

Paeonia saueri (Paeoniaceae), a new species from the Balkans

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Paeonia saueri D. Y. Hong, X. Q. Wang et D. M. Zhang is described as a new species from Greece and Albania based on population sampling, statistics, chromosome observation, and SEM observations. It is characterized by tuberous roots, a high number of leaflets/segments, adaxially setulose along the veins on leaves, and a chromosome number of $2n = 20$. It is closely related to *P. peregrina*. However, as shown by cluster analysis (Fig. 3) and principal coordinate analysis (Fig. 4), it differs distinctly from the latter in having mostly entire leaflets/segments that are hispidulous beneath, and red stigmas. From *P. officinalis* it differs in having the leaves setulose along the veins above and sparsely hispidulous below, and the stems, petioles and sepals always glabrous. Furthermore, it differs from those two species by its relatively short and wide follicles. The somatic chromosome number of the new species, $2n = 20$, is reported here for the first time.

KEYWORDS: Balkan, chromosome number, multivariate statistics, Paeoniaceae, *Paeonia saueri*, *P. peregrina*, *P. officinalis*, SEM

INTRODUCTION

Dr. Wilhelm Sauer of Tübingen University, Germany, kindly sent the senior author as a gift in 1991 three specimens of *Paeonia* from Greece, collected in 1979 and 1985. They all belonged to the same taxon and resembled *P. peregrina* and *P. officinalis* in having a relatively high number of leaflets/segments, and particularly *P. peregrina* with setulose hairs along the veins on the upper side of the leaves. This new taxon, however, differed from *P. peregrina* in having the leaflets/segments mostly entire instead of dentate-lobed and from *P. officinalis* in having stems, petioles and sepals entirely glabrous (vs. pilose), and with leaves mostly sparsely hispidulous (vs. pilose) beneath.

According to Hong & al. (1994, 1998) and subsequent extensive field observations, the shape of the roots, whether tuberous or carrot-shaped, is a very stable characteristic of species in *Paeonia*. Among the 22 species so far recognized in *Paeonia* sect. *Paeonia* (i.e., all Eurasian herbaceous species; Hong & al., unpubl.), only six have so far been found to possess tuberous roots: *P. arietina*, *P. intermedia* (Hong & Pan, unpubl.), *P. officinalis*, *P. parnassica*, *P. peregrina* and *P. tenuifolia*. They all possess high or relatively high numbers of leaflets/segments. However, two species, *P. anomala* and *P. clusii*, with a high number of leaflets/segments, possess carrot-shaped roots.

Because all three collections lacked roots and the sample is so small, it was hard to judge precisely their relationships with other groups and to determine their

taxonomic status. The authors had an opportunity to visit the localities of Dr. Sauer's material and its allies in 2001 and 2002. Based on field observations, population sampling, chromosome preparations, SEM observations, and statistical analysis, *Paeonia saueri* is described as a new species in the present article.

MATERIALS AND METHODS

Population sampling. — We sampled one population (D. Y. Hong, D. M. Zhang & X. Q. Wang H02227) on the Mountain Pangeon above Eleftheroupolis, Kavala, Greece, at the same locality as one of Sauer's collections. Eight individuals were randomly collected, from which four were dug up with their roots for observation and transplantation and only the aerial parts of the other four were harvested. In addition, a lower leaf was collected from each of six different individuals. In the same way, three populations were sampled for *P. arietina*, one for *P. parnassica*, two each for *P. officinalis* and for *P. peregrina* (see details in the Appendix). Ecological parameters of the populations were documented. The vouchers are preserved in A, CAL, K, MO, PE and UPA.

Chromosome observation. — Root tips were harvested from the individuals of H02227 transplanted to the Beijing Botanical Garden, Chinese Academy of Sciences, and pretreated in an aqueous solution of 0.05% colchicine and 0.002M 8-oxyquinoline for five hours. The material was washed and fixed in Carnoy I fixative (absolute alcohol and acetic acid 3:1) overnight, and then

Table 1. Comparison of diagnostic characters of leaflets/segments between the new species (*P. saueri*) and its closest allies. Number of individuals observed in parentheses. Mean and standard deviation given for number and width of leaflets.

