

A karyomorphological study on *Actaea acuminata*, *A. asiatica*, and *A. erythrocarpa* (Ranunculaceae)

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Academic editor: R. Yakovlev | Received 1 October 2024 | Accepted 21 November 2024 | Published 8 December 2024

<http://zoobank.org/2197246A-320E-4574-BB04-85B67DE7BE7A>

Citation: Mitrenina EYu, Erst AS, Krivenko DA, Veklich TN, Leonova TV, Chernyagina OA, Yakubov VV, Xiang K, Ling Yu-Yu, Wang W (2024) A karyomorphological study on *Actaea acuminata*, *A. asiatica*, and *A. erythrocarpa* (Ranunculaceae). Acta Biologica Sibirica 10: 1445–1460. <https://doi.org/10.5281/zenodo.14280678>

Abstract

A comparative karyomorphological analysis was performed on *Actaea acuminata*, *A. asiatica*, and *A. erythrocarpa*. The karyotype of *A. acuminata* was re-examined; its formula proved to be $2n = 16 = 10m + 2sm + 2st + 2t$. This species' karyotype was found to differ from that of *A. spicata* ($2n = 16 = 10m + 4sm + 2t$), thus allowing us to propose a species status of *A. acuminata* in contrast to the subspecies status (previously suggested by Compton): *A. spicata* var. *acuminata*. Karyotype structure of the analyzed *A. asiatica* from Shandong Province matches previously obtained data for Yunnan Province in China and corresponds to $2n = 16 = 10m + 4sm + 2t$. The chromosome set of *A. erythrocarpa* was investigated in five regions of Russia; its structure proved to be similar among all the analyzed populations and is described by formula $2n = 16 = 10m + 4sm + 2t$, consistently with previous findings about this species in Western Siberia. There are differences among the populations in the number and localization of secondary constrictions. A conclusion was made about conservatism of karyotype structure within the genus *Actaea*.

Keywords

Actaea, Cimicifugeae, China, chromosomes, karyotype, Russia

Introduction

The tribe Cimicifugeae Torr. & A. Gray belongs to the family Ranunculaceae Juss. and includes four plant genera and more than 49 species (Compton et al. 1998a; Wang et al. 2005; Yuan and Yang 2006). This tribe contains *Anemonopsis* Siebold & Zucc. (one species), *Actaea* L. (32 species), *Eranthis* Salisb. (14 species), and *Beesia* Balf. f. & W.W. Sm. (two species), which have a circumboreal distribution (Compton and Culham 2002; Ling et al. 2023). Two genera are endemic to some regions, e.g., *Anemonopsis* grows only in Japan, and *Beesia* occurs only in China and N. Myanmar. Traditionally, the genus *Actaea* has been thought to contain only eight species with fleshy berries (Tamura 1995). Compton et al. (1998a) were the first to use molecular data (ITS) to investigate its circumscription, where the sampled species of *Actaea*, *Cimicifuga* L. ex Wernisch., and *Souliea* Franch. formed a clade. On the basis of two molecular and morphological datasets, those authors (Compton et al. 1998b) have then confirmed this result and accordingly redefined *Actaea* to include *Cimicifuga* and *Souliea*, and this approach has been accepted by subsequent investigators (Xiang and Wang 2018). Three genera (*Actaea*, *Cimicifuga*, and *Souliea*) are currently recognized as belonging to the genus *Actaea*, and this notion is based on a perennial habitus, racemose inflorescences, actinomorphic flowers with free carpels, follicular or baccate, multiseeded fruits, and follicles with distinct transverse or reticulate venation (Compton et al. 1998a). According to integrative morphological and molecular data, the genus is divided into eight sections: *Actaea*, *Chloranthae* J.P. Luo, Q. Yuan & Q.E. Yang, *Cimicifuga* (L. ex Wernisch.) DC., *Dichanthera* (P.K. Hsiao) J. Compton, *Oligocarpae* (Tamura) J. Compton, *Pityrosperma* (Siebold & Zucc.) J. Compton, *Podocarpae* J. Compton, and *Souliea* (Franch.) J. Compton. Nonetheless, taxa with wide geographic ranges, such as *A. cimicifuga* L., *A. rac-*

emosa L., *A. rubra* (Aiton) Willd., and *A. spicata* L. may include different species, for example cryptic species (Erst et al. 2020). In contrast, some subspecies listed in Compton's revision (Compton et al. 1998b) may have the taxonomic rank of a species, e.g., *A. spicata* var. *acuminata* (Wall. ex Royle) H. Hara. In the present work, we accept generally recognized names of the following species: *Actaea acuminata* Wall. ex Royle, *A. asiatica* H. Hara, and *A. erythrocarpa* (Fisch.) Freyn (Luferov 2004).

