The agamic complex of *Pilosella* (Asteraceae) in Bulgaria and SW Romania: variation in ploidy level and breeding systems

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Abstract. Chromosome numbers and breeding systems are given for a set of *Pilosella* species occurring in Bulgaria and SW Romania (Banat). All diploids were found sexual, most of polyploids apomictic.

Key words: agamic complexes, breeding systems, Bulgarian flora, chromosome numbers, *Hieracium* s.l., *Pilosella*, ploidy levels, Romanian flora

Introduction

Pilosella Vaill. (syn. *Hieracium* subgen. *Pilosella*) is notorious for its taxonomic complexity caused by co-occurrence of hybridization, polyploidy and apomixis. Taxonomic treatment of the genus varies greatly, depending on the understanding of the adopted group and the species concept (cf. Zahn 1923; Sell & West 1975, 1976). In order to reveal the causes of the great morphological variation within the genus and the microevolutionary processes, a series of studies have been carried out at local population level in Central Europe during the past two decades (e.g. Krahulec & al. 2004, 2008; Fehrer & al. 2005). It has been confirmed that from biosystematic point of view the genus is a huge agamic complex involving few sexual – all diploid and some polyploid species – and many facultatively apomictic polyploid taxa (Fehrer & al. 2007). This complex turned out to be a very suitable model for investigation into the breeding systems, hybridization and ploidy level differentiation at population level and, thus, for better understanding of the microevolutionary processes (Krahulcová & al. 2009).

Literature data suggest that in Europe more diploid species of *Pilosella* are concentrated in the southern parts of the continent, especially in the Balkan and Iberian Peninsulas and adjacent territories (Merxmüller 1975; Vladimirov 2000; Szeląg 2008; Šingliarová & Mráz 2009). The aim of the present work was to study the breeding systems, ploidy levels and hybridization between co-occurring taxa in selected localities in Bulgaria and Southwest Romania with higher share of diploid species and to compare the results with the observed patterns from Central Europe.

Materials and methods

During our studies of *Pilosella* populations in Bulgaria and Romania we have accepted the same approach as we used in Central Europe (Krahulec & Krahulcová 2006). We have studied selected populations in

which we sampled all morphologically distinguishable plants, preferring to collect them with more specimens. This approach allowed us to treat the whole population as one entity. The plants were transported to the experimental gardens in Průhonice (Czech Republic) and Sofia (Bulgaria). We determined either their DNA ploidy level using flow cytometry, or the chromosome number (chromosome counts done by AK or VV). The methods used have been described in detail in Krahulcová & al. (2004) for the flow cytometry and in Krahulcová & Krahulec (1999) and Vladimirov & Szeląg (2001) for the chromosome counting. The breeding system was determined by comparison of the seed production in emasculated versus open pollinated plants (Krahulcová & Krahulec 1999). All plants coming from Bulgaria were collected by F. Krahulec, V. Vladimirov and A. Krahulcová during common field trips in 2005, 2006 and 2007. The plants from Romanian Banat were collected in 2006 by Zdeněk Skála and Hana Skálová (Průhonice). The localities of the Bulgarian (Bu) and Romanian (Ro) populations are given in the Appendix: they are numbered consecutively, according to their position within each country from the north to the south and from the west to the east. For local names in Banat (Romania) we used the Czech version of the map of Banat (Banát, 2004. Turistická mapa 1:100 000. Edice klubu českých turistů. 2. vydání 2005).

The taxonomic concept of *Pilosella* as a separate genus, as well as the species and hybrid concept (incl. names) corresponds to the classification by Bräutigam & Greuter (2007, 2008). There is a problem concerning the names of recent hybrids and of the hybridogenous species: for distinguishing between these two categories see also Krahulec & al. (2008), Krahulcová & al. (2009). The names of hybrids within the genus *Pilosella* are used at present (Bräutigam & Greuter 2007) in the form of hybridogenous species, without the symbol „×“ indicating their hybrid origin. This approach is not completely correct, because many of them were described as recent hybrids. We think that all types of hybrid origin found by us in the field in Bulgaria were recent hybrids. For that reason we have used hybrid formulae and not the names of hybridogenous species.