Taxa & populations ¹	Number of leaflets	Width (cm) of leaflets	Number of dentate lobes (≤ 1 cm long)	Indumentum on upper surface		Indumentum on lower surface		
				Type ²	Density ³	Type ²	Density ³	
<i>P. arietina</i>								
<i>H02204</i>	17.9 ± 3.3 (9)	4.2 ± 0.8 (11)	0 (11)	pilose	0 (8), 1 (3)	pilose	1 (1), 2 (2), 3 (8)	
<i>H02216</i>	21.4 ± 7.2 (9)		0 (8), 1 (1)	pilose	0 (1), 1 (8)	pilose	3 (4), 4 (5)	
<i>H02217</i>	17.1 ± 5.1 (10)		0 (9), 2 (1)	pilose	1 (10)	pilose	3 (7), 4 (3)	
<i>P. parnassica</i>								
<i>H02224</i>	12.4 ± 3.5 (16)	4.4 ± 1.2 (16)	0 (13), 1 (2), 2 (1)	pilose	0 (15), 1 (1)	pilose	3 (10), 4 (6)	
<i>P. peregrina</i>								
<i>H02201</i>	66.6 ± 12.7 (10)	2.1 ± 0.3 (10)	28.9 ± 8.9(10)	–	0 (10)	–	0 (10)	
<i>H02223</i>	101.7 ± 15.3 (6)	2.7 ± 0.6 (6)	49.3 ± 15.1(6)	setulose	0 (4), 1 (2)	–	0 (6)	
<i>P. officinalis</i>								
<i>H01029</i>	25.7 ± 5.6 (12)	2.3 ± 0.6 (12)	0 (10), 1 (2)	–	0 (12)	pilose	2 (1), 3 (9), 4 (2)	
<i>H01009</i>	45.7 ± 11.2 (12)	2.0 ± 0.4 (16)	2.1 ± 1.9 (13)	pilose	0 (10), 1 (3)	pilose	3 (1), 4 (8), 5 (4)	
<i>P. saueri</i>								
<i>H02227</i>	32.6 ± 8.7 (14)	2.9 ± 0.6 (14)	0 (10), 1 (3), 2 (1)	setulose	2 (13), 3 (1)	hispidulous	0 (1), 1 (11), 2 (2)	

¹ See appendix for details.² See Fig. 2.³ See text for explanation.**Table 2. A comparison of diagnostic characters of flower and fruit between the new species (*P. saueri*) and its closest allies. Parentheses and footnotes as in Table 1.**

Taxa	Indumentum on stems & petioles		Indumentum on sepals		Follicle shape & mean ratio of length/width
	Type ²	Density ³	Type ²	Density ³	
<i>P. arietina</i>					
<i>H02204</i>	pilose	2 (3), 3 (8)	pilose	2 (2), 3 (3), 4 (3)	ellipsoid, 2.5 (4)
<i>H02216</i>	pilose	4 (9)	pilose	3 (1), 4 (5)	
<i>H02217</i>	pilose	4 (10)	pilose	4 (5), 5 (1)	
<i>P. officinalis</i>					
<i>H01029</i>	pilose	1 (10), 2 (2)	pilose	4 (5), 5 (2)	ellipsoid, 2.2 (6)
<i>H01009</i>	pilose	3 (5), 4 (8)	pilose	4 (7)	
<i>P. parnassica</i>					
<i>H02224</i>	pilose	2 (1), 3 (9), 4 (6)	pilose	3 (4), 4 (2)	
<i>P. peregrina</i>					
<i>H02201</i>	–	0 (10)	–	0 (6)	
<i>H02223</i>	–	0 (6)	–	0 (6)	ellipsoid, 2.0 (3)
<i>P. saueri</i>					
<i>H02227</i>	–	0 (14)	–	0 (8)	ovoid, 1.53 (8)

transferred to 70% alcohol. For chromosome preparation, the material was washed and macerated for 1.5 minutes in a mixed solution of 1N HCl and absolute alcohol at room temperature, and was then stained in carbol fuchsin diluted with acetic acid before being squashed (Fig. 1).

SEM observation. — A preliminary observation of the indumentum of the material under study and its allies found that there were three major types of indu-

mentum: (1) pilose on the stems, petioles, abaxial surface of the leaves and the sepals, and occasionally adaxially at the base of the main veins of the leaves; (2) setulose adaxially along the veins of leaves; and (3) hispidulous abaxially on the leaves. For a detailed description of hair types, we conducted SEM observations. The material was directly coated and observed under a Hitachi S-800 SEM (Fig. 2).