Actaea acuminata, *A. asiatica*, and *A. erythrocarpa* are included in the type section *Actaea*. Black-fruited *A. asiatica* occurs in Russia (Amur Region, ewish Autonomous Region, Khabarovsk Territory, and Primorye Territory), China (Gansu, Hebei, Heilongjiang, Hubei, Jilin, Liaoning, S. Nei Mongol, Qinghai, Shaanxi, Shanxi, Sichuan, E Xizang, and Yunnan), Japan, and Korea (Liangqian and Tamura 2001; Luferov 2004). Black-fruited *A. acuminata* grows mainly in Tibet and the Himalayas (People's Republic of China, Nepal, and N. India); the western border of its range reaches the Hindu Kush in N. Afghanistan and Pakistan, and the eastern border extends to Chinese provinces Sichuan and Qinghai (Liangqian and Tamura 2001; Luferov 2004). In Russia, this species is widespread in the Far East. *Actaea asiatica* differs from *A. acuminata* by the presence of narrower pointed leaflets, a less dense inflorescence, pedicels and peduncles more strongly bent aside; the latter are thicker: 0.8–1.3 mm (in *A. acuminata*: 0.4–0.9 mm thick). The widespread species *A. erythrocarpa* grows in Russia (Altai, Kamchatka, Khabarovsk, Krasnoyarsk, Primorye, and Trans-Baikal Territories; Amur and Irkutsk Regions; Buryatia, Sakha (Yakutia), and Tuva Republics; and the Sakhalin and Kuril Islands), China (Hebei, Heilongjiang, Jilin, Liaoning, Nei Mongol, and Shanxi), Japan, Kazakhstan, Korea and Japan, and as an introduced plant in Europe (Estonia, Finland, and Sweden) (Liangqian and Tamura 2001). In Kamchatka, special form f. *kamtschatica* Kom. occurs, which is distinguished as a distinct variety by glabrous leaves, except for the veins, which bear appressed hairs above, and by appressed ciliate hairs beneath. A white-fruited form, f. *leucocarpa* Ledeb., is also known to occur in different parts of this area, growing together with the typical form. Red-fruited *A. rubra* (Aiton) Willd. is often erroneously claimed to grow for Russia (Compton et al. 1998a). This species is widespread in North America and is distinguished by much-thickened peduncles and mostly emarginate, quasibilobate petal-nectaries (Shipczinsky 1937).

At present, a comparative analysis of karyotypes plays an important role within the framework of an integrative approach to plant taxonomy, as evidenced by a large number of modern studies devoted to this analysis (Haider 2018; Mráz et al. 2019; Erst et al. 2020; Vimala et al. 2021). Although the research on karyotypes of representatives of the tribe Cimicifugeae began in the mid-20th century, several species remain poorly investigated or not studied at all (Kurita 1956; Kurita 1956). Most species of *Actaea*, *Anemonopsis*, and *Beesia* are diploid with basic chromosome number $x = 8$ and somatic chromosome number $2n = 16$. The chromosome set is usually represented by five pairs of large metacentric chromosomes and three pairs of shorter unequal-armed chromosomes of two or three morphological types (Kurita 1956; Kawano et al. 1966; Blair et al. 1975; Lee and Park 1998; Yang 1999;

Yang 2002; Yuan and Yang 2006; Luo et al. 2016). Exceptions are the tetraploid species *Actaea kashmiriana* J. Compton having $2n = 32$ (Rashid et al. 2022) as well as *A. cimicifuga* L. and *Beesia calthifolia* (Maxim. ex Oliv.) Ulbr., for which tetraploid cytotypes with $2n = 32$ have been described along with diploid cytotypes characterized by $2n = 16$ (Hasegawa 1969; Yang 1999). For the genus *Eranthis* Salisb., greater diversity has been revealed in both chromosome numbers and karyotype structure (Mitrenina et al. 2021; Mitrenina et al. 2023).

