Acta Bot. Croat. 69 (1), 71–82, 2010

CODEN: ABCRA 25
ISSN 0365–0588

# Multilocus genomic associations among selected taxa of genus *Potentilla* (Rosaceae) in Poland using RAPD analysis

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Nine taxa of *Potentilla* species from Poland representing *P.* sect. *Terminales* (Dőll.) Gren et Godr. and *P.* sect. *Aureae* (Wolf) Juz. were analyzed via a series of random amplified polymorphic DNA (RAPD) analyses to test (1) the hypothesis that the six species representing *P.* subsect. *Collinae* Juz. of the *P. Terminales* sect., i.e. *P. collina* Wibel, *P. thyrsiflora* Zimmeter, *P. wimannania* Günther et Schummel, *P. leucopolitana* P. J. Müll., *P.* ×*gabarae* Kolodziejek, *P. koernickei* Zimmeter, are genetically differentiated enough to be considered as separate taxa, and (2) the position of populations of *P. thyrsiflora* and *P. collina* with respect to the *Terminales* sect. (*P. argentea* L) and the *Aureae* sect. (*P. tabernaemontani* Ascherson and *P. incana* P. Gaertner, B. Meyer et Scherb.). RAPD-based genetic similarity values using the UPGMA method and corresponding dendrogram exhibited incomplete accordance between RAPD and morphological variations. According to 'overgenomic' associations based on a series of genomic loci selected at random, *P. thyrsiflora* and *P. collina* are closely related and similarly related to the species: *P. argentea*, *P. tabernaemontani* and *P. incana* hypothesized as being 'parental'. Within *Terminales* sect., our dendrogram shows *P. argentea* to be relatively isolated from the other members of the section analysed, *P. thyrsiflora* and *P. collina*.

**Key words:** average similarity, multilocus diversity, *Potentilla,* RAPD, *Rosaceae*, taxonomy

**Abbreviations**: RAPD – random amplified polymorphic DNA; UPGMA – unweighted pairs group method using mathematical averages

## Introduction

The genus *Potentilla* L. comprises approximately 490 species distributed mainly in the northern hemisphere (Eriksson et al. 1998, Soják 2005). Within *Potentilla* seven subgen-

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era have been distinguished, i.e. *Schistophyllidium* Juz., *Micropogon* Bge., *Fragariastrum* Ser., *Closterostyles* Torr. et Gr., *Hypargyrium* Fourr., *Dynamidium* Fourr. and *Argentina* (Lam.) Jepson (JUZEPCZUK 1941, SOJÁK 2005).

This study included seven species of the *P.* sect. *Terminales* (Dőll.) Gren. et Godr. (*P. argentea* and six species of *P.* subsect. *Collinae*) and two species of the *P.* sect. *Aureae* (Wolf) Juz. (*P. tabernaemontani* Ascherson and *P. incana* P. Gaertner, B. Meyer et Scherb.) within *P.* subgen. *Hypargyrium*.

In Europe, a total of 25 taxa have been described in the *P.* subsect. *Collinae*, most of them being not very well defined (as separate species, subspecies or varietas). This divergence in taxonomic viewpoints is accompanied by problems in distinguishing them and mistakes in the use of synonyms. Species identification strictly on the basis of morphological features is however difficult, because all species are closely related and morphologically similar. The high degree of polymorphism is partly caused by apomictic reproduction (pseudogamy) in conjunction with polyploidy and hybridization, as first pointed out by MÜNTZING (l928, 1931). In Poland, there are 12 taxa of *P.* subsect. *Collinae*, i.e. *P. collina* Wibel, *P. thyrsiflora* Zimmeter, *P. silesiaca* R. Uechtr., *P. wimannania* Günther et Schummel, *P. leucopolitana* P. J. Müll., *P. microdons* Schur, *P. schultzii* F. W. Schultz, *P. koernickei* Zimmeter, *P. leucopolitanoides* Błocki, *P. scholziana* Callier, *P. tynieckii* Błocki and *P.* ×*gabarae* Kołodziejek (*P. leucopolitana* P. J. Müll. × *P. incana* P. Gertner, B. Meyer et Scherb) (KOŁODZIEJEK unpublished).