Voucher specimens of all plants studied at Průhonice by A. Krahulcová and F. Krahulec are deposited in the Herbarium of the Institute of Botany, Průhonice (PRA); voucher specimens of plants studied by V. Vladimirov are deposited in the Herbarium of the Institute of Botany, Sofia (SOM).

Results and discussion

An annotated list of species and hybrids is provided below. The symbols for locality and floristic region of the Bulgarian plants are supplemented for each cytotype. Full description of the localities is given in Appendix 1. The studied specimens are numbered by AK & FK and kept in PRA.

Appendix 1. List of localities and co-occurring species. Ploidy levels of the particular taxa and hybrids are given in parentheses.

Bulgaria (collected by *F. Krahulec*, *A. Krahulcová* and *V. Vladimirov*, determined by *S. Bräutigam*, *F. Krahulec* and *V. Vladimirov*):

Bu 1 Forebalkan (*Western*): meadow on the western slope at the wood margin near the Chernila Dam, *ca.* 3 km S of Montana town, 43°22'29.8"N, 23°14'37.5"E, 180–185 m, 12.05.2005, 27.06.2006.

Recorded taxa: *P. piloselloides* subsp. *magyarica* (5*x*, 6*x*), *P. piloselloides* subsp. *bauhinii* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (4*x*, 5*x*, 6*x*).

Bu 2 Forebalkan (*Western*): Mt Vrachanska, meadows along the road from Vratsa to Parshevitsa chalet near the Kiparis Hotel, *ca.* 6 km SW of Vratsa town, 43°10'33.9"N, 23°28'54.8"E, 1054 m, 11.05.2005, 26.06.2006.

P. piloselloides subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. onegensis* (2*x*), *P. officinarum* (6*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*).

Bu 3 Forebalkan (*Western*): Mt Vrachanskat, meadow above the road from Vratsa to Parshevitsa chalet, *ca.* 7.5 km SW of Vratsa town, 43°10'14.3"N, 23°28'50.1"E, 1159 m, 11.05.2005, 26.06.2006.

P. piloselloides subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. onegensis* (2*x*), *P. officinarum* (5*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*).

Bu 4 Balkan Range (*Western*): wet meadow below the road in the Petrohan saddle between Mt Berkovska and Mt Koznitsa, *ca.* 12 km S of Berkovitsa town, 43°07'06.4"N, 23°07'36.4"E, 1394 m, 12.05.2005, 27.06.2006.

P. hoppeana subsp. *testimonialis* (2*x*), *P. onegensis* (2*x*), *P. officinarum* (4*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*).

Bu 5 Sofia Region: Sofia, grassy place in the campus of the Academy of Sciences, close to the building of the Institute of Botany, 42°40'25.2"N, 23°22'06.2"E, 556 m, 30.06.2006.

P. piloselloides subsp. *magyarica* (6*x*).

Bu 6 Vitosha Region: in the valley E of Mt Vitosha, grassy place along the road near Zheleznitsa village, 42°32'44.1"N, 23°21'49.1"E, 1042 m, 08.06.2007.

P. piloselloides subsp. *magyarica* (6*x*), *P. officinarum* (5*x*, 6*x*);

hybrid: *P. officinarum* × *P. piloselloides* (6*x*).

In addition, *P. onegensis* (the ploidy level not studied) was also recorded at this locality.

Bu 7 Vitosha Region: in the valley E of Mt Vitosha, grassy places and road margin at the highest point of the road between Kovachevitsa village and the road fork to Plana village, 42°30'35.5"N, 23°20'00.2"E, 1296 m, 08.06.2007.

P. piloselloides subsp. *magyarica* (6*x*), *P. officinarum* (6*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*).

Bu 8 Vitosha Region: abandoned pasture above the road N of Kovachevitsa village, in the valley between Vitosha and Verila Mts, 42°27'39.9"N, 23°19'48.2"E, 1074 m, 30.06.2006, 08.06.2007.