This study is aimed at a comparative analysis of karyotype structure in *Actaea acuminata*, *A. erythrocarpa*, and *A. asiatica* (Figure 1). The chromosome set of *A. asiatica* has been investigated in Yunnan Province of China (Yang 1998; Yang 2002). As for *A. erythrocarpa*, the karyotype of plants from only one region of Russia (Western Siberia) has been studied (Malakhova et al. 1976; Dubrova 1981). For *A. acuminata* was previously described karyotype which was somewhat untypical of the *Actaea* (Dubrova 1985), so it needed to be restudied.

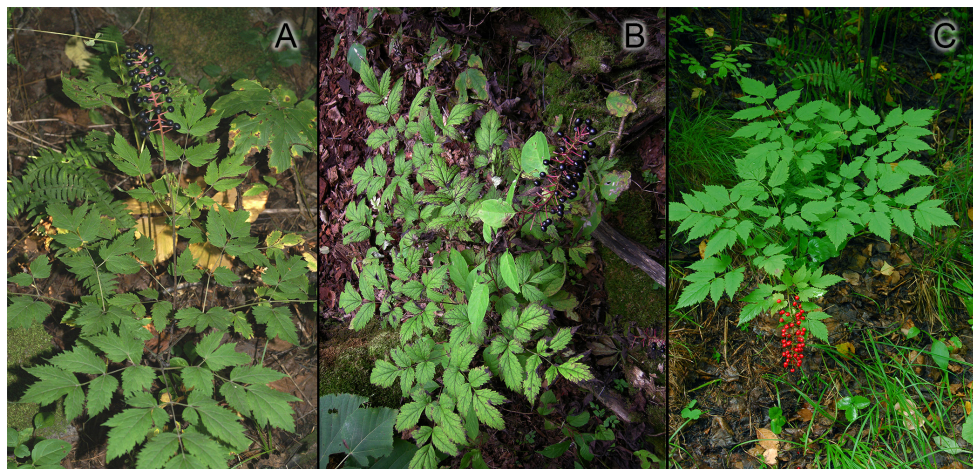


Figure 1. General view of *Actaea acuminata* (A), *A. asiatica* (B), and *A. erythrocarpa* (C).

Materials and methods

Plant samples

Plant materials (rootstocks) of *A. acuminata*, *A. asiatica*, and *A. erythrocarpa* were collected during field research in Russia and China during 2023. The list of the examined specimens is presented in Table 1. Herbarium specimens were deposited in NS herbaria (herbarium acronyms according to Thiers 2023, continuously updated).

Karyotype analysis

Karyotype analysis was performed on seven populations. Somatic chromosomes of the three *Actaea* species were prepared from root meristems. The rootstocks were germinated at room temperature for 1–3 months. Newly formed 1–2-cm-long roots were excised and pretreated with a 0.5% colchicine solution for 3–4 h. Roots were fixed in a mixture of 96% ethanol and glacial acetic acid (3:1). Chromosomes stained with 1% aceto-hematoxylin were used for morphometric analyses. Staining and karyotyping were performed according to standard protocols (Smirnov 1968; Mitrenina et al. 2021). Mitotic metaphase chromosome plates were examined under an Axio Star microscope (Carl Zeiss, Munich, Germany) and photographed using an Axio Imager A.1 microscope (Carl Zeiss, Germany) equipped with an AxioCam MRC5 CCD camera (Carl Zeiss, Germany) at 1000× magnification by means of AxioVision 4.7 software (Carl Zeiss, Munich, Germany) in the Laboratory for Ecology, Genetics and Environmental Protection (Ecogene), National Research Tomsk State University (Tomsk, Russia). The KaryoType 2.0 software (Altnordu et al. 2016) was employed for the karyotyping, and Adobe Photoshop CS5 (Adobe Systems, USA) and Inkscape 0.92 (USA) for image editing.

