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The Chromosome Numbers of *Waldsteinia* (*Rosaceae-Colurieae*) in North America

By

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With 11 Figures

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Summary

TEPPNER H., SCHUEHLY W. & WEAKLEY A. S. 2009. The chromosome numbers of *Waldsteinia* (*Rosaceae-Colurieae*) in North America. – *Phyton* (Horn, Austria) 48 (2): 225–238, with 11 figures.

For *Waldsteinia fragarioides* (MICHAX) TRATTINNICK subsp. *fragarioides* the ten chromosome counts known till now are reported; clones with $2n = 2x = 14$, $2n = 3x = 21$ and $2n = 6x = 42$ do exist. For *W. fragarioides* subsp. *doniana* (TRATT) TEPPNER comb. nova ($2n = 14$), *W. lobata* (BALDWIN in ELLIOTT) TORR. & GRAY ($2n = 14$), and *W. idahoensis* PIPER ($2n = 4x = 28$) the chromosome numbers are reported for the first time from one population each. The chromosome morphology is discussed in relation to the literature. The virtual „*Waldsteinia pendula*“ from Puerto Rico of some U. S. websites is a mistake based on a read error for *Wallenia pendula* (URBAN) MEZ (*Myrsinaceae*).

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Zusammenfassung

TEPPNER H., SCHUEHLY W. & WEAKLEY A. S. 2009. The chromosome numbers of *Waldsteinia* (Rosaceae-Colurieae) in North America. [Die Chromosomenzahlen von *Waldsteinia* (Rosaceae-Colurieae) in Nordamerika]. – Phytton (Horn, Austria) 48 (2): 225–238, mit 11 Abbildungen.

Für *Waldsteinia fragarioides* (MICHAX) TRATTINICK subsp. *fragarioides* werden die 10 bisher bekannt gewordenen Chromosomenzählungen zusammengestellt; es gibt Klone mit $2n = 2x = 14$, $2n = 3x = 21$ und $2n = 6x = 42$. Für *W. fragarioides* subsp. *doniana* (TRATT) TEPPNER comb. nova ($2n = 14$), *W. lobata* (BALDWIN in ELLIOTT) TORR. & GRAY ($2n = 14$) und *W. idahoensis* PIPER ($2n = 4x = 28$) werden die Chromosomenzahlen – von je einer Population ermittelt – erstmals mitgeteilt. Die Chromosomenmorphologie wird in Relation zur vorhandenen Literatur diskutiert. Die virtuelle „*Waldsteinia pendula*“ von Puerto Rico, die auf so manchen U. S. Websites zu finden ist, stellt einen Fehlgriff statt *Wallenia pendula* (URBAN) MEZ (*Myrsinaceae*) dar.

1. Introduction

The text of this paper is written by the first author, thus the maintenance of a distinct genus *Waldsteinia* falls in his responsibility; he has some doubts, whether the last word on the delimitation of the genera within the *Colurieae* RYDBERG (former *Geeae* JUEL) is spoken already. Contrary, WEAKLEY & GANDHI 2008 accepted the molecular results of SMEDMARK 2006 and her forerunner papers and include the genus *Waldsteinia* within *Geum*.

With the efforts of the coauthors, it was possible to procure the remaining *Waldsteinia* species for which no chromosome numbers are known up to now. This justified the reason for presenting the new results together with all previous chromosome number counts.

2. Material and Methods

The plants collected from natural habitats were grown in the cool greenhouse of the Botanic Garden at the Institut fuer Pflanzenwissenschaften der Universitaet Graz and field-grown in the private gardens of H. TEPPNER (*W. idahoensis*), W. SCHUEHLY (*W. lobata*, *W. idahoensis*) and A. DRESCHER (*W. fragarioides* subsp. *doniana*). From the live plant collection used by the author in TEPPNER 1968, *W. fragarioides* subsp. *fragarioides* from Esperance, N. Y., is still present in the Botanic Garden.