Since it is widely accepted (ASCHERSON and GRAEBNER 1904, WOLF 1908, ASKER and FRŐST 1970, KURTTO et al. 2004) that the species of *P.* subsect. *Collinae* has been derived from natural hybridization between different *Potentilla* species, we attempted to use RAPD analysis to deduce the putative parents of this species. In morphology, the taxa of *P.* subsect. *Collinae* shows traits both from the *Terminales* (*P. argentea*) and the *Aurea* sections (*P. tabernaemontani* and *P. incana*). The characteristic features of the *P.* subsect. *Collinae* are primarily style conical shape with a few papillae at base, slightly clavate at apex; leaflets 5–7 oblong-obovate or oblanceolate, variously toothed, sparsely pubescent to white-tomentose or sericeous beneath with mixture of four types of hairs, i.e. crispate, straight, curved and imperfectely stellate. In contrast, *P. argentea* has a subcylindrical style, a dense crispate indumentum on the lower side of leaves and leaves with inrolled margins. The *P.* subsect. *Collinae* differs from the *P. tabernaemontani* and *P. incana* by its mixed (straight, curved, flexuous and incomplete stellate) hairs on the upper and lower side of the leaves. *P. tabernaemontani* have only straight hairs. From *P. incana* it differs by the lack of the stellate hairs. Apart from by the different indumentum, the members of the *Collinae* subsect. can easily be separated from species of the *Aureae* sect. (*P. tabernaemontani* and *P. incana*) by their style shape. In species of the *Aureae* sect., the styles towards the apex are widened.

The members of the *Collinae* subsect. occurs in isolated sites in the plains of Poland and have slightly different habitats. The species prefer open habitats, mostly on dry sandy soils and calcareous rendzinas. *Potentilla argentea* and *P. incana* can be found growing abundantly on dry mineral soils over the whole territory, while *P. tabernaemontani* occurs locally on dry soils in West, Central and South Poland. However, in a few localities, for instance on the Wyżyna Częstochowska upland the seven taxa, i.e. *P. collina*, *P. thyrsiflora*, *P. wimannania*, *P. leucopolitana*, *P. tabernaemontani*, *P. incana* and *P. argentea* occurred side by side on gravelly or sandy, not or only slightly calcareous ground (KOŁODZIEJEK 2004).

In the present paper we explore the value of a detailed, molecular (RAPDs) approach for unravelling complex variation at low taxonomic level, in order to use this approach to (1) test the hypotheses whether the morphologically defined species of the *Collinae* subsect. represent genetically distinct units. (2) secondly, we consider the position of *P. thyrsiflora* and *P. collina* with respect to the *Terminales* (*P. argentea*) and *Aureae* sections (*P. tabernaemontani* and *P. incana*), in order to obtain a better taxonomic classification of *Potentilla*.

## Material and methods

### Plant material and genomic DNA isolation

In this study, we investigated six species of *P.* subsect. *Collinae*, i.e. *P. collina, P. thyrsiflora, P. wimannania, P. leucopolitana, P. koernickei, P.* ×*gabarae* and a group representing the *Terminales* and *Aureae* sections, i.e. *P. argentea, P. tabernaemontani* and *P. incana* considered (ASCHERSON and GRAEBNER 1904, WOLF 1908, ASKER and FRŐST 1970) as the potential parental species for the group. The geographic localities and information on the population samples are given in table 1. Some of the plants investigated were preserved in the form of herbarium specimens and deposited in the Department of Geobotany and

**Tab. 1.** Collection data from *Potentilla* material sampled from cytogenetic analyses. The chromosome counts after ILNICKI and KOLODZIEJEK (2008).