Table 1. The list of the studied *Actaea* specimens

Species	Specimen number	Voucher information
<i>A. acuminata</i>	PR2023-3	Russia, Primorye Territory, outskirts of Vladivostok city, near Akademicheskaya railway station, broadleaf forest, 59 m, 43°11'37.5"N, 131°55'30.1"E, 16.06.2023, V.V. Yakubov
<i>A. asiatica</i>	CH2023-26	China, Shandong Province, Tai'an City, Doumugong, Mount Tai, 2385 m, 34°02'48.6"N, 107°42'37.8"E, 15.07.2023, G.L. Cao & G.Q. Liu
<i>A. erythrocarpa</i>	AM2023-6	Russia, Amur Region, Zeysky district, Zeysky Nature Reserve, mixed (<i>Betula platyphylla</i> , <i>Populus tremula</i> and <i>Larix gmelinii</i>) forest, 352 m, 53°51'56.8"N, 127°20'36.3"E, 22.08.2023, T.N. Veklich
<i>A. erythrocarpa</i>	IRK2023-3	Russia, Irkutsk Region, Shelekhovsky district, 2.5 km SSE of Rassokha settlement, left bank of the Bolshaya Olkha River, aspen-birch green moss forest, 552 m, 52°00'29.53"N, 104°06'21.17"E, 30.08.2023, D.A. Krivenko
<i>A. erythrocarpa</i>	KAM2023-1	Russia, Kamchatka Territory, Ust-Kamchatsky district, right bank of the Kamchatka River, vicinity of Mayskoe village, fern (with <i>Pteridium aquilinum</i>) forest of <i>Betula ermanii</i> , 56°13'39.7"N, 160°06'35.2"E, 04.09.2023, O.A. Chernyagina
<i>A. erythrocarpa</i>	KR2023-4	Russia, Krasnoyarsk Territory, Minusinsky district, vicinity of Znamenka village, 461 m, 53°32'43.4"N, 91°56'11.1"E, 15.07.2023, T.V. Leonova
<i>A. erythrocarpa</i>	NS2023-1	Russia, Novosibirsk Region, Iskitimsky district, near Sosnovka village, pine forest, 150 m, 54°39'59.1"N, 82°53'41.4"E, 02.07.2023, A.S. Erst

The symbols used to describe the karyotypes matched those coined by Levan et al. (1964): m = a median centromeric chromosome with an arm ratio (r) of 1.0–1.7 (metacentric chromosome); sm = a submedian centromeric chromosome with an arm ratio of 1.7–3.0 (submetacentric chromosome); st = a subterminal centromeric chromosome with arm ratio 3.0–7.0 (subtelocentric chromosome); t = a terminal centromeric chromosome with arm ratio 7.0 and more (telocentric chromosome); T = a chromosome without an obvious short arm. Mean values of the arm ratio (r), centromeric index (CI), and relative chromosome length (RL) for each chromosome pair and total haploid length (THL) were determined. In addition, we calculated the coefficient of variation of chromosome length (CV_{cl}) (Paszko 2006), the coefficient of variation of the centromeric index (CV_{ci}) (Paszko 2006), and mean centromeric asymmetry (M_{ca}) (Peruzzi and Eroğlu 2013).

Results

Karyotypes of three *Actaea* spp.

A comparative karyomorphological analysis of *Actaea acuminata*, *A. asiatica*, and *A. erythrocarpa* was carried out. For *A. acuminata*, specimens from one population from the Primorye Territory of Russia were examined. For *A. asiatica*, specimens from one population in China (Shandong Province) were studied. For *A. erythrocarpa*, specimens from five populations were investigated that grow in Russia in Amur, Irkutsk, and Novosibirsk Regions and in Kamchatka and Krasnoyarsk Territories (Table 1). All the studied specimens were found to be diploid with $2n = 16$ (Figures 2 and 3). Their chromosome sets contain five pairs of large metacentric chromosomes, gradually decreasing in length, and three pairs of shorter unequal-armed chromosomes (Table 2). Values of the parameters characterizing levels of intrachromosomal asymmetry (mean centromeric asymmetry) and interchromosomal asymmetry (the coefficient of variation of chromosome length) proved to be similar among all the studied specimens (Table 3).

Actaea acuminata

The first three pairs of metacentric chromosomes of *A. acuminata* represent a group having an average relative length of 6.98–8.14% and similar arm ratios of ~1.07–1.09. The fourth and fifth pairs of metacentrics are less symmetrical and have an arm ratio of 1.27 and 1.29, respectively, on average. The sixth (by length) pair of chromosomes is subtelocentric, and the seventh one is submetacentric, having relative lengths of 5.59% and 4.62%, respectively, on average. The eighth pair is represented by telocentric chromosomes with a poorly distinguishable short arm; its relative length is on average 4.06% (Figure 2A and 3; Table 2).

Actaea asiatica

Chromosome pairs 1–5 of *A. asiatica* gradually decrease in length, and their relative length varies on average from 6.07% to 7.40%. Up to six metacentric chromosomes have secondary constrictions in subtelomeric regions. The sixth and seventh pairs of chromosomes are submetacentric. They have similar arm ratios but are clearly distinguishable by length; their relative lengths are 5.60% and 4.59%, respectively, on average. The shortest pair of chromosomes is telocentric having a relative length of 4.33% on average; its short arm is almost indistinguishable (Figure 2B and 3; Table 2).