P. piloselloides subsp. *magyarica* (5*x*, 6*x*), *P. piloselloides* subsp. *bauhinii* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (4*x*, 5*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*).

Bu 9 Pirin Mts: rocky places along the hiking trail above the Vihren chalet, 41°45'23.7"N, 23°24'55.3"E, 2014 m, 28.06.2006.

P. alpicola (3*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (6*x*).

Bu 10 Pirin Mts: on the northwestern shore of lake Bezbog, 41°43'50.4"N, 23°31'23.8"E, 2254 m, 29.06.2006.

P. pseudopilosella (2*x*).

Bu 11 Pirin Mts: along the hiking trail from the uppermost lift station at Bezbog chalet to the lowermost lift station, 41°44'22.3"–41°45'32.2"N, 23°31'37.6"–23°32'21.5"E, 2150–1656 m, 29.06.2006.

P. pseudopilosella (2*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. alpicola* (2*x*), *P. onegensis* (2*x*), *P. pavichii* (2*x*, 4*x*), *P. officinarum* (6*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (6*x*), *P. pavichii* × *P.* sect. *Pilosellina* (6*x*).

Bu 12 Rhodopi Mts (*Central*): along the road from Lyaskovo village to Asenovgrad town, 41°59'34.8"N, 24°49'45.3"E, 840 m, 05.06.2007.

P. hoppeana subsp. *testimonialis* (2*x*).

Bu 13 Rhodopi Mts (*Central*): along the road from Lyaskovo village to Asenovgrad town, 41°59'30.4"N, 24°51'11.5"E, 698 m, 05.06.2007.

P. hoppeana subsp. *testimonialis* (2*x*), *P. piloselloides* subsp. *magyarica* (6*x*).

Bu 14 Rhodopi Mts (*Central*): in the vicinity of Bachkovo Monastery, the meadow and wood along the hiking trail to peak Chervenata Stena, *ca.* 12 km S of Asenovgrad town, 41°56'15.5"–41°56'28.1"N, 24°51'45.4"–24°51'25.9"E, 469–506 m, 13.05.2005.

P. piloselloides subsp. *magyarica* (5*x*, 6*x*), *P. officinarum* (6*x*), *P. pavichii* (5*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*).

Bu 15 Rhodopi Mts (*Central*): abandoned pasture and wood margin above the road at Ionchovoto hamlet, *ca.* 3 km N of Komuniga village, 41°50'18.9"N, 25°11'39.0"E, 770 m, 14.05.2005, 06.06.2007.

P. piloselloides subsp. *magyarica* (6*x*), *P. piloselloides* subsp. *bauhinii* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. pavichii* (2*x*), *P. officinarum* (6*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*), *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (6*x*), *P. pavichii* × *P. piloselloides* (6*x*) corresponding to *P. georgieffiana*, *P. pavichii* × *P. hoppeana* subsp. *testimonialis* (6*x*), *P. pavichii* × ? (3*x*).

Bu 16 Rhodopi Mts (*Eastern*): rocky grassland above the road *ca.* 2 km E of Kardzhali town, 41°38'09.1"N, 25°25'50.7", 260 m, 14.05.2005.

P. piloselloides subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (6*x*).

Romania (plants collected by *Zdenek Skála* and *Hana Skálová*, determined by *S. Bräutigam* and *F. Krahulec*):

Ro 1: Banat, Gârnic, valley NE of the village near Petrův Mlýn Mill, pastures along the brook, 44°45.59'N, 21°47.94'E, 580 m, 17.05.2006.

P. officinarum (5*x*, 6*x*).

Ro 2: Banat, Gârnic, valley on the NW part of the village near Filipova Díra cave, 44°45.27'N, 21°46.8'E, 610 m, 18.05.2006.

P. officinarum (6*x*).

Ro 3: Banat, Gârnic, pastures on the karst plateau SW of the village, near the road to Sfânta Elena, 44°44.73'N, 21°46.84'E, 650 m, 18.05.2006.

