions of *Randia* from Mexico, Central America and the Antilles reveals only a single morphological character to distinguish Central American taxa from South American ones. When studying data of both sequences we found two, well separated groups between Central American *Randia* species selected for this research. The molecular differences seem to be well in line with the size of leaves combined with the texture of exocarp. The big-leaved *Randia* species with lignified exocarp are: *R. tetracantha*, *R. induta*, *R. guerrerensis* and *R. capitata*. The small-leaved species with thin exocarp are: *R. nelsonii*, *R. aculeata*, *R. cookii* and *R. malacocarpa*. However, an exception based on the above morphological classification was found among the studied taxa, since *R. thurberi* is a small-leaved species but with a fruit with strongly lignified exocarp. Meanwhile, the exocarp seems to be the only decisive character between the 2 separated groups among *Randia* species.

4.2.3. Study of the ITS, rps16 and trnL-F sequences of *Hintonia latiflora* and *H. standleyana*

Examination of three DNA sections (the whole ITS region, *rps*16 chloroplast intron, *trn*F-L chloroplast intron and spacer) of *Hintonia latiflora* and *Hintonia standleyana* was completed. Following amplification and sequencing, the *rps*16 intron was found quite variable in length in the studied species: 746 bp in *H. latiflora* and 738 bp in *H. standleyana*. The reason for this length difference is the sequencing error of *rps*16 sequence in *H. standleyana* between 193 and 198 bp. The divergence was 1.1 %, but without the erroneous region only two well determined position differences were detected. These changes occurred between pirimidin bases (T or C), so they were transition.

The length of ITS region was 599 bp in *H. standleyana* collected from Mexico, Guerrero, Rio Zopilote and 577 bp in *H. latiflora* from Mexico, Guerrero, San Valentino. After the optimization of the alignment, the divergence between the two sequences of the above taxa was only 0.9%. In this case, the main, well determined nucleotide changes were a transition (T or C) and a transversion (A or C). These changes can be considered as the initial steps of separation in the course of evolution. Two ITS sequences were chosen from GenBank, these species were collected from Guatemala (*H. standleyana*) and from Mexico, Sonora, Rio Mayo (*H. latiflora*). The length of the two ITS sequences was 620 bp and divergent from each other with 1,5 %. The *trn*L-F sequences were also chosen from GenBank. The 935 bp-long *trn*L-F sequences showed 99.4 % identity. The sequence regions with differences involved both transition (T and C) and transversion (A and T, C and A, G and T).

Sequence differences were determined between *rps*16, ITS and *trn*L-F DNA regions of two *Hintonia* taxa. The level of differences in the *rps*16 sequences in the studied taxa was similar to that of all other, widely accepted species of the Cinchonideae subfamily and the *Randia* species analysed by us. Therefore we propose to treat *Hintonia latiflora* and *H. standleyana* as separate species. The ITS sequence differences between the two species are in connection with the place of origin. Although low, the level of divergence is the same as that between other, widely accepted species belonging to the Catesbaeeae-Chiococceae complex, to which also *Hintonia* taxa belong.

5. SHORT SUMMARY OF NEW RESULTS

The examination of micromorphological leaf venation of some species in the *Exostema* and *Rondeletia* genera revealed that these characters vary within a single leaf in the majority of cases. So the taxonomical significance of these features must be handled with care. These variations, however, may be typical of a species, in this case we can use these features as collateral information on the characterization of the species and some taxonomic divisions.

The number of veinlet terminations per unit area — if taking into consideration the average values of studied taxa — showed diagnostic variance on the generic level. In a similar approach there was no difference between the genera in the number of vein islets per unit area.

The vein islets which comprise 2, 3 and 4 vein terminations are typical of species in the *Rondeletia* genus, while in *Exostema* species areoles which contain many (even 10-12) veinlets dominate besides the previously mentioned categories (the latter type of vein islets is totally absent in *Rondeletia* species).

Quadrangular or pentagonal areoles and simple, linear or curved veinlets without branching are typical of the studied species in the *Rondeletia* genus, whereas triangular or quadrangular and circular vein islets, and once or twice branching veinlets are characteristic of the *Exostema* genus. The morphology of areoles and veinlets showed uniform aspect at section level only within the *Exostema* genus.

In the study of the Rondeletia complex we introduced ITS, as a new sequence into the molecular analysis. The results confirmed the genus level separation of three groups (*Rondeletia*, *Rogiera*, *Arachnothryx*) within the complex.

The phylogenetic analysis of two DNA sections, the ITS region and the chloroplast *rps*16 intron, was completed in order to clarify the inner division of the *Randia* genus. It was proved that the studied species can be divided into two groups, whose existence is supported also by morphological differences (leaf size and exocarp).

Within the *Hintonia* genus species level differences were discovered between *H. latiflora* and *H. standleyana*, by studying various DNA sections.

6. LIST OF CANDIDATE'S PUBLICATIONS

Scientific papers serve as a basis of dissertation:

STRANCZINGER SZ., BORHIDI A., SZENTPÉTERI J. L., JAKAB F. (2007): The phylogenetic relationships among some *Randia* (Rubiaceae) taxa. – *Acta Biologica Hungarica* **58** (2). in press.

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BORHIDI A., DARÓK J., KOCSIS M., **STRANCZINGER SZ.**, KAPOSVÁRI F. (2004): El *Rondeletia* complejo en México. – *Acta Botanica Hungarica* **46** (1-2): 91-135.

STRANCZINGER SZ., DARÓK J. (2002): *Exostema* fajok (*Rubiaceae*) levélerezetének makro- és mikromorfológiai vizsgálata. – In: SALAMON-ALBERT É. (szerk.): Magyar Botanikai kutatások az ezredfordulón. Tanulmányok Borhidi Attila 70. születésnapja tiszteletére. PTE Növénytani Tanszék Pécs, 227-234.

STRANCZINGER SZ., DARÓK J. (2001): *Exostema* (*Rubiaceae*) fajok levélerezetének összehasonlító vizsgálata. – In: DARÓK J.: Taxonómiai és anatómiai tanulmányok a *Rubiaceae* családban. Pécs, 129-149.

Scientific poster and oral presentations serve as a basis of dissertation:

STRANCZINGER SZ., SZENTPÉTERI J. L., BORHIDI A. (2005): The molecular approach of different taxonomical levels in Rubiaceae. XVII International Botanical Congress Vienna, Austria, 439. o.

STRANCZINGER SZ., SZENTPÉTERI J. L., BORHIDI A. (2004): Molecular phylogenetic studies of the Rubiaceae. 14th FESPB Congress. Cracow, Poland, 63. o.

STRANCZINGER SZ. (2003): Molekuláris filogenetikai és levélerezet vizsgálatok a Rubiaceae családban. A PAB Sejtbiológiai Munkabizottságának Doktorandusz Szimpóziuma.

STRANCZINGER SZ., SEBERG O., BORHIDI A. (2003): DNS-szekvenciaelemzés a növény-rendszertan szolgálatában. A DNS 50 éve. A PAB Sejtbiológiai Munkabizottságának rendezvénye.

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