Morphological observations and statistical analysis. — The roots of all the four individuals of *H02227* were tuberous (Figs. 3 and 4), lower leaves with 19–45 leaflets/segments, leaflets/segments mostly entire, only rarely lobed, each lower leaf with lobes varying from 0 to 15 in number, these setulose adaxially along the veins, and hispidulous abaxially, the stems, petioles and sepals always glabrous, and a chromosome number of $2n = 20$ (see below). With these characters, Sauer's collections and our sampled population *H02227* should have a position near the six tuberous-rooted species. *Paeonia intermedia* and *P. tenuifolia* have the highest number of leaflets/segments (70–350), which are filiform to linear, less than 1.5 cm wide. Furthermore, *P. intermedia*, is a diploid (Hong & al., unpubl.), found in thickets or grasses on sunny slopes in Central Asia and Georgia, and *P. tenuifolia* is also a diploid (Stebbins, 1938; Punina, 1989; Zhang & Sang, 1999; Hong & al., unpubl.) found in meadows or bushes in the Caucasus, Ukraine, Bulgaria, Romania and Serbia. Therefore, these two species are distinctly different from the material under study. According to our observations (Tables 1 and 2), Sauer's and our collections are most similar to the remaining four species, *P. peregrina*, *P. officinalis*, *P. ari-*

etina and *P. parnassica*. They are all tetraploid with $2n = 20$ (see Barber, 1941 and Hong & al., unpubl. for *P. arietina*; Dark, 1936, Barber, 1941, Gajewski, 1948, Löve & al., 1974, and Hong & al., unpubl. for *P. officinalis*; Tzanoudakis, 1983 for *P. parnassica*; Tzanoudakis, 1983, Uspenskaya & Solovyeva, 1991, and Hong & al., unpubl. for *P. peregrina*). A statistical analysis was carried out in the present investigation to understand how the new taxon differs from its allies, and to evaluate the justification for describing it as a new species.

Seven populations in total were included in the analysis, two each for *P. peregrina* and *P. officinalis*, and one each for *P. parnassica*, *P. arietina* and the putative new taxon. A total of 11 characters (Tables 1 and 2) were used in the analysis: four binary (the type of indumentum on stems and petioles, and the upper and lower surfaces of leaflets/segments, and on the back side of sepals); two ordinal (numbers of leaflets/segments and of dentate lobes); one continuous (width of leaflets/segments). The density of indumentum was treated as ordinal. To describe density of indumentum we divide the density into six grades with 0 expressing absolute absence of

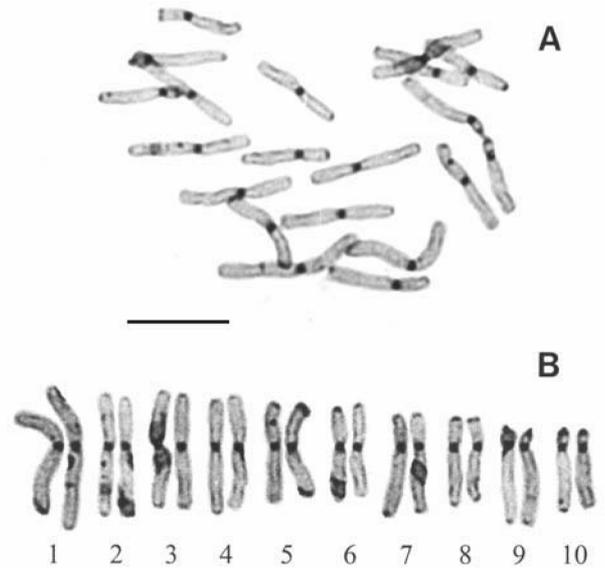


Fig. 1. Chromosomes of *Paeonia saueri*. A, micrograph of somatic chromosomes at mitotic metaphase, scale bar = 10 μm ; B, karyogram.