P. hoppeana subsp. *testimonialis* (2*x*), *P. officinarum* (5*x*, 6*x*).

Ro 4: Banat, Sfânta Elena, valley E of the road to Gârnic, N-NE of the village along the trail to the Kulhavá Skála rock, 44°42.1'N, 21°43.3'E, 330 m, 19.05.2006.

hybrid: *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (2*x*) corresponding to *P. hypeurya*.

Ro 5: Banat, Sfânta Elena, valley S of the village to Dunarea, in the valley of the Ali Beg brook, 44°40.38'N, 21°42.2'E, 350 m, 20.05.2006.

P. hoppeana subsp. *testimonialis* (2*x*), *P. piloselloides* subsp. *magyarica* (5*x*, 6*x*), *P. officinarum* (5*x*);

hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. piloselloides* (5*x*, 6*x*).

Basic species

Pilosella alpicola (Steud. & Hochst.) F.W. Schultz & Sch. Bip.

2*n* = 2*x*, sexual, almost sterile in experimental condition

Bu 11, Pirin Mts.: 1 plant no. 1118.

2*n* = 3*x* = 27, sexual, almost sterile

Bu 9, Pirin Mt.: 1 plant 1106.

The low seed-set recorded in these sexual biotypes under garden conditions need not necessarilly stand for their sterility in the natural populations. This mountain species, represented by a single plant (i.e. one cultivated genotype) per each cytotype, flowered earlier than other *Pilosella* types in the lowland garden. For this reason, the plants there probably lacked genetically compatible counterparts for fertilization needed to ensure the seed-set.

The plants from Bulgaria were recently evaluated by Šingliarová & Mráz (2009). They named all Bulgarian populations as *P. rhodopea* (Griseb.) Szeląg. Our results correspond to their unpublished data (2n = 2x–5x). The triploid plants have already been published by Vladimirov & Szeląg (2001) from the same locality.

Pilosella hoppeana (Schult.) F.W. Schultz & Sch. Bip. subsp. ***testimonialis*** (Peter) P.D. Sell & C. West (*P. hoppeana* subsp. *macrantha* auct. non Ten. 1830)

2*n* = 2*x*, sexual

Bu 1, Forebalkan (*Western*): 2 plants 969 (perished), 1100; **Bu 2**, Forebalkan (*Western*): 2 plants 948 (perished), 1094; **Bu 3**, Forebalkan (*Western*): 2 plants 962/1, 1081/1; **Bu 4**, Balkan Range (*Western*): 1 plant 1103; **Bu 8**, Vitosha Region: 7 plants 1136, 1146, 1274, 1275, 1280, 1286 and one plant with chromosome number determined as 2*n* = 18 (VV); **Bu 9**, Pirin Mts: 2 plants 1107, 1108; **Bu 11**, Pirin Mts: 6 plants 1114 (the breeding system yet unknown), 1116, 1117, 1120, 1123, 1129; **Bu 12**, Rhodopi Mts (*Central*): 1 plant 1234; **Bu 13**, Rhodopi Mts (*Central*): 1 plant 1232; **Bu 15**, Rhodopi Mts (*Central*): 4 plants 1238, 1252, 1258, 1262; **Bu 16**, Rhodopi Mts (*Eastern*): 2 plants 1008/1 and one plant with chromosome number determined as 2*n* = 18 (VV); **Ro 3**: 3 plants 1059, 1060 (perished), 1065; **Ro 5**: 3 plants 1055, 1056, 1057.

Pilosella officinarum Vaill.

2*n* = 4*x*, apomictic

Bu 4, Balkan Range (*Western*): 5 plants 976, 977, 978/2, 1102, 1104; **Bu 8**, Vitosha Region: 2 plants 1288/1, 1288/2 (the morphology is possibly slightly influenced by *P. piloselloides* subsp. *bauhinii*).

The morphological characters of all tetraploids correspond to *P. officinarum*. However, these plants are apomictic, unlike the widespread tetraploid cytotype of *P. officinarum* in Central Europe, which is sexual. Rare exceptions to this rule are the apomictic tetraploids influenced by the gene flow from coexisting species towards *P. officinarum* (e.g. Mráz & al. 2008).