Root tips or tips of creeping shoots were pre-treated for c. 3 hours in a solution of 8-hydroxyquinoline. Fixations were made in ethanol : chloroform : acetic acid 5 : 3 : 1 and the material was stained in acetic acid carmine for squash preparations in the usual way (e. g., DARLINGTON & LA COUR 1963, SHARMA & SHARMA 1965).

The microscopic investigations were made using a Zeiss Photomikroskop III (with a camera lucida) provided 1978 by the Fonds zur Förderung der wissenschaftlichen Forschung (Austria). The negatives were scanned with Epson Perfection 2400 Photo and the figures were edited with Adobe Photoshop CS3.

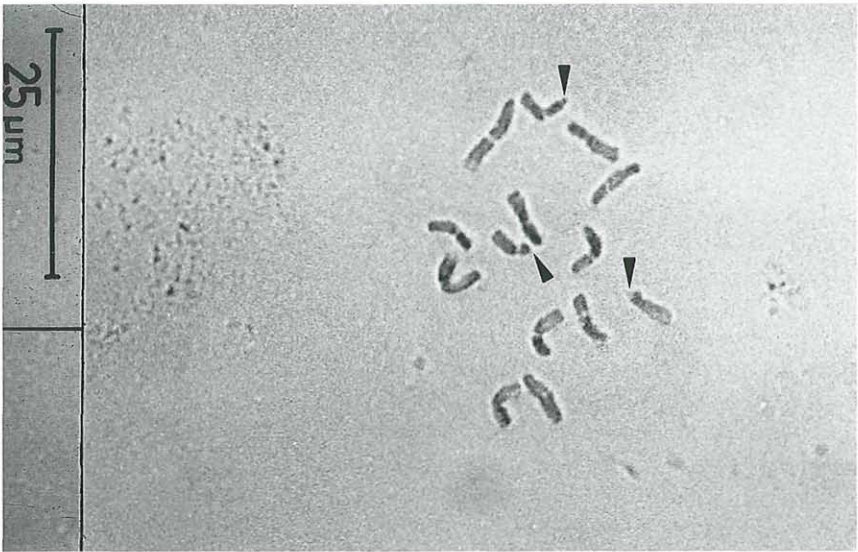


Fig. 1. *Waldsteinia lobata*, mitotic metaphase plate with $2n = 14$ chromosomes and an interphase nucleus with small chromocentres. Three of four satellites visible (arrowheads). – Scale bar equals 25 μm.



Fig. 2. *Waldsteinia lobata*, nucleus in mitotic prophase, strongly squashed, with $2n = 14$ chromosomes, small sections of heterochromatin and two distinct (arrows) and two presumably satellites (arrowheads; one a little out of the optical plane).

3. Results

3. 1. *Waldsteinia lobata* (BALDWIN in ELLIOTT) TORREY & GRAY [*Geum lobatum* (BALDW. in ELLIOTT) SMEDMARK]

U. S. A., South Carolina, Oconee County, along S.-side of Brasstown Creek N.NE. of Forest Road 751, 4 Mi W of RT 76, c. 700-800 m, scrub of *Rhododendron maximum* together with *Podophyllum peltatum*, *Xanthorhiza simplicissima*, *Collinsonia canadensis* (veg.), *Tiarella cordifolia*, *Stachys latidens*, *Sambucus canadensis* (in flower), *Aesculus* cf. *octandra*, *Polystichum acrostichoides* and *Dioscorea* sp., 12.6.2007, leg. W. SCHUEHLY & S. CROCKETT – Grown in the Bot. Garden at the Inst. of Plant Sciences, University of Graz, live plant, received 19.6.2007. (Vouchers in GZU) 2n = 14

The diploid set of 2n = 14 chromosomes contains three pairs of metacentrics, the others are more heterobrachial (Fig. 1). In this preparations satellite chromosomes or satellites, respectively, were a little better discernible than in the other species. Especially in the mitotic prophase one pair of large satellites and one pair of small ones can be seen (Fig. 2).