Actaea erythrocarpa

Karyometric parameters were found to be similar among the studied *A. erythrocarpa* specimens from different regions (Figure 2C–F and 3; Table 2). Five pairs of large metacentric chromosomes vary in relative length on average from 5.94% to 8.17% and in the arm ratio on average from 1.06 to 1.32. In metacentric chromosomes of the specimens from Kamchatka and Krasnoyarsk Territories, up to four secondary constrictions were successfully visualized in subtelomeric regions. In the specimens from Amur Region, Novosibirsk and Irkutsk Regions, there were up to ten secondary constrictions in metacentric chromosomes in different metaphase plates. The sixth and seventh pairs of chromosomes are submetacentric with similar arm ratios but differ considerably in length. In the specimen from Krasnoyarsk Territory, smaller submetacentric chromosomes have secondary constrictions in subtelomeric regions of the long arms. The eighth pair of telocentric chromosomes in specimens from four regions (Amur, Irkutsk and Novosibirsk Regions, and Kamchatka Territory) has a poorly distinguishable short arm. In the specimen from Krasnoyarsk Territory, the short arms of the eighth pair were visualized better and carry small satellites.

Table 2. Karyomorphological parameters in three *Actaea* spp.

Species	Pair	CL, μm	r	CI, %	RL, %	Type
<i>A. acuminata</i>	1	8.90 ± 0.52	1.07 ± 0.05	48.37	8.14	m
	2	8.21 ± 0.34	1.09 ± 0.06	47.91	7.52	m
	3	7.62 ± 0.41	1.09 ± 0.03	47.91	6.98	m
	4	7.50 ± 0.67	1.27 ± 0.11	44.05	6.87	m
	5	6.79 ± 0.42	1.29 ± 0.08	43.77	6.22	m
	6	6.11 ± 0.32	3.28 ± 0.18	23.45	5.59	st
	7	5.04 ± 0.44	2.21 ± 0.14	31.14	4.62	sm
	8	4.43 ± 0.46	11.27 ± 1.11	8.20	4.06	t

Species	Pair	CL, μm	r	CI, %	RL, %	Type
<i>A. asiatica</i>	1	7.40 ± 0.52	1.09 ± 0.06	48.03	7.82	m
	2	7.05 ± 0.56	1.09 ± 0.05	47.91	7.44	m
	3	6.76 ± 0.58	1.15 ± 0.07	46.48	7.14	m
	4	6.32 ± 0.36	1.25 ± 0.08	44.56	6.68	m
	5	6.07 ± 0.34	1.23 ± 0.09	45.07	6.41	m
	6	5.30 ± 0.49	1.94 ± 0.13	34.18	5.60	sm
	7	4.35 ± 0.37	2.01 ± 0.15	33.32	4.59	sm
	8	4.10 ± 0.32	12.63 ± 1.76	7.42	4.33	t
<i>A. erythrocarpa</i> (Am)	1	8.65 ± 0.42	1.10 ± 0.06	47.71	8.11	m
	2	7.87 ± 0.31	1.07 ± 0.07	48.41	7.38	m
	3	7.61 ± 0.41	1.16 ± 0.06	46.38	7.14	m
	4	7.08 ± 0.49	1.06 ± 0.05	48.65	6.64	m
	5	6.93 ± 0.36	1.28 ± 0.05	43.87	6.50	m
	6	5.77 ± 0.18	1.91 ± 0.20	34.47	5.41	sm
	7	4.66 ± 0.38	1.99 ± 0.22	33.67	4.37	sm
	8	4.75 ± 0.35	11.50 ± 2.49	8.24	4.46	t
<i>A. erythrocarpa</i> (Irk)	1	8.59 ± 0.26	1.10 ± 0.05	47.68	7.99	m
	2	7.88 ± 0.29	1.07 ± 0.04	48.35	7.33	m
	3	7.60 ± 0.24	1.23 ± 0.12	44.88	7.07	m
	4	7.27 ± 0.60	1.04 ± 0.04	48.86	6.76	m
	5	6.99 ± 0.29	1.24 ± 0.04	44.76	6.50	m
	6	5.99 ± 0.28	1.91 ± 0.14	34.46	5.57	sm
	7	4.85 ± 0.32	1.93 ± 0.09	34.15	4.52	sm
	8	4.59 ± 0.19	15.02 ± 3.38	6.46	4.27	t
<i>A. erythrocarpa</i> (Kam)	1	8.27 ± 0.81	1.10 ± 0.05	47.63	8.06	m
	2	7.71 ± 0.48	1.10 ± 0.04	47.69	7.52	m
	3	7.28 ± 0.50	1.07 ± 0.04	48.34	7.10	m
	4	7.20 ± 0.59	1.18 ± 0.04	45.87	7.02	m
	5	6.09 ± 0.57	1.24 ± 0.08	44.81	5.94	m
	6	5.74 ± 0.50	1.88 ± 0.11	34.92	5.60	sm
	7	4.62 ± 0.37	2.33 ± 0.18	30.16	4.50	sm
	8	4.37 ± 0.39	18.74 ± 4.73	5.22	4.26	t
<i>A. erythrocarpa</i> (Kr)	1	9.34 ± 0.48	1.11 ± 0.03	47.49	7.68	m
	2	8.93 ± 0.29	1.07 ± 0.06	48.50	7.34	m
	3	8.57 ± 0.31	1.07 ± 0.03	48.37	7.05	m
	4	8.27 ± 0.22	1.32 ± 0.09	43.14	6.80	m
	5	7.85 ± 0.41	1.31 ± 0.10	43.36	6.46	m
	6	6.92 ± 0.35	1.94 ± 0.21	34.14	5.70	sm