2*n* = 5*x*, apomictic

Bu 3, Forebalkan (*Western*): 2 plants 1086, 1091; **Bu 6**, Vitosha Region: 1 plant 1297; **Bu 8**, Vitosha Region: 3 plants 1142, 1143, 1283; **Ro 1**: 4 plants 1063, 1064, 1068, 1069; **Ro 3**: 3 plants 1066, 1071, 1072; **Ro 5**: 4 plants 1043, 1045, 1046, 1050.

2*n* = 6*x*, apomictic

Bu 2, Forebalkan (*Western*): 2 plants 957, 1093; **Bu 6**, Vitosha Region: 1 plant 1300; **Bu 7**, Vitosha Region: 2 plants 1295 and one plant with chromosome number determined as 2*n* = 54 (VV); **Bu 11**, Pirin Mts: 4 plants 1111 (the breeding system yet unknown), 1113, 1122, 1125; **Bu 14**, Rhodopi Mts (*Central*): 2 plants 984 and one plant with chromosome number determined as 2*n* = 54 (VV); **Bu 15**, Rhodopi Mts (*Central*): 4 plants 1247, 1248 (perished), 1249, 1272; **Ro 1**: 1 plant 1075; **Ro 2**: 5 plants 1058, 1061, 1062, 1067, 1070; **Ro 3**: 1 plant 1076.

Pilosella onegensis Norrl.

2*n* = 2*x*, sexual

Bu 2, Forebalkan (*Western*): 2 plants 946 and one plant with chromosome number determined as 2*n* = 18 (VV); **Bu 3**, Forebalkan (*Western*): 2 plants 1082 and one plant with chromosome number determined as 2*n* = 18 (VV); **Bu 4**, Balkan Range (*Western*): 5 plants 975, 978/1, 979 and two plants with chromosome number determined as 2*n* = 18 (VV); **Bu 11**, Pirin Mts: 1 plant 1126.

Pilosella pavichii (Heuff.) Arv.-Touv.

2*n* = 2*x*, sexual, with reduced seed fertility

Bu 11, Pirin Mts: 1 plant 1127; **Bu 15**, Rhodopi Mts (*Central*): 4 plants 1004, 1237, 1239 (perished), 1240 (perished).

2*n* = 4*x*, sexual

Bu 11, Pirin Mts: 1 plant 1119.

2*n* = 5*x*, apomictic

Bu 14, Rhodopi Mts (*Central*): 3 plants 988, 989 and one plant with chromosome number determined as 2*n* = 45 (VV).

Pilosella pavichii is a relatively unknown species. For that reason it has not been considered as a separate species, for example, in *Flora Europaea* (Sell & West 1976), where it was considered as a taxon close (maybe identical) to *H. piloselloides*. However, in *Hieracium* monographs (e.g. Zahn 1922-30), as well as in local Floras (Nyárády 1965, Delipavlov 2003) it is fully accepted as a separate taxon. Its morphological characters are precisely given by Bräutigam (1985). This species is well characterized with respect to its habitats: eroded slopes in forests and slopes along forest trails.

Pilosella piloselloides (Vill.) Soják subsp. ***bauhinii*** (Peter) S. Bräut. & Greuter

2*n* = 6*x*, apomictic

Bu 1, Forebalkan (*Western*): 1 plant 970; **Bu 8**, Vitosha Region: 4 plants 1130, 1135, 1137, 1292; **Bu 15**, Rhodopi Mts (*Central*): 3 plants 992, 1000 (2*n* = 54, the morphology slightly influenced by *P. officinarum*), 1007 (the morphology slightly influenced by *P. officinarum*).

Pilosella piloselloides (Vill.) Soják subsp. ***magyarica*** (Peter) S. Bräut. & Greuter

2*n* = 5*x*, apomictic

Bu 1, Forebalkan (*Western*): 1 plant 1097; **Bu 8**, Vitosha Region: 1 plant 1285 (the morphology slightly influenced by *P. officinarum*); **Bu 14**, Rhodopi Mts (*Central*): 1 plant 986; **Ro 5**: 1 plant 1044.