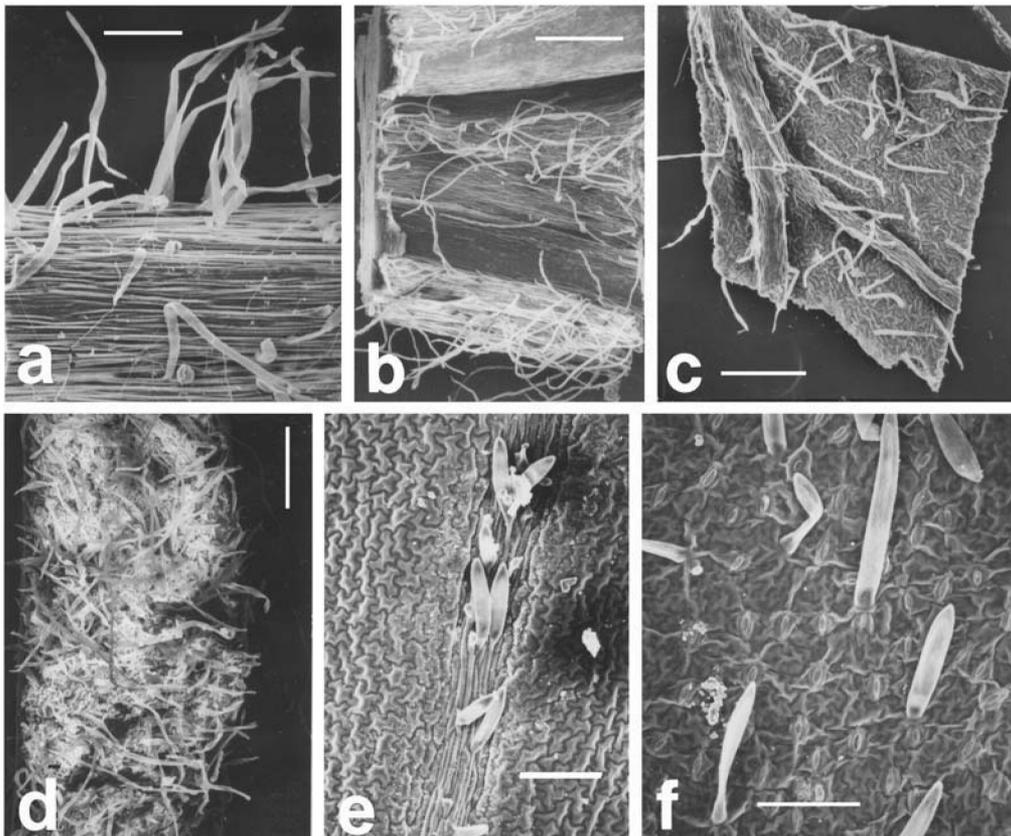


Fig. 2. SEM photographs of indumentum types in *Paeonia*. a, pilose hairs on stems (*P. officinalis* subsp. *villosa*, H01009); b, pilose hairs on the main vein at base of leaves (*P. officinalis* subsp. *officinalis*, H01029); c, pilose hairs on lower surface of leaves (H01029); d, pilose hairs on the back side of sepals (H01029); e, setulose hairs along main veins of leaves (*P. saueri*, H02227); f, hispidulous hairs on lower surface of leaves (H02227). a, e & f, scale bar = 200 μm ; b, c & d, scale bar = 500 μm .



Fig. 3. The holotype of the new species, *Paeonia saueri* D. Y. Hong, X. Q. Wang et D. M. Zhang, consisting of sheets a and b.

hairs, 1 extremely sparsely hairy and 5 the most densely hairy (completely covering surface), with 2, 3 and 4 in between.

A data matrix using 11 characters was made for each population with each individual as an OTU (Tables 1 and 2). Although we sampled nine populations in total (Tables 1 and 2), only seven were used in our analysis because individuals in the populations *H02216* and *H02217* were too young, with their leaves not fully developed. In Tables 1 and 2, 12 characters are listed, but follicle shape and width were not used in the analysis due to the inadequate sample size. The Gower general similarity coefficient for mixed datasets was used in the analysis. Both cluster analysis and principal coordinate analysis were conducted using MVSP-Version 3.13b analysis software.

RESULTS

The somatic chromosomes were shown to be twenty ($2n = 20$; Fig. 1A), and thus the plant was a tetraploid. It can be seen from Fig. 1B that the tetraploid has diploidized in karyotype, as shown in the obvious differ-

entiation, e.g., between the first and the second pair, and between the ninth and the tenth pair.

The observations of the indumentum are shown in Fig. 2. The hairs on the stems, petioles, leaves (upper and lower side) and sepals in *P. officinalis*, *P. arietina* and *P. parnassica* are nearly the same, filiform, soft and bent, 0.6–1.0 mm long, and thus could be called pilose (Fig. 2 a, b, c & d). The hairs along the adaxial surface of the main veins of leaves in the material under study, and less frequently in *P. peregrina* are straight, pointed at both ends, only ca. 0.2 mm long, and thus could be called setulose (Fig. 2e). The hairs on the lower surface of the leaves under study are mostly straight, pointed at the apex, ca. 0.2–0.4 mm long, and are called hispidulous (Fig. 2f). The three types of hairs are rather distinct.