Species	Pair	CL, μm	r	CI, %	RL, %	Type
	7	5.31 ± 0.23	2.28 ± 0.13	30.54	4.37	sm
	8	5.60 ± 0.25	7.39 ± 0.68	12.07	4.61	t
<i>A. erythrocarpa</i> (Ns)	1	10.71 ± 0.37	1.06 ± 0.03	48.69	8.17	m
	2	10.07 ± 0.63	1.08 ± 0.08	48.08	7.68	m
	3	9.34 ± 0.77	1.09 ± 0.06	47.93	7.13	m
	4	8.76 ± 0.75	1.21 ± 0.11	45.20	6.68	m
	5	8.31 ± 0.46	1.24 ± 0.10	44.79	6.34	m
	6	6.96 ± 0.19	1.91 ± 0.10	34.35	5.31	sm
	7	5.63 ± 0.27	2.12 ± 0.20	31.87	4.30	sm
	8	5.75 ± 0.39	13.04 ± 1.91	7.19	4.39	t

Notes: Pair – chromosome pair; CL – chromosome length ($M \pm SD$); r – arm ratio ($M \pm SD$); CI – centromeric index; RL – relative chromosome length; m – metacentric chromosome; sm – submetacentric chromosome; st – subtelocentric chromosome; t – telocentric chromosome.