2*n* = 6*x*, apomictic

Bu 1, Forebalkan (*Western*): 3 plants 966, 967, 974; **Bu 2**, Forebalkan (*Western*): 3 plants 945, 956, 1095; **Bu 3**, Forebalkan (*Western*): 5 plants 963, 962/2, 1081/2, 1087, 1088/2; **Bu 5**, Sofia Region: 6 plants 1148, 1149, 1150, 1151 and two plants with chromosome number 2*n* = 54 (VV); **Bu 6**, Vitosha Region: 2 plants 1298, 1299/2; **Bu 7**, Vitosha Region: 1 plant 1294; **Bu 8**, Vitosha Region: 2 plants 1131, 1277, **Bu 13**, Rhodopi Mts (*Central*): 1 plant 1233; **Bu 14**, Rhodopi Mts (*Central*): 1 plant 987; **Bu 15**, Rhodopi Mts (*Central*): 7 plants 993, 994, 996, 997, 1244, 1267, 1264; **Bu 16**, Rhodopi Mts (*Eastern*): 2 plants 1005, 1006; **Ro 5**: 1 plant 1054.

The subspecific identity of *P. piloselloides* is occasionally uncertain, because in addition to the more common *P. piloselloides* subsp. *magyarica* (Peter) S. Bräut. & Greuter we also found *P. p*. subsp. *bauhinii* (Schult.) S. Bräut. & Greuter. Additional hexaploid (2*n* = 6*x*) plants were sampled, which perished before subspecies determination: **Bu 3**, Forebalkan (*Western*): one plant 1096 and one plant with chromosome number determined as 2*n* = 54 (VV); **Bu 8**, Vitosha Region: 1134, 1139; **Bu 14**, Rhodopi Mts (*Central*): one plant with chromosome number determined as 2*n* = 54 (VV); **Bu 15**, Rhodopi Mts (*Central*): one plant with chromosome number determined as 2*n* = 54 (VV).

Pilosella pseudopilosella (Ten.) Soják

2*n* = 2*x*

Bu 10, Pirin Mts: 1 plant 1110; **Bu 11**, Pirin Mts: 2 plants 1112, 1115.

The expected sexual reproductive system was not confirmed in all diploid plants because of their infrequent flowering. All three specimens showed a rath-

er good seed-set after open pollination. The chromosome number agrees with the number from another locality in the Pirin (Vladimirov & Szeląg 2001) and Rila Mts (Pavlova 1999), and with most other localities in the Mediterranean area, except for Morocco, where tetraploid plants were found (Galland 1988, Schuhwerk & Lippert 1998, Vogt & Oberprieler 1993, 1994).

Plants of hybrid origin

The plants were classified in this category with respect to their own morphological characters, irrespective of either the dominating morphological characters of one of the putative parents (both usually co-occurring at the locality), or the intermediate morphological characters between the parental taxa.

P. hoppeana subsp. ***testimonialis* / *P. officinarum* × *P. piloselloides*** (partly corresponding to *P. ruprechtii* (Boiss.) Dostál and to *P. brachiata* (DC.) F.W. Schultz & Sch. Bip.)

The morphological similarity of *P. hoppeana* and *P. officinarum,* as well as their co-occurrence at most localities did not allow us to distinguish with certainty their hybrids with *P. piloselloides*, which represent the most common hybrid combination recorded in Bulgaria. Whenever it was possible to identify the parental species, this was reflected in parenthesis after the specimen number ('**testimonialis*' stands for *P. hoppeana* subsp. *testimonialis*). In our opinion hybridization of *P. piloselloides* (most commonly hexaploid, seldom pentaploid) with *P. officinarum* (most commonly hexaploid or pentaploid, seldom tetraploid) is more common there than hybridization with the diploid *P. hoppeana*. This is supported by (almost) synchronous flowering of *P. piloselloides* and *P. officinarum* (*P. hoppeana* flowers later) and by the extent of variation in ploidy level in the putative parental counterparts and in their hybrids.