An account on the ecology and distribution is given in PATRICK & al. 1995 (see Georgia DNR Wildlife Resource Division Home Page <http://georgiawildlife.dnr.state.ga.us/assets/documents/waldlo.pdf> and DOUGLASS 1980. Phytogeographical aspects of endemism in the southeastern U. S. A. are discussed by ESTILL & CRUZAN 2001.

In addition to the description in TEPPNER 1968: 27–29, it will be mentioned that in the cultivated plants at the terminal flowers up to three epicalyx-leaves were present, all other flowers lack an epicalyx. The diameter of fresh flowers measures c. 11–12 mm, the petals were c. 4–4.2 × 1.7–2 mm, the stamen number reached up to 72 per flower and the inner side of the receptacle was glabrous throughout.

3. 2. *Waldsteinia idahoensis* PIPER [*Geum idahoense* (PIPER) SMEDMARK]

U. S. A., Idaho, Idaho Co, Lochsa River near Powell, white sand (Hwy 12), with *Linnaea borealis* and *Cornus canadensis*; 4.7.2004; leg. W. SCHUEHLY & S. CROCKETT (GZU). – Grown in the Bot. Garden at the Inst. of Plant Sciences, University of Graz, live plants, received 15.7.2004 and 11.8.2004. (Vouchers in GZU) 2n = 28

In the tetraploid set of 2n = 28 chromosomes the proportion of the chromosome types is the same as in the diploids: six pairs (the largest included) are metacentrics, whereas the other eight are stronger heterobrachial (Fig. 3).

For a long time *W. idahoensis* was only known to occur sporadically through three counties in north-central Idaho (see, e. g., http://fishandgame.idaho.gov/cms/tech/CDC/spp_accounts_plants/walida.cfm, 18.10.2005).

In 1987 LACKSCHEWITZ (LACKSCHEWITZ & al. 1988: 358) discovered a population in Missoula Co, Montana, near the frontier to Idaho (see, e. g.,

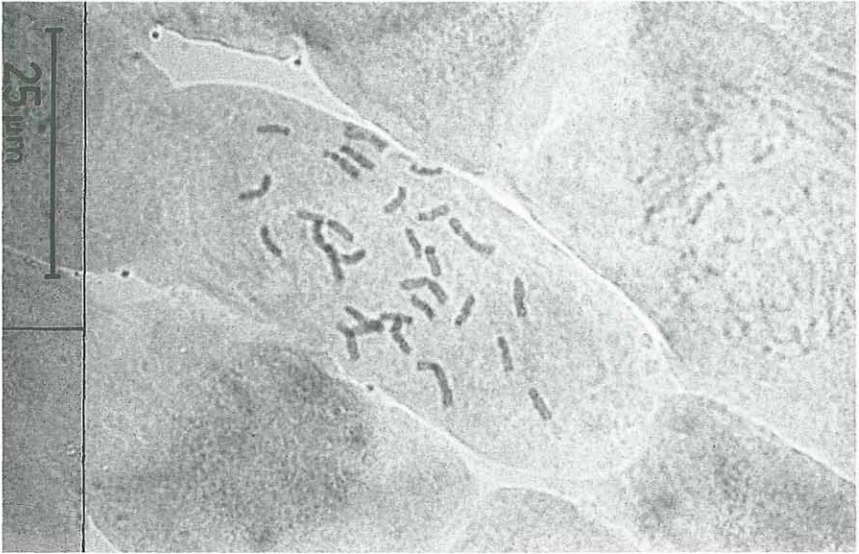


Fig. 3. *Waldsteinia idahoensis*, mitotic metaphase plate with $2n = 4x = 28$ chromosomes; a prophase nucleus (right) and interphase nuclei.

Montana Natural Heritage Program, http://fieldguide.mt.gov/detail_PDROS_1_S020.aspx, 10.10.2008).

In our grown plants the size of the petals was $8.2\text{--}9.2 \times 8.3\text{--}9.1$ mm including a claw of 0.5–1mm; the apex of the petals was rounded, emarginate or somewhat narrowed.