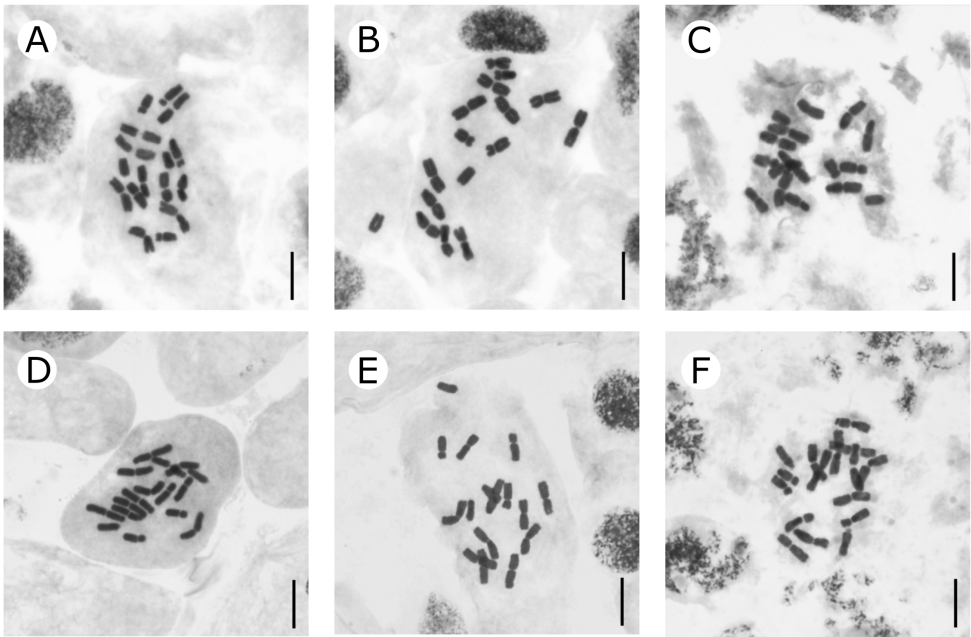


Figure 2. Mitotic metaphase chromosomes of three *Actaea* spp. **A** – *A. acuminata* (PR2023-3). $2n = 16$; **B** – *A. asiatica* (CH2023-26), $2n = 16$; **C** – *A. erythrocarpa* (AM2023-6), $2n = 16$; **D** – *A. erythrocarpa* (IRK2023-3), $2n = 16$; **E** – *A. erythrocarpa* (KAM2023-1), $2n = 16$; **F** – *A. erythrocarpa* (KR2023-4), $2n = 16$. Scale bars = 10 μm .

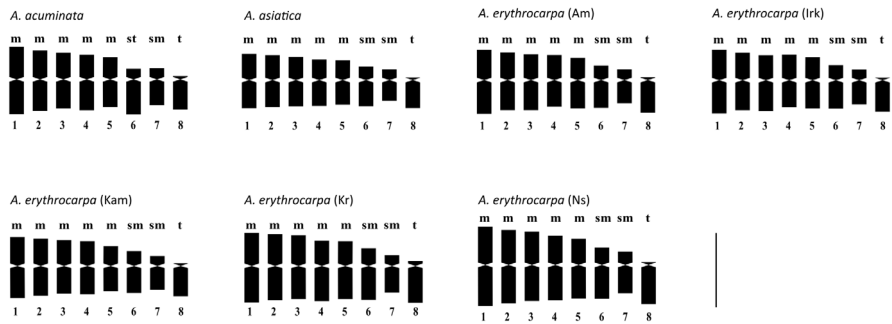


Figure 3. Haploid idiograms of three *Actaea* spp. m – metacentric chromosome; sm – sub-metacentric chromosome; st – subtelocentric chromosome; t – telocentric chromosome; 1–8 – chromosome pairs. Scale bar = 10 μ m.

Table 3. Karyotype parameters in three *Actaea* spp.

Species	2n	PL	Karyotype formula	THL, μ m	CV _{CL}	M _{CA}	CV _{CI}
<i>A. acuminata</i>	16	2x	10m + 2sm + 2st + 2t	54.60 $\pm$ 2.57	21.83 $\pm$ 0.63	25.98 $\pm$ 1.73	38.45 $\pm$ 1.06
<i>A. asiatica</i>	16	2x	10m + 4sm + 2t	47.34 $\pm$ 3.26	20.31 $\pm$ 0.75	23.25 $\pm$ 0.76	34.92 $\pm$ 0.84
<i>A. erythrocarpa</i> (Am)	16	2x	10m + 4sm + 2t	53.32 $\pm$ 1.22	21.89 $\pm$ 1.69	22.67 $\pm$ 2.07	34.19 $\pm$ 1.84
<i>A. erythrocarpa</i> (Irk)	16	2x	10m + 4sm + 2t	54.75 $\pm$ 0.78	21.09 $\pm$ 0.27	22.59 $\pm$ 0.60	35.85 $\pm$ 0.91
<i>A. erythrocarpa</i> (Kam)	16	2x	10m + 4sm + 2t	51.34 $\pm$ 4.02	22.30 $\pm$ 2.74	24.20 $\pm$ 0.53	37.68 $\pm$ 1.03
<i>A. erythrocarpa</i> (Kr)	16	2x	10m + 4sm + 2t	60.78 $\pm$ 1.73	20.75 $\pm$ 2.80	22.68 $\pm$ 0.40	32.03 $\pm$ 1.64
<i>A. erythrocarpa</i> (Ns)	16	2x	10m + 4sm + 2t	65.53 $\pm$ 3.29	22.80 $\pm$ 1.28	22.95 $\pm$ 0.95	35.79 $\pm$ 0.90

Notes: 2n – somatic chromosome number; PL – ploidy level; m – metacentric chromosome; sm – sub-metacentric chromosome; st – subtelocentric chromosome; t – telocentric chromosome; THL – total haploid length (M $\pm$ SD); CV_{CL} – coefficient of variation of chromosome length (M $\pm$ SD); M_{CA} – mean centromeric asymmetry (M $\pm$ SD); CV_{CI} – coefficient of variation of centromeric index (M $\pm$ SD).