The result of our observations on the sampled populations is shown in Tables 1 and 2, and the results of cluster analysis and principal coordinate analysis are shown in Figs. 5 and 6, respectively. The two figures both show three distinct groups, *P. saueri* (the new species), *P. peregrina* and a group of the other three species, *P. officinalis*, *P. arietina* and *P. parnassica*. The new species seems to be a natural group and distinctly different from all of its allies.

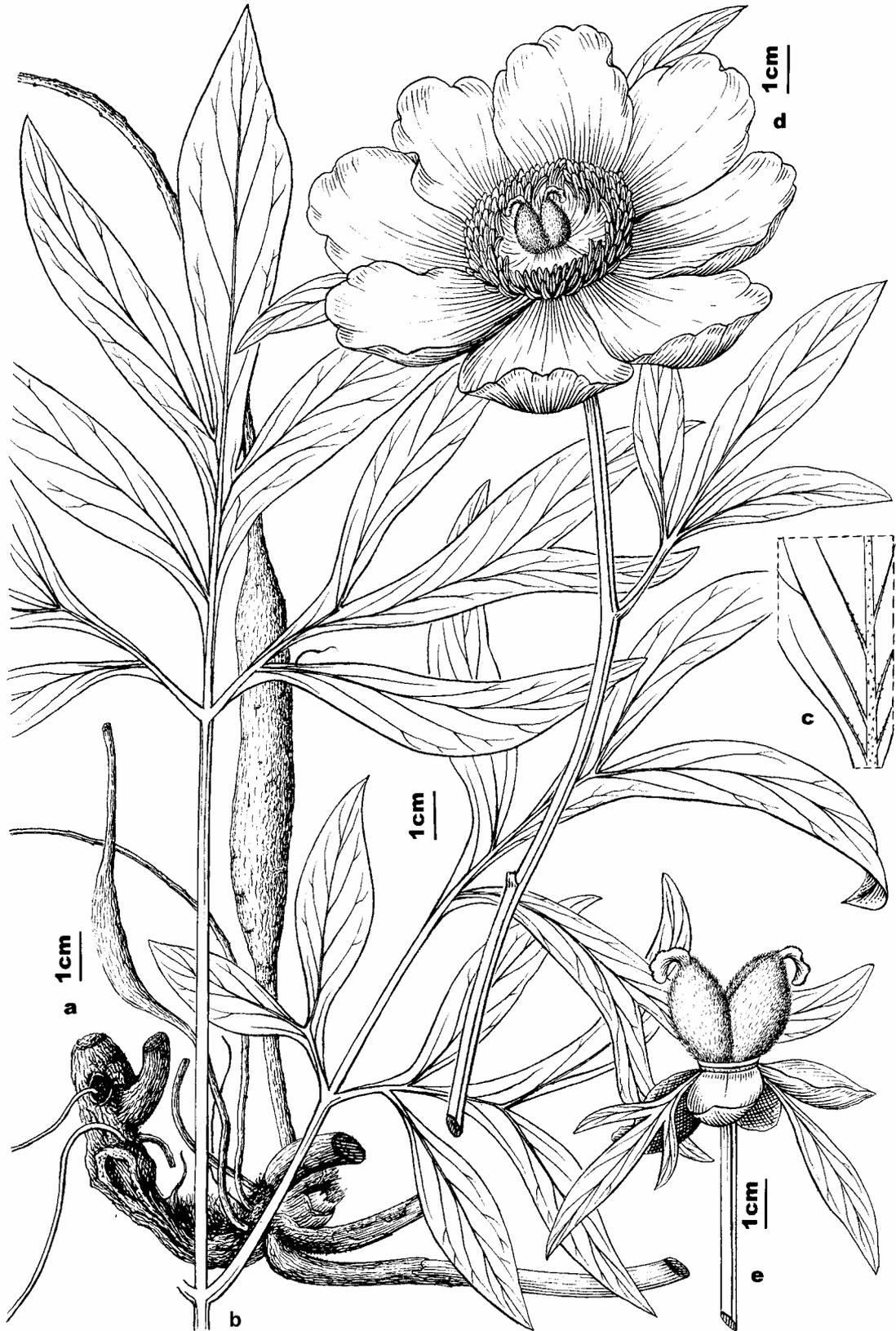


Fig. 4. *Paeonia saueri*. a, rhizome and roots; b, lower leaf; c, part of leaf, showing minute setulose hairs along veins above; d, upper part of stem with a single flower; e, flower after anthesis, showing two involucral bracts, four sepals, disc and two carpels (young follicles) from outer to inner.