3. 3. *Waldsteinia fragarioides* (MICHAX) TRATT. subsp. *fragarioides*
[*Geum fragarioides* (MICH.) SMEDMARK p. p.]

In this taxon three ploidy levels are known till now. TEPPNER 1968:41–42 checked the modest morphological differences between a $2x$ - and a $3x$ -clone. For the other clones investigated in the meantime, this remains to be done. For the seven clones investigated by MULLIGAN & BAUM, till now for two only the locality of origin has been published.

3. 3. 1. $2n = 14$

Canada, Ontario, bei Roblin, north of Napanee, leg. W. J. CODY, Plant Research Institute, Department of Agriculture, Ottawa
..... (TEPPNER 1968: 39–40, Fig. 69–72, incl. meiosis, PAGITZ 1974)

[Canada, Ontario,] Ottawa, Britannia, near filtration plant; June 15, 1967; leg. G. A. MULLIGAN, B. R. BAUM & C. FRANKTON. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3552
..... (MULLIGAN & BAUM 1970: 609 and in herb. DAO)

[Canada,] Fitzroy Harbour Provincial Park, Ontario; June 15, 1967; leg. G. A. MULLIGAN & B. R. BAUM. Large flowers. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3553 (MULLIGAN & BAUM 1970 in herb. DAO)

[Canada,] Fitzroy Harbour Provincial Park, Ontario; June 15, 1967; leg. G. A. MULLIGAN & B. R. BAUM. Small flowers. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3554 (MULLIGAN & BAUM 1970 in herb. DAO)

[U.S.A.,] North slope of shale ridge along Sacony Creek, 11/2 mi. NNE of Kutztown, Berks County, Pennsylvania; Aug. 2, 1967; leg. H. WILKENS. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3556 (MULLIGAN & BAUM 1970 in herb. DAO)

3. 3. 2. $2n = 21$

U.S.A., New York, surroundings of Esperance, George Landis Arboretum..... (TEPPNER 1968: 40–41, Fig. 73–78, incl. meiosis)

[Canada,] Gatineau Park, Ottawa District, Quebec; Oct. 5, 1967; leg. J. GILLET. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3551..... (MULLIGAN & BAUM 1970: 609 and in herb. DAO)

[Canada,] Otter Lake Sanctuary, 12 miles N. of Sydenham, Ontario; June 29, 1967; leg. R. BESCHEL. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3550 (MULLIGAN & BAUM 1970 in herb. DAO)

[U.S.A.,] about 11 mi. NNW of Ottsville, Bucks County, Pennsylvania; Aug. 23, 1967; leg. A. E. SCHUYLER & E. WHERRY. – Grown in greenhouse at Ottawa; March 1970; leg. G. A. MULLIGAN & B. R. BAUM 3555 (MULLIGAN & BAUM 1970 in herb. DAO)

3. 3. 3. $2n = 42$

U.S.A., Virginia, Franklin County, Smart View – Blue Ridge Parkway, moist habitat in open forest; leg. Carrol WOOD. – Received 1971 by Arnold Arboretum, via von Ch. PUFF (h. l. Fig. 4, TEPPNER 2003: 14, Fig. 2c)



Fig. 4. *Waldsteinia fragarioides* subsp. *fragarioides*, 6x clone from Virginia, mitotic metaphase plate with $2n = 42$ chromosomes.

3. 4. *Waldsteinia fragarioides* subsp. *doniana* (TRATT.) TEPPNER comb. nova

Basionym: *Waldsteinia doniana* TRATTINNICK, Rosacearum Monographia 3: 109 (1823). The combination *W. f.* subsp. *doniana* was published in TEPPNER 1968:49 and was effectively published according the rules of the 1966 Code. Due to the retroactive Article 30.5 in the Vienna Code 2006 it was becoming ineffectively published. Thus, a new combination is necessary once more.

[*Geum donianum* (TRATT.) WEAKLEY & GANDHI].