$2n = 4x$, sexual, semisterile

Bu 1, Forebalkan (*Western*): 1 plant 1099.

$2n = 5x$, apomictic

Bu 1, Forebalkan (*Western*): 3 plants 971, 972 (both accessions *P. *testimonialis* × *P. piloselloides)*, 1101 (perished); **Bu 2**, Forebalkan (*Western*): 1 plant 947 (perished); **Bu 3**, Forebalkan (*Western*): 6 plants 959, 960, 1083/1, 1083/2 (perished), 1088/1, 1089 (*P. brachiata*); **Bu 4**, Balkan Range (*Western*): 1 plant 1105; **Bu 7**, Vitosha Region: 2 plants 1293/1, 1293/3; **Bu 8**, Vitosha Region: 6 plants 1141, 1289, 1290, 1291, 1303 (*P. officinarum* × *P. piloselloides*) and one plant with chromosome number determined as $2n = 45$ (VV); **Bu 14**, Rhodopi Mts (*Central*): 3 plants 980 (*P. *testimonialis* × *P. piloselloides*), 985, and one plant with chromosome number determined as $2n = ca.$ 45 (VV); **Bu 15**, Rhodopi Mts (*Central*): 5 plants 1002, 1251 (*P. *testimonialis* × *P. piloselloides*), 1254, 1269 and one plant with chromosome number determined as $2n = 45$ (VV); **Ro 5**: 2 plants 1048 (*P. brachiata*), 1049 (*P. ruprechtii*).

$2n = 6x$, apomictic

Bu 1, Forebalkan (*Western*): 3 plants 965, 973 (*P. *testimonialis* × *P. piloselloides*), 1098; **Bu 2**, Forebalkan (*Western*): 7 plants 953 (*P. officinarum* × *P. piloselloides*), 954 (perished), 955 (*P. *testimonialis* × *P. piloselloides*), 958 (perished), 1092/1, 1092/2 and one plant with chromosome number determined as $2n = 54$ (VV); **Bu 3**, Forebalkan (*Western*): 1 plant 961; **Bu 6**, Vitosha Region: 1 plant 1301 (*P. officinarum* >*P. piloselloides*; **Bu 8**, Vitosha Region: 13 plants 1133, 1144 (perished), 1145, 1138, 1140, 1147 (the morphology corresponds to backcross with *P. piloselloides*), 1273, 1276, 1278, 1279, 1281, 1282 (*P. officinarum* × *P. piloselloides*), 1287 (*P. officinarum* × *P. piloselloides*); **Bu 9**, Pirin Mts: 1 plant 1109 ($2n = 54$); **Bu 11**, Pirin Mts: 1 plant 1121; **Bu 14**, Rhodopi Mts (*Central*): 3 plants 981, 982, 983 (*P. *testimonialis* × *P. piloselloides*); **Bu 15**, Rhodopi Mts (*Central*): 23 plants 991, 998, 999 ($2n = 54$), 1001, 1003 ($2n = 54$), 1235, 1242, 1245, 1246, 1253, 1256 (*P. officinarum* × *P. piloselloides*), 1257, 1259, 1260, 1263, 1265, 1266, 1268, 1270, 1271 (*P. *testimonialis* × *P. piloselloides*) and 3 plants with chromosome number determined as $2n = 54$ (VV); **Bu 16**, Rhodopi Mts (*Eastern*): 6 plants 1009 ($2n = 54$), 1010, 1011, 1012 ($2n = 54$) and 2 plants with chromosome number determined as $2n = 54$ (VV); **Ro 5**: 7 plants 1041, 1040 (perished), 1042 (perished), 1047, 1051, 1052, 1053 (the last four accessions corresponded to *P. brachiata*).