| Population code | Taxon | 2n | Sample collection site |
|---|---|---|---|
| A | *P. collina* | 28,35 | Silesia prov., Kłobuck 50°57'N/18°59'E |
| B | *P. collina* | 28,35 | Silesia prov., Rzędkowice 50°35'N/19°27'E |
| C | *P. collina* | 28,35 | Silesia prov., Mirów 50°37'N/19°28'E |
| D | *P. thyrsiflora* | 21,28,35 | Silesia prov., Cisowa near Pilica 50°26'N/19°42'E |
| E | *P. thyrsiflora* | – | Silesia prov., Ostra Góra near Siewierz 50°27'N/19°09'E |
| F | *P. wimannania* | 35 | Silesia prov., Mirów 50°37'N/19°28'E |
| G | *P. leucopolitana* | 35 | Pomerania prov., Chałupy 54°43'N/18°23'E |
| H | *P.* ×*gabarae* | – | Silesia prov., Jaroszów village near Żarki 50°39'N/19°21'E |
| I | *P. koernickei* | 28,35,42 | Pomerania prov., Czarna Woda near Czersk 53°51'N/18°08'E |
| J | *P. argentea* | 35 | Silesia prov., Cisowa near Pilica 50°26'N/19°42'E |
| K | *P. argentea* | 35 | Łódź prov., Bolimów Landscape Park 52°05'N/20°10'E |
| L | *P. tabernaemontani* | – | Małopolska prov., Szaflary 49°25'N/19°33'E |
| M | *P. tabernaemontani* | – | Silesia prov., Jaroszów village near Żarki 50°39'N/19°21'E |
| N | *P. incana* | 28 | Ojców Nationale Park 50°14'N/19°50'E |
| O | *P. incana* | 28 | Sląsk prov., Kusięta village nar Olsztyn 50°45'N/19°16'E |

Plant Ecology of the University of Lodz. Others were transplanted into the experimental garden for further studies. The selected species of *Collinae* are the most common taxa of the subsection occurring in Poland. Two of them, i.e. *P. collina* and *P. thyrsiflora* are encountered in relatively the greatest number of localities and their populations are the richest, consisting of more than fifty individuals. The relative abundance and incidence of the accessions observed for these two species suggests more frequent genetic material exchange between them and the species of the *Terminales* and *Aureae* sections.

The studied plant material was collected during field trips in Poland. For RAPD analysis we used five plants representing each population. Each plant selected was separated from another by at least 0.5 m. All of them were dug in May – July. Leaves were dried in fine-grained silica gel.

The samples of the plant genomic DNA were extracted from approximately 0.25 g of fresh and young leaves from field-collected plants. The frozen material, prior to the cell-lysis, was disrupted by grinding in liquid nitrogen, then digested by RNase-A in a lysis buffer at 65°C. The extraction itself, performed using DNeasy Plant Mini Kit (Qiagen), was followed by the spectrophotometrical quantification at 260 nm. The UV-spectrophotometer Hitachi U-2000, Japan was used for determination of DNA purity and concentration.

Similar to the other experimental work (e.g. ROMÁN et al. 2003, SAKOWICZ and CIEŚLIKOWSKI 2006), ours followed the template-mixing strategy where equal amounts of working solution DNAs from each group of individuals of the same species were pooled as the 'species-template DNA' prior to the PCR reaction. DNA was extracted from single plants and each population was represented by the bulk of a variable number of individuals, depending on the availability of samples after collection. That was to increase the relative contribution of the common target sequences and consequently to highlight the species-specific features (amplicons) at the step of amplification, before starting numerical analysis.

**RAPD amplification**

The random polymorphic DNA amplification was performed according to the subtle modified (temperature profile) procedure of WILLIAMS at al. (1990). The reaction mixture included 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ (Finnzyme, Finland), 0.2 mM dNTP (Promega, USA). The aliquot of 25 μL contained: the reaction mixture, 30 ng of primer (SIGMA-ARK, Germany), 5 ng of genomic DNA and 2 units of termostable polymerase Dynazyme™ (Finnzyme, Finland) and Milli-Q water (Millipore, Austria). The temperature profile was as follows: initial denaturation at 95 °C for 1 min., followed by 35 cycles (denaturation at 94 °C for 1 min., annealing for 1 min. and extension at 72 °C for 2 min.) with a final extension on the step at 72 °C for 10 min. The annealing temperature was primer-specific and was each time 5°C below its melting temperature supplied by the manufacturer – SIGMA-ARK (Tabs. 2, 3). The amplification was performed in thin-wall vials (MJ Research, USA) with a thermocycler Uno II (Biometra, Germany). The amplification products were separated against molecular-weights marker (100 bp DNA ladder, MBI Fermentas) in 1.5% agarose gel and TAE buffer (40 mM TRIS-acetate, 1 mM EDTA, pH 7.8) at 80 V in MGU 602T electrophoresis unit (CBS Scientific, USA). The agarose gel stained with ethidium bromide was visualized under ultra-violet light and documented using computer image system (Vilber Lourmat, France, Agarose, TAE, EtBr from SERVA –

Germany). The gel stained with ethidium bromide was visualized under ultra-violet light and documented using computer image system (Vilber Lourmat, France).