U. S. A., North Carolina, Montgomery County, Uwharrie National Forest, banks of Barnes Creek, ca. 3 km due east of Ophir; 12. May 2008; leg. Alan S. WEAKLEY & Andy WALKER. – Grown in the Bot. Garden at the Inst. of Plant Sciences, University of Graz, live plant, received 2.6.2008. (Vouchers in GZU) 2n = 14

The diploid chromosome set contains one pair of large chromosomes with nearly median centromere, a medium sized and a smaller metacentric chromosome, the other four pairs are more heterobrachial (Fig. 5). Interphase nuclei and late prophase (Fig. 6) show relatively small amounts of heterochromatin.

4. Discussion

The presence of apomictic embryo sacs beside the meiotic one has been shown by CZAPIK 1985 in the diploid *Waldsteinia* (subg. *Waldsteinia*) *geoides* WILLD. The possibility of the development of functional embryos from the apomictic ESs remains to be investigated. There is also a suspicion for the possibility of apomictic embryo sacs in the other species of this genus [subg. *Comaropsis* (RICHARD in NESTLER) TEPPNER], but this is not proven till now. However, at least at the local scale, the different chromosome numbers can be maintained by the very effective, vegetative propagation by creeping shoots.

W. fragarioides subsp. *fragarioides* is the only American taxon for which a number of chromosome counts (ten) is available. Three ploidy levels, 2x, 3x and 6x have been found; nevertheless no geographical pattern is recognizable except probably for the fact that the 6x plants come from the southern part of the distribution area (for its distribution see TEPPNER 1968: 42–48, Fig. 79 and HILL 2003: 28–31). The Old World *W. ternata* (STEPHAN) FRITSCH shows a similar phenomenon: in all partial areas, except one, more than one ploidy level occur: Carinthia/Slovenia 4x, 5x, Slovakia 4x, 5x, Romania 6x, Central Asia 2x, 6x and Eastern Asia 4x, 6x (TEPPNER 2003: 15–14). Such a pattern makes the independent, convergent evolution of the different ploidy levels probable.

Local populations investigated till now, usually showed one ploidy level only. But one mixed population is known from Europe: *W. ternata*



Fig. 5. *Waldsteinia fragarioides* subsp. *doniana*, mitotic metaphase plate, strongly squashed, with $2n = 14$ chromosomes.



Fig. 6. *Waldsteinia fragarioides* subsp. *doniana*, nucleus in late mitotic prophase, strongly squashed, small sections of heterochromatin in the chromosomes, and interphase nuclei with small chromocentres.

subsp. *trifolia* (ROCHEL ex KOCH) TEPPNER, population 'a' near St. Luzia in Carinthia, TEPPNER 1974: 293, map. Fig. 3, 4x and 5x. Such a possibility can never be excluded.

For the three taxa for which the new chromosome counts are presented (*W. lobata*, *W. idahoensis*, *W. fragarioides* subsp. *doniana*), only one clone each could be investigated. From the measurements of pollen grain size (TEPPNER 1968) there are no indications for different ploidy levels within the taxa; nevertheless some more counts would be desirable for a final picture. TEPPNER 1968: 32 postulated from the pollen grain volume and the number of colpi, that *W. idahoensis* should possess a higher ploidy level than *W. lobata*; this is really true (2x versus 4x).

As a result, for all *Waldsteinia* taxa worldwide at least one chromosome count is now published.

The phantom "*Waldsteinia pendula* (URBAN) MEZ" from Puerto Rico, which can be found on many U. S. websites does not indicate a *Waldsteinia*, this name is apparently a mistake for *Wallenia pendula* (URBAN) MEZ (*Myrsinaceae*), see MEZ 1901: 413 and URBAN 1910: 477 (H. W. LACK, Berlin, in litt.). Obviously, around the wrong name, one has constructed an imaginary, not existing *Waldsteinia*.