Discussion

The karyotype of *A. acuminata* was previously described for specimens from the Russian Far East (Vladivostok city) and its formula was defined as 2n = 16 = 10m + 4sm + 2t. The peculiarity of this karyotype was the presence of heteromorphic seventh pair of submetacentric chromosomes, namely one homologue was significantly shorter than the other. The longer homologue was similar to the chromosomes

of pair 6 in length and morphology. On this basis the author of the work has assumed hybrid origin of *A. acuminata* (Dubrova 1985). In our study we determined the karyotype formula for *A. acuminata* from the same region as $2n = 16 = 10m + 2sm + 2st + 2t$. The sixth and seventh pairs of chromosomes were subtelocentric and submetacentric respectively, as well as homomorphic and well differentiated in length (Table 1). It can be assumed that the specimens from the previous study were hybrid taxon, or had heterozygous chromosome rearrangement. Heteromorphism of chromosome pair occurs within the tribe Cimicifugeae. For example, heteromorphic chromosome pairs were detected in *Eranthis byunsanensis* B.Y. Sun, in one of the populations of *E. pinnatifida* Maxim. (Mitrenina et al. 2021) and in *E. bulgarica* (Stef.) Stef. (Mitrenina et al. 2023). The origin of this heteromorphism in *Eranthis* is not yet clear.

According to Compton's revision (1998b), *Actaea acuminata* has a subspecies rank: *A. spicata* var. *acuminata*. The karyotype of *A. spicata* has been described earlier for specimens from a population in Kemerovo Region (Western Siberia, Russia) (Malakhova et al. 1976). According to this evidence, the karyotype formula of *A. spicata* is $2n = 16 = 10m + 4sm + 2t$. In our work, it was determined that the karyotype formula for *A. acuminata* is $2n = 16 = 10m + 2sm + 2st + 2t$. Considering low variation of karyomorphological parameters among representatives of the genus *Actaea*, it can be hypothesized that the difference in the karyotype formula between *A. acuminata* and *A. spicata* in one pair of chromosomes is an essential feature that allows to discriminate these taxa. On the other hand, the taxonomic status of *A. acuminata* can undoubtedly be determined unambiguously via a comprehensive study that includes anatomical-morphological and molecular-phylogenetic approaches.

The chromosome set of *A. asiatica* has been previously investigated by some researchers, and its structure has been presented as different formulas. In the first studies, the chromosome set was described as $2n = 16 = 10m + 6sm$ (Kurita 1957; Wang et al. 1994). This has subsequently led to an incorrect conclusion that the karyotype of *A. asiatica* is the most symmetrical and primitive among the Ranunculaceae representatives that had been studied by then (Tamura 1995). Later, the karyotype structure has been revised, and it has been shown that the karyotype formula of *A. asiatica* growing in Yunnan Province of China corresponds to $2n = 16 = 10m (3sat) + 4sm + 2T$ (Yang 1998). In a later work, same researcher presented the formula as $2n = 16 = 10m(6sat) + 2sm + 2st + 2T$ (Yang 2002). The presence of subtelocentric chromosomes in this karyotype in our opinion is reported erroneously, judging by the microphotograph of chromosomes presented in that paper. It should be clarified that the generally accepted classification of chromosomes according to Levan et al. (1964) was used in that work. In our study, we analyzed the karyotype of *A. asiatica* from Shandong Province of China and noted its similarity with the one described earlier in the study by Yang (Yang 1998) for Yunnan Province. Because we were able to visualize the small arm of the eighth pair of chromosomes, which was visible on not all metaphase plates, we describe the karyotype formula as $2n = 16 = 10m(6sat) + 4sm + 2t$.

A detailed analysis of the *A. erythrocarpa* karyotype has been previously performed on plants from Tomsk Region (Western Siberia, Russia) (Malakhova et al. 1976; Dubrova and Malakhova, 1980; Dubrova 1981). According to these articles, the karyotype has formula $2n = 16 = 10m(10sat) + 4sm + 2t(2sat)$. The number of secondary constrictions in the examined samples varied among metaphase plates. Accordingly, the reported formula indicates the maximal number of chromosomes carrying secondary constrictions. We evaluated karyotypes of *A. erythrocarpa* specimens from five regions of Russia. Overall, our results are very similar to the findings of the articles cited above. The general karyotype formula of our specimens can be described as $2n = 16 = 10m + 4sm + 2t$. The plants studied here – also growing in Western Siberia (Novosibirsk Region), Eastern Siberia (Irkutsk Region