### Numerical analysis of DNA fingerprinting electrophoretic patterns

The computer assisted analysis was carried out with the aid of BioNumerics programme (Applied Maths, Kortijk, Belgium), and the unweighted pairs group method using mathematical averages – UPGMA was used for the data cluster analysis. Numbers at branches are bootstrap values (%) generated after 1000 replications. According to specificity of the RAPD method, the electrophoretic patterns were compared on the basis of the whole densitometric curve shape and the Pearson's product-moment correlation coefficient has been applied. The final dendrograms describing averaged phenetic similarities were calculated according to UPGMA algorithm and euclidean distances (Statistica 5.1, Statsoft). The bootstrap method (FELSENSTEIN 1985) employed to evaluate the reliability of tree topology was evaluated after 1000 samples. The calculations were performed with the MEGA 4.0. software (TAMURA et al. 2007).

## Results

### RAPD-based genetic diversity among the six species of *P.* subsect. *Collinae*

RAPDs generated a total of 110 bands using 13 decamer primers (an average of 8.5 bands per assay) of which 39.1% were polymorphic. The size of amplification products ranged between 300–800 bp (Tab. 2).

The dendrograms formed (not shown) as a result of 13 experiments were diverse in their shape and global homology level. For seven out of the thirteen RAPD tests, *P. leucopolitana* and *P. koernickei* were rather similar and their electrophoretic patterns

**Tab. 2.** Names, percentage of polymorphic bands and melting temperatures (Tm) of different primers used to RAPD analysis for six species of *P.* subsect. *Collinae*.

| Code | Sequence 5' to 3' | Percentage of polymorphic bands | Tm (°C) |
|---|---|---|---|
| J-01 | TGGGTCCCTC | 42.9 | 34 |
| J-02 | CGGCGGACTA | 40.0 | 38 |
| J-03 | GGCGGATAGC | 50.0 | 34 |
| J-04 | GAGTCAGCAG | 36.4 | 32 |
| J-05 | GTCAGGGCA | 37.5 | 32 |
| J-06 | GTCAGGGCAA | 33.3 | 32 |
| J-07 | TCACGTCCAC | 40.0 | 32 |
| J-08 | CAGGGGTGGA | 41.7 | 34 |
| J-09 | CAGGGGTGGA | 40.0 | 34 |
| J-10 | GGGCCGTTTA | 28.6 | 32 |
| J-11 | GCCCTCGGAT | 44.4 | 32 |
| J-12 | GTGCGCGACC | 37.5 | 29 |
| J-13 | TGAGGGTCCC | 36.4 | 34 |
| | | 39.1 (average) | |

formed common subcluster. These two species were weakly associated with *P.* ×*gabarae*. In 8 experiments, three populations of *P. collina* were clustered altogether, while in 3 RAPD tests they were clustered together with *P. wimannania*.

In an analysis of the total RAPD data set, three very distinct clusters of RAPD phenotypes (Fig. 1) appeared. The first distinct minor cluster consisted of *P. leucopolitana* and *P. koernickei*. These species showed a similarity coefficient of 0.43 with the cluster supporting by a bootstrap value of 82%. The middle cluster included *P. thyrsiflora* (population D). The third cluster was further divided into two, somewhat less distinct, subclusters of which the left included population of *P. collina* located in Mirów (population C), *P. wimannania*, *P.* ×*gabarae*, and while the right subcluster was formed by two populations (A, C) of *P. collina* and *P. thyrsiflora* (population E).