Detailed investigations of chromosome morphology and idiograms are only available for *Coluria geoides* (PALL.) LEDEB., *W. geoides* and *W. fragarioides* subsp. *fragarioides* (PAGITZ 1974). The chromosome set of *W.* (subg. *Waldsteinia*) *geoides* (Fig. 7) is very similar (only a little less symmetrical) to that of *W.* (subg. *Comaropsis*) *fragarioides* subsp. *fragarioides* (Fig. 8). In the latter, the largest chromosome possesses a median centromere, the two next ones show centromeres in the median region with $r = 1.3$ and 1.5 . The other four are more asymmetric with an arm-ratio of $r = 1.60$ or more and the most asymmetric chromosome bears a distinct satellite (terminology: TEPPNER 1980).

For the other species of subg. *Comaropsis*, from good mitotic metaphase plates it can be said that the chromosome morphology is very similar to the above idiograms in having also three metacentric chromosomes and the other four more heterobrachial per haploid set. The SAT-chromosomes were not easy to detect and often not discernible with the method used. From some plates and prophase stages found in *W. lobata* it can be concluded, that the pair of satellite chromosomes shown in the idiograms is present in the other species, too; in the prophase (Fig. 2) small dots at the nucleoli have been seen, so that probably a further pair of SAT chromosomes with very small satellites may be present (see also Fig. 1).

A confusing issue is the taxonomic status of "*W. doniana*". TEPPNER 1968 promoted the idea, that, supposing *Waldsteinia* as being monophyletic, in *W.* subg. *Comaropsis* the simple-leaved species (*W. lobata*, *W. idahoensis*) should form a subclade (which may be the more primitive

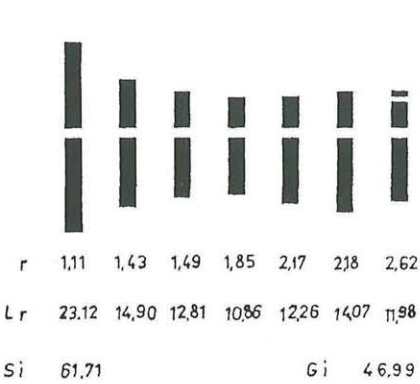


Fig. 7. *Waldsteinia* (subg. *Waldsteinia*) *geoides*, idiogram of the haploid chromosome set, mean of four mitotic metaphase plates. - r = arm ratio, Lr = relative chromosome length (%), Si = symmetry index, Gi = gradient of chromosome length index. Order of chromosomes follows r. - From PAGITZ 1974: Fig. 16).

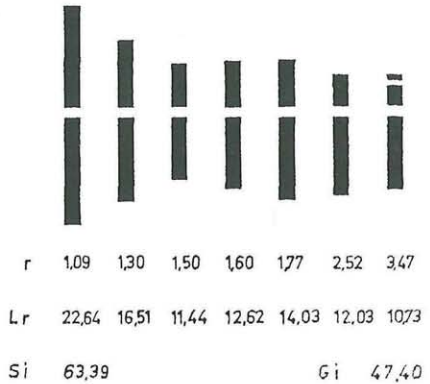


Fig. 8. *Waldsteinia* (subg. *Comaropsis*) *fragarioides* subsp. *fragarioides* (from Roblin, Napanee, Ontario), idiogram of the haploid chromosome set, mean of four mitotic metaphase plates. Order of chromosomes follows r. - From PAGITZ 1974: Fig. 24).