Only pentaploid plants ($2n$=45) were reported from Bulgaria (the Pirin Mts) by Vladimirov & Szeląg (2001). The variation in ploidy level recorded in our hybrids corresponds to two cytotypes (pentaploid and hexaploid, respectively) in two of the putative parents, *P. officinarum* and *P. piloselloides*. The tetraploid semisterile hybrid plant seems to originate from the cross between the diploid *P. hoppeana* subsp. *testimonialis* and *P. piloselloides*.

P. hoppeana subsp. ***testimonialis*** × ***P. officinarum***
(partly corresponding to *P. hypeurya* (Peter) Soják)
2*n* = 2*x*, sexual
Ro 4: 2 plants 1073, 1074 (*P. hypeurya*).
2*n* = 6*x*, apomictic

Bu 15, Rhodopi Mts (*Central*): 3 plants 990 (*P. hypeurya*), 1250 (closer to *P. officinarum*) and one plant with chromosome number determined as 2*n* = 54 (VV).

As hybridization between the putative parental taxa would have been heteroploid, especially the diploid hybrids probably originated from multiple crosses (backcrosses), which possibly resulted in introgression to *P. hoppeana* subsp. *testimonialis*.

P. pavichii* × *P. piloselloides (corresponding to *P. georgieffiana* (Zahn) Soják)
2*n* = 6*x*, apomictic

Bu 15, Rhodopi Mts (Central): 1 plant 1243.

P. pavichii* × *P. hoppeana subsp. ***testimonialis***
2*n* = 6*x*, apomictic

Bu 15, Rhodopi Mts (Central): 1 plant 1261 (prevailing morphological characters of *P. pavichii*)

unclassified hybrids of *P. pavichii*
2*n* = 3*x*, sterile

Bu 15, Rhodopi Mts (*Central*): 1 plant 1255 (perished plant).
2*n* = 6*x*, apomictic

Bu 11, Pirin Mts: 1 plant 1128 (a species of the *P.* section *Pilosellina* is presumed as the second putative parent).

Unfortunately, the triploid plant perished early and thus we had no herbarium specimen available for its revision. The triploid level, sterility and morphological characters of this putative hybrid, however, may have corresponded to a (multiple) cross involving almost with certainty the diploid *P. pavichii* and possibly some of the pentaploid hybrids between *P. hoppeana* subsp. *testimonialis* / *P. officinarum* and *P. piloselloides*, which coexist in the respective locality.

In general, our results have supported the expectations, but also gave new details. Diploid plants (*P. hoppeana*, *P. alpicola*, *P. onegensis*, *P. pavichii*, and probably *P. pseudopilosella*) were found as sexuals. A triploid sexual (*P. alpicola*) was recorded but the plant was almost sterile under experimental conditions. The polyploid basic species (excepting the triploid *P. alpicola* and tetraploid *P. pavichii*) and almost all polyploid hybrids were found as apomicts. In only one case a sexual tetraploid hybrid plant was recorded (*P. hoppeana* / *P. officinarum* × *P. piloselloides*) but it was semisterile under experimental conditions. A sexual diploid hybrid (*P. hoppeana* × *P. officinarum*) was found in Romania. An unclassified triploid hybrid of *P. pavichii* was found to be sterile.

All hybrids recorded in the field should be considered as recent. During our study we did not find any stabilized, widespread hybridogenous species which would grow independently, without the coexisting parental species. As far as we know, only *P. guthnickiana* could be considered among the *Pilosella* taxa in Bulgaria as such stabilized hybridogenous taxon. Another difference of the *Pilosella* populations in Central Europe is the absence of sexual higher-polyploids in Bulgaria. In Central Europe, the sexual tetra-, penta- and hexaploid biotypes of the basic species (*P. echioides, P. piloselloides, P. officinarum*) occur along with some sexual polyploid hybrids. The pivotal role of the sexual *P. officinarum* (4*x*, 6*x*) seems to be particularly important due to production of numerous interspecific stabilized hybrids in Central Europe (e.g. Suda & al. 2007). Such hybrids, commonly having diverse ploidy levels and breeding system, exercise an essential influence on the structure of the agamic complex in Central Europe.

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