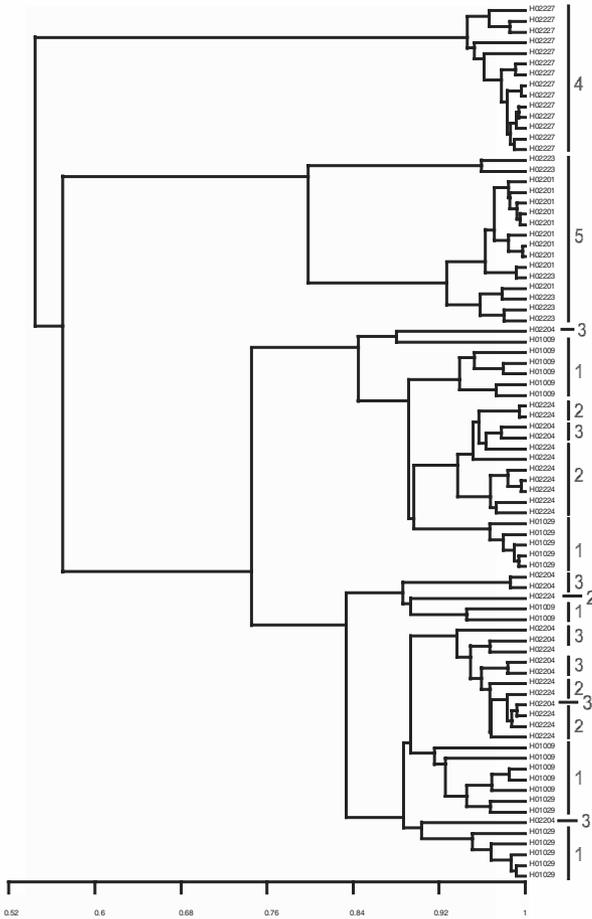


Fig. 5. Cluster analysis (UPGMA). OTUs and characters used are explained and described in the text and Table 1. 1, *P. officinalis*; 2, *P. parnassica*; 3, *P. arietina*; 4, *P. saueri*; 5, *P. peregrina*. The scale at the bottom indicates Gower general similarity coefficient.

Furthermore, in addition to the characters used in the statistics there are other characters that distinguish our new species from its allies. The stigmas are yellow in *P. peregrina*, while they are always red in *P. saueri*. The follicles are ellipsoid with a mean length/width ratio of 2.0 in *P. peregrina*, 2.2 in *P. officinalis* and 2.5 in *P. arietina*, whilst they are ovoid with a length/breadth ratio of 1.53 in *P. saueri*. Thus, all data so far available indicate that the material under study is a distinct species, and it is justifiable to describe it as new.

The differences and relationships between the new species and the four related species are shown by the following key:

- 1. Stems, petioles and sepals always pilose; leaflets/segments adaxially glabrous, occasionally pilose at base of main vein, abaxially pilose..... 2
- 1. Stems, petioles and sepals always glabrous; leaflets/segments adaxially setulose along veins or glabrous, abaxially glabrous or sparsely hispidulous

- 4
- 2. Leaflets/segments 9–25(–32), elliptic, ovate-lanceolate or oblong, 3–6 cm wide 3
- 2. Leaflets/segments 19–60(–90), linear to narrow-elliptic, 0.8–3.5 cm wide..... *P. officinalis*
- 3. Leaflets/segments mostly more than 13; petals red or rose *P. arietina*
- 3. Leaflets/segments mostly 9–13; petals dark purple ...
..... *P. parnassica*
- 4. Leaflets/segments always dentate-lobed (dentate lobes = 1.0 cm long), abaxially glabrous; stigmas yellow..... *P. peregrina*
- 4. Leaflets/segments entire, rarely lobed, abaxially sparsely hispidulous; stigmas red *P. saueri*

DISCUSSION AND CONCLUSION

Figs. 5 and 6 show that the new taxon (*P. saueri*) forms an independent group. *Paeonia peregrina* is also distinctly different from the other three. However, the other three species, *P. officinalis*, *P. arietina* and *P. parnassica*, are not distinct and their elements are intermingled. Within this group, *P. arietina* is different but not distinctly so from *P. officinalis*, and it is hardly distinguishable from *P. parnassica*. The relationships and taxonomical treatment of these three species are currently under study (Hong & al., unpubl.).