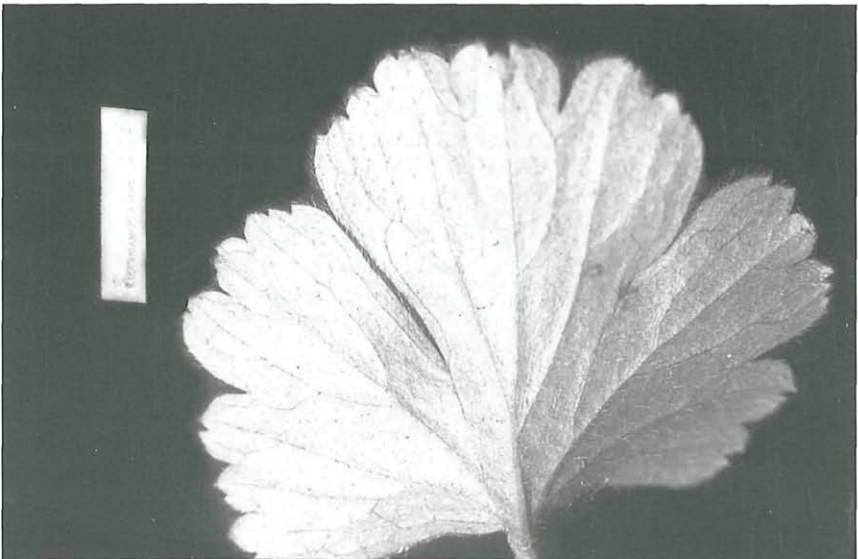


Fig. 9. *Waldsteinia ternata* subsp. *trifolia* from Slovakia, Hliník n. Hronom (5x); an exceptionally simple leaf, from the lower side. - Scale bar 1 cm.

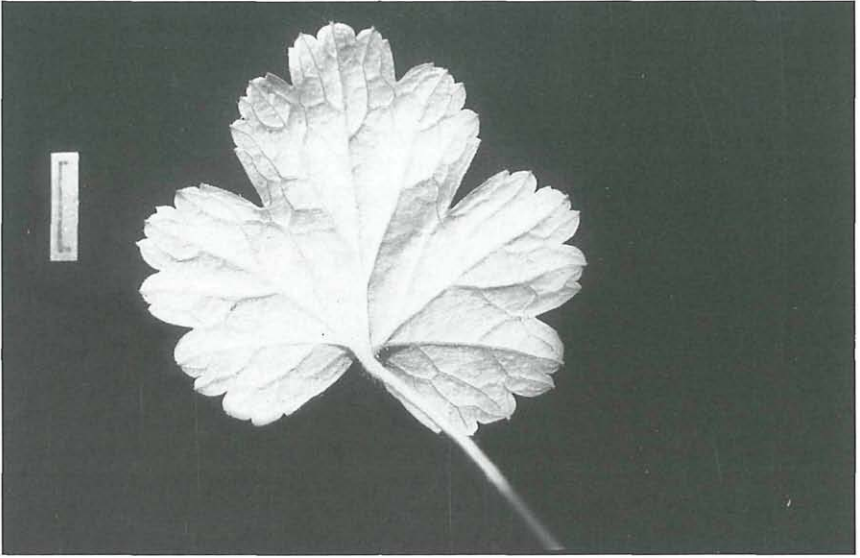


Fig. 10. *Waldsteinia lobata*, leaf from the lower side. – Scale bar 1 cm.