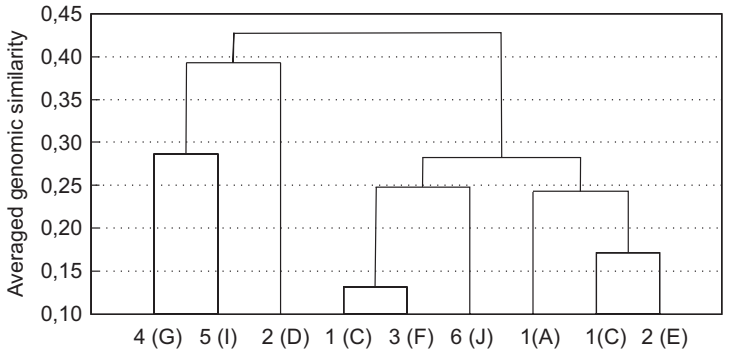


**Fig. 1.** Taxonomic similarity among the *Potentilla* subsect. *Collinae* Juz. species – the final dendrogram. The averaged similarity between nine populations found on the basis of thirteen RAPD analyses.

In the present study, significant divergence was found between two *P. thyrsiflora* populations, and the genetic distance values between populations was 0.48. Populations from different sites did not cluster together. One RAPD phenotype of *P. thyrsiflora* observed in plants from the Cisowa population clustered at a high level (0.49) with the *P. leucopolitana* and *P. koernickei* cluster, and the other phenotype of *P. thyrsiflora* observed in the Ostra Góra population from clustered well within the *P. collina* subcluster. The associations determined with RAPD markers among *P.* subsect. *Collinae* showed genetic similarity coefficients ranging from 0.22 (population C of *P. collina* and *P. wimannania*) to 0.55 (*P. leucopolitana*, *P. koernickei* and *P. thyrsiflora*) revealing medium levels of genetic variation among the species studied.

### RAPD-based genetic diversity among *P. collina*, *P. thyrsiflora*, and the putative parents

Using 19 decamer primers, a total of 168 RAPD marker loci were scored (an average of 8.8 bands per primer) and 43.4% were polymorphic over all accessions (Tab. 3).

For most of the RAPD tests the electrophoretic patterns of *P. collina* and *P. thyrsiflora* formed common cluster (15/19 RAPDs) or were weakly connected in a stair shape manner (4/19 RAPDs), while the species representing *Aureae* sect. remained separated (not

**Tab. 3.** Names, percentage of polymorphic bands and melting temperatures (Tm) of different primers used to RAPD analysis for *Potentilla collina* and *P. thyrsiflora*, and the putative parents, i.e. *P. argentea, P. tabernaemontani* and *P. incana*.

| Code | Sequence 5' to 3' | Percentage of polymorphic bands | Tm (°C) |
|---|---|---|---|
| S-01 | GTCAGGGCAA | 37.5 | 32 |
| S-02 | GGGCTCGTGA | 54.5 | 34 |
| S-03 | GAGTCAGCAG | 44.4 | 32 |
| S-04 | TGGGGGTCCC | 33.3 | 36 |
| S-05 | GGCGGATAGC | 50.0 | 34 |
| S-06 | CAGGGGTGGA | 28.6 | 34 |
| S-07 | CCTGGGCCAC | 44.5 | 36 |
| S-08 | CCCGCCTCCC | 50.0 | 38 |
| S-09 | GAGCACTAGC | 45.5 | 34 |
| S-10 | GAGCACGGGA | 44.4 | 34 |
| S-11 | ATCTGCGAGC | 50.0 | 32 |
| S-12 | TCCGATGCTG | 42.9 | 32 |
| S-13 | ACCGTCGGCA | 37.5 | 34 |
| S-14 | GCTTGCACCG | 45.5 | 35 |
| S-15 | CTACCGTGGC | 58.3 | 36 |
| S-16 | AGGGGCGGTC | 33.3 | 36 |
| S-17 | GTCCACACGG | 42.6 | 32 |
| S-18 | GTGTGAGAGA | 37.5 | 31 |
| S-19 | CCAGTGCATG | 44.4 | 31 |
| | | 43.4 (average) | |

shown). The internal homology level of *P. collina* and *P. thyrsiflora* ranged between 75% and 30% (in stair-shaped dendrograms). The separation of *P. collina* and *P. thyrsiflora* was distinct in two tests. These two species were occasionally included into the *Aureae* sect. In 11 experiments, *P. tabernaemontani* and *P. incana* of the *Aureae* sect. were gathered together, while *P. argentea* clustered out of this section, or was weakly associated with it.