***Paeonia saueri* D. Y. Hong, X. Q. Wang & D. M. Zhang, sp. nov.** (Figs. 3 & 4). Type: Greece, Kavala Province, Mt. Pangeon, above Eleftheroupolis, 40°55' N, 24°12' E, 960 m, at edges of *Fagus sylvestris* forest with sparse *Juniperus* sp., limestone, 29 May 2002, D. Y. Hong, D. M. Zhang & X. Q.

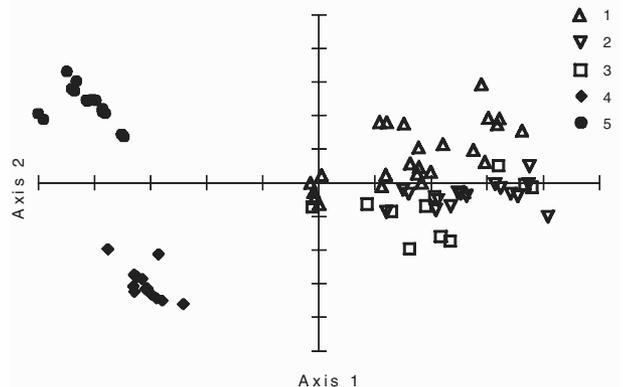


Fig. 6. Principal coordinate analysis. OTUs and characters used are the same as for Fig. 5. Axis 1 expresses 38.7% of the total variation, while axis 2 represents 14.6% of the variation. Numbers refer to taxa given in legend of Fig. 5.

Wang H02227 (holotype: PE; isotypes: A, CAL, K, MO, UPA).

Herba perennis, parte subterranea tuberosa. Caulis 45–65 cm altus, glaber. Folia inferiora biternata, aliqua segmentata; foliola/segmenta 23–43(–54), omnino integra vel aliquot fissa, elliptica vel anguste elliptica, basi cuneata, apice acuto, 3.3–10(–12.5) cm longa, 1.1–3.5(–5.4) cm lata, infra sparsim hispida vel glabra, supra setulosa secus venas. Bracteae involucreatae 2–3, segmentis foliorum similes. Sepala 3–5 (6), rotundata, mucronata, 2.5–3.0 cm longa, 2.0–2.5 cm lata; petala 7–10, atro-rubra, obtriangulato-rotundata, basi cuneata, apice rotundata et varie erosa vel incisa, 5.0–5.5 cm longa, 3.2–4.0 cm lata; filamenta atro-purpurea; carpella (1)–2–4(–6), tomentosa, stylis absentibus, stigmatibus valde recurvis, c. 2.0 mm latis. Fl. Majo.

Perennials. Roots tuberous. Stems 45–65 cm tall, green, rarely purple, glabrous. Lower leaves biternate, with some leaflets segmented, leaflets/segments 19–45 in total, all entire or very few lobed, elliptic or narrow-elliptic, cuneate at the base, acute at the apex, 3.3–11 cm long, 1.0–4.2 cm wide, with setulose hairs along veins above, sparsely hispidulous, very occasionally glabrous beneath. Involucral bracts 2–3, similar to leaflets/segments. Sepals glabrous, 3–5, rarely 6, rounded or mucronate at the apex, 2.5–3.0 cm long, 2.0–2.5 cm wide; petals 7–10, red, obovate, cuneate at the base, rounded or variously incised at the apex, 5.0–5.5 cm long, 3.2–4.0 cm wide; filaments dark-purple; disc slightly wavy or dentate, ca. 1 mm high; carpels mostly 3 or 2, less frequently 4, very rarely 1, 5 or 6, whitish tomentose; styles absent; stigmata red, strongly recurved, about 2.0 mm wide; ovules 14–20. Young follicles ovoid, 2.8–3.0 cm long, 1.9 cm in diameter. Flowering in May.

Chromosome number $2n = 20$.