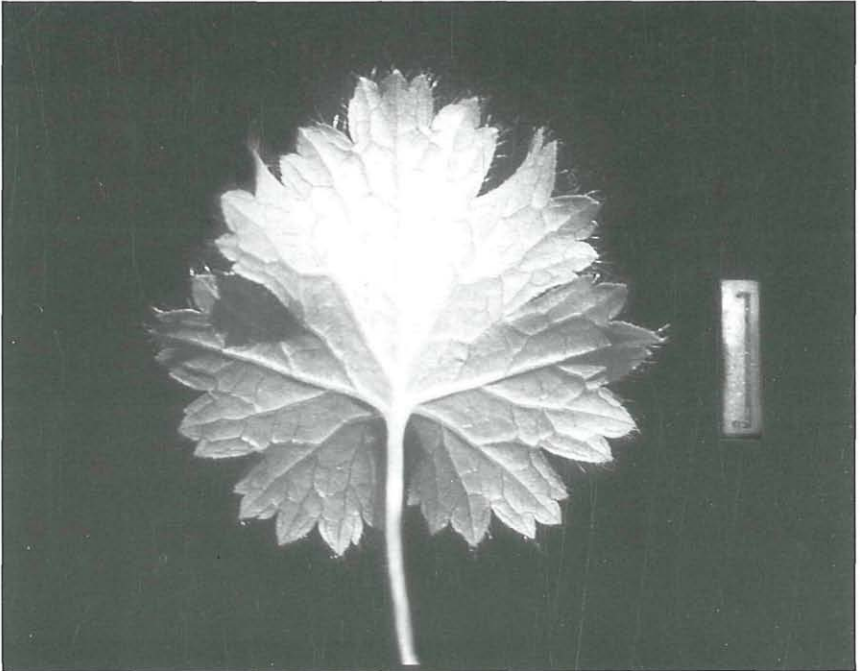


Fig. 11. *Waldsteinia idahoensis*, leaf from the lower side. – Scale bar 1 cm.

one), and the trifoliolate-leaved an other one. Furthermore, many transitions between both trifoliolate taxa occur, especially also in the three most apparent characteristics, the size of the petals, the petiole of the terminal leaflet, and the angle at the base of the terminal leaflet. Plants with small petals, up to 4 mm length only, occur in the area of subsp. *fragarioides* (New York, Pennsylvania, Virginia and West Virginia); on the other hand plants with 4–5 mm long petals can be found in the southern area (North Carolina) (TEPPNER 1968: 42). The petiole of the terminal leaflet is 1–4(–5) mm (mean 2,1 mm) in subsp. *fragarioides* versus 1–5 mm (mean 3 mm) in subsp. *doniana*. The basal angle of the terminal leaflet measures 63°–115° (mean 91°) in subsp. *fragarioides* and 60°–130° (mean 81°) in subsp. *doniana* (TEPPNER 1968: 39, 49). In view of the many and wide overlaps of the characteristics the author regarded the two trifoliolate taxa of N. America as typical examples for the rank of subspecies. In contrast, WEAKLEY & GANDHI 2008:416 were more emphasizing on the petal size than to the leaf character (simple versus compound), thus reaching another opinion: „Indeed, the morphological relationships of the three taxa make the closer affinity of Taxon B [*doniana*] problematic (if leaf lobing is the more phylogenetically revealing character, the Taxon B may be more closely related to Taxon A [*fragarioides* s.str.], but lacking molecular data it is plausible that the floral characters are more phylogenetically revealing, and taxa B and C [*lobata*] are more closely related, forming a southern, small-petaled clade.“ So they treated the trifoliolate, small flowered taxon at the rank of species as *Geum donianum* (TRATT.) WEAKLEY & GANDHI. The decision between the two opinions will be difficult and probably not possible from morphology alone, because practically no sufficiently distinctive morphological characteristics are available. For e. g., in *W. fragarioides* the petioles of the nutlets are free to base or grown together for half of their length, in *W. lobata* grown together for half of their length and free to base in *W. idahoensis*. Unfortunately, there is no significant difference in the nervature of leaves between principally simple leaved species (Fig. 10, 11) and occasionally simple leaves in trifoliolate species (Fig. 9). Leaves with secondarily connected leaflets occur very rarely in *W. fragarioides* subsp. *fragarioides* (documented only once: TEPPNER 1968: Fig. 58, from Pennsylvania); in the clone of *W. fragarioides* subsp. *doniana* grown in Graz we detected one small leaf, in which the terminal leaflet is connected with one lateral for 3 mm. Simple leaves are regularly formed beside of normal ones in a clone of *W. ternata* subsp. *trifolia* (5x) from Slovakia (TEPPNER 1974: 286; h. l. Fig. 9) and apparently also in *W. ternata* subsp. *ternata* (2x) (STEPANOV 1994: Fig. 2, as *W. tanzybeica*). Since connected leaflets sporadically occur in all trifoliolate species, even far from any simple leaved ones, this character is a case of teratology and can not be a sign for close affinity or hybridization. Because of the transitions between *fragarioides* s. str. and *doniana* and no such transitions known between *doniana* and *W. lobata*,

the first author tends still to recognize the subspecies as used in this paper, whereas the third one advocates the species rank.

5. Acknowledgements

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