Cluster analysis, presented in the form of a final dendrogram grouped all species into two groups (Fig. 2). The first group is represented by two species, *P. collina* and *P. thyrsiflora* with the high genetic similarity of 0.88. The second, mixed cluster is formed by three species, i.e. *P. argentea*, *P. tabernaemontani* and *P. incana*. (supported by a 64% BS). Out of these species, *P. tabernaemontani* and *P. incana* are joined as a distinct minor subcluster with a low genetic similarity (0.35), supported by a bootstrap value of 89%.

Interestingly, the analysis of species similarity showed that RAPD markers placed *P. argentea* (*Terminales* sect.) closer to *P. tabernaemontani* and *P. incana* (*Aureae* sect.) than to *P. collina* and *P. thyrsiflora*, both from the *Terminales* section (Fig. 2). The association of *P. argentea* and the subcluster containing *P. tabernaemontani* and *P. incana* was supported by a moderate bootstrap (BS) value of 64%. *P. tabernaemontani* and *P. incana* (classified in the same section) showed a similarity coefficient of 0.35 with the cluster supported by a bootstrap value of 81%.

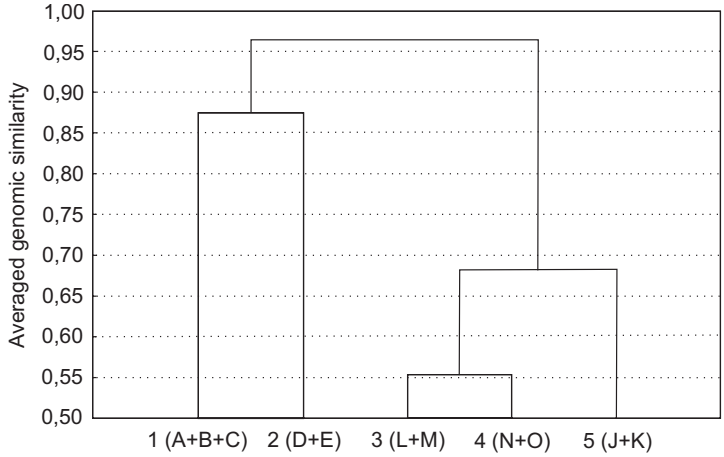


**Fig. 2.** Taxonomic similarity among *Potentilla collina*, *P. thyrsiflora*, and the putative parents, i.e. *P. argentea*, *P. thyrsiflora* and *P. incana* – the final dendrogram. The averaged similarity among five species found on the basis of nineteen RAPD analyses.

## Discussion

In the investigation presented here, 39.1% of the RAPD fragments were polymorphic. Compared to other plant species, the members of *Collinae* subsect., therefore, showed a medium level of genetic variability. In a study of the widespread *Tanacetum vulgare*, 85% polymorphic bands were observed (), in the rare *Vicia pisiformis* 7.2% () and in apomictic species of *Rosa* sect. *Caninae* only 3% (). These differences are not astonishing, since the level of genetic variability strongly depends on the plant's life history traits (). The species of the subsection are perennial, facultatively apomictic plants with a narrow ecological amplitude and these biological characteristics all contribute to create and maintain the observed medium level of genetic variability.

Within *Terminales* sect., our dendrogram shows *P. argentea* to be relatively isolated from the other members of the section analysed, *P. collina* and *P. thyrsiflora* (Fig. 2). Previous morphological studies have also found the same differentiation between *P. argentea* and other members of the section (LEHT 1997).

In earlier studies (KOŁODZIEJEK 2007, KOŁODZIEJEK and GABARA 2007, 2008), based on morphological analyses in *P.* subsect. *Collinae* material, i.e. *P. collina, P. thyrsiflora*, *P. wimannania*, *P. leucopolitana*, *P.* ×*gabarae* and *P. koernickei*, was identified at the species level. However, these conclusions do not fit our observations based on RAPD markers. The clustering obtained with morphological characters after research based largely upon herbarium material (KOŁODZIEJEK data not shown) was not altogether compatible with the dendrogram based on RAPD marker. The absence of a relationship between the morphological and genetic similarities was also found for wild populations of other plants (GREENE et al. 2004, STEINER and SANTOS 2001). Several reasons may account for the discordance between the morphological traits and RAPD marker. First, molecular markers represent a sample of the plant genome, and even so, are used to make an inference concerning the whole genome. Second, morphological variation is strongly associated with environmental

variation; the morphological similarities observed may be due to different combinations of alleles producing similar phenotypes that might result in morphological similarities or differences that are not proportional to the underlying genetic differences. Third, the *P.* subsect. *Collinae* is a very variable species complex in some respects appearing intermediate between *P. argentea*, *P. tabernaemontani*, *P. incana* and regarded by some authors as derived from hybridisation between them, the occurrence of polyploids and ability to reproduce both sexually and apomictically (MÜNTZING 1928, ASKER and FRŐST 1970, HOLM and GHATNEKAR 1996, GREGOR at al. 2002). Apparently, taxa of the *Collinae* subsect. occasionally hybridise with each other and with their parents (KURTTO et al. 2004).

The similarity between *P. leucopolitana* and *P. koernickei* has been a matter of debate. These two species have been traditionally considered to be closely related, and *P. koernickei* have even been treated as forms within *P. leucopolitana* by some authors (e.g. WOLF 1908). In contrast, other authors have classified both taxa as separate species (ZIMMETER 1887). However, recent morfological studies (data not shown), clearly support the separation of *P. leucopolitana* from *P. koernickei*. Our results also support these findings since the dendrogram separated both species.

The RAPD variation in four other species of *P.* subsect. *Collinae*, i.e. *P. praecox*, *P. alsatica*, *P. leucopolitana* and *P. alpicola* and the putative parents, i.e. *P. argentea*, *P. incana* and *P. tabernaemomtani* has been investigated earlier GREGOR et al. 2002). According to their research, *P. argentea* is probably a parental species of *P. praecox*, *P. alsatica*, *P. leucopolitana* and *P. alpicola*, and *P. tabernaemontan* might be the parent species of *P. lindackeri*. Based on chemotaxonomical studies, ASKER and FRŐST (1970) showed a close relationship between taxa from *P.* subsect. *Collinae* and *P. argentea* and *P. tabernaemontani*. According to theim, the taxa of *P.* subsect. C*ollinae* could have emerged from crosses between *P. argentea* of the *Terminales* sect. on one side, and *P. tabernaemontani* or *P. incana* of the *Aureae* sect. on the other side. Even other taxa from the *Aureae* sect., such as *P. tommasiniana* F. W. Schultz and *P. gaudinii* Gremli (= *P. pusilla* Host), can participate in the emergence of new species belonging to *P. collina* (GUSTAFSSON 1947). The molecular data thus are consistent with the supposed intermediacy in morphological features, which has been cited to support the hybrid origin of the taxa of *P.* subsect. C*ollinae* (ASCHERSON and GRAEBNER 1904, WOLF 1908, BALL et al. 1968). As has been noted to be the case for many hybrids and hybrid derivatives (RIESEBERG 1995), the morphology of the taxa of *P.* subsect. C*ollinae* is not strictly intermediate between its putative parental species, but rather consists of a mixture of qualititative characters that match one or the other parental species as well as intermediacy in quantitative characters.

## Conclusion

Morfological differences and traditional taxonomy practice seem to justify recognition of the six taxa *P. collina*, *P. thyrsiflora*, *P. wimannania*, *P. leucopolitana*, *P.* ×*gabarae* and *P. koernickei* as a separate taxa at species level. However, the grouping of nine populations representing the six species within *Collinae* subsect. in the RAPD trees, as partitioned in traditional taxonomic treatments, was not altogether compatible with the well defined species.

The authors state (ASKER and FRŐST 1970, GREGOR et al. 2002). that molecular data agree with the intermediacy of species of *Collinae* subsect.between *P. argentea* (*Termi-*

*nales* sect.), *P. tabernaemontani* and *P. incana* (*Aureae* sect.), but our own results do not agree with this.

Further analyses are needed to determine the correct infrageneric taxonomic treatment of the *Collinae* subsect. as it is outside the *Terminales* sect. in our and LEHT (1997) analyses.

To obtain a deeper insight into the position of this species, it will be necessary to increase both the number of *Potentilla* populations and the number of specimens analysed.

## Acknowledgements

Part of this work was supported by grant No. 2 P04F 040 26 from the Ministry of Science and Information Society Technologies (KBN).

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