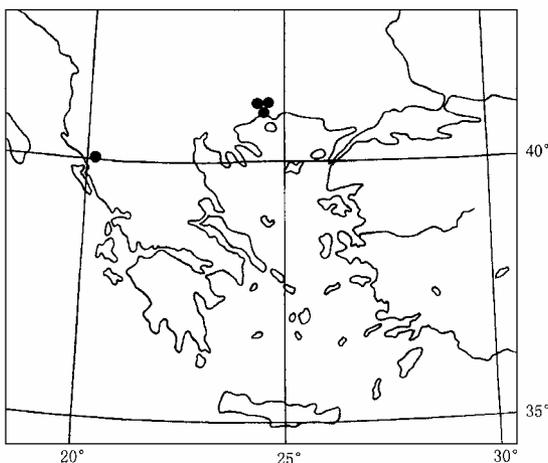


Fig. 7. Distribution of *Paeonia saueri*.

Paeonia saueri is found in NE. Greece and S. Albania (Fig. 7). It grows usually near mountain summits, in clearings and with limestone or granite, 460–1220 m.

Additional specimens examined: ALBANIA: S. Albania, Melesine, above Leskovik, 1220 m, near summits, limestone, 19 Jun 1933, *Alston & Sandwith 1775* (K). GREECE: Makedonia, Kavala Province, Mt. Pangeon, above Eleftheroupolis, 1040 m, in *Fagus sylvestris* forest or clearings, limestones, 27 May 1985, *G. & W. Sauer 31212* (PE, SA); the same locality, *Fagus-Quercus-Carpinus-Fraxinus* mixed forests, clearings with *Pteridium aquilinum*, ca. 910 m, limestone, 27 May 1985, *G. & W. Sauer 31198* (PE, SA); Mt. Pangeon, W of Palaiochiori or Akrovounion, *Betula* forests, granite and Mediterranean brown loess, 900 m, 23 Apr 1979, *G. & W. Sauer 24108* (PE, SA); Mt. Pangeon, 30 km W of Kavala, 460 m, irrigated grass meadows, 24 May 1959, *Stainton 7536* (K).

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Appendix. Origins of sampled populations of *Paeonia saueri* and relatives.

- | | |
|-----------------------|---|
| <i>P. arietina</i> | <i>H02204.</i> Turkey, Balikesir Province, Mt. Ida (Kaz Dag), from Ayazma to Kobakli, 39°45'N, 26°52'E, <i>Pinus nigra</i> forest, limestone, 1000 m, 13 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i>
<i>H02216.</i> Turkey, Sivas Province, Zara, 10 km from Zara to Serefiye, 39°58'N, 37°44'E, pasture with scattered shrubs, 1700 m, 19 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i>
<i>H02217.</i> Turkey, Sivas Province, Zara, 25 km from Zara to Sesehri, 39°58'N, 37°58'E, sparse <i>Quercus</i> woods, limestone, 1850 m, 19 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i> |
| <i>P. officinalis</i> | <i>H01029</i> (subsp. <i>officinalis</i>). Switzerland, Lugano, Mt. Generoso, steep rocky herbs, limestone, 1450–1600 m, 22 Jun 2001, <i>D. Y. Hong & X. Q. Wang.</i>
<i>H01009</i> (subsp. <i>villosa</i>). France, Nice Province, above Grasse, 43°43'N, 6°52'E, <i>Quercus pubescens</i> with shrubs, limestone, 1130 m, 25 May 2001, <i>D. Y. Hong, X. Q. Wang & A. Fridlender.</i> |
| <i>P. parnassica</i> | <i>H02224.</i> Near village Ertalophos, Mt. Parnassos, Greece, 38°35'N, 22°31'E, edges of <i>Abies</i> forest, limestone, 1220 m, 25 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i> |
| <i>P. peregrina</i> | <i>H02201.</i> Gozelleme Observation Station, above Baglica village, Nallihan, Ankara Province, Turkey, 40°13'N, 31°18'E, <i>Pinus nigra</i> forest, limestone, 1250 m, 11 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i>
<i>H02223.</i> Near Selalmaz village, 20 km to Eflani from Daday, Kastamonu Province, Turkey, 41°28'N, 33°10'E, grasses, at edges of forest, 1060 m, 22 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i> |
| <i>P. saueri</i> | <i>H02227.</i> Above Eleftheroupolis, Mt. Pangeon, Kavala Province, Greece, 40°55'N, 24°12'E, edges of <i>Fagus sylvatica</i> with sparse <i>Juniperus</i> sp., limestone, 960 m, 29 May 2002, <i>D. Y. Hong, D. M. Zhang & X. Q. Wang.</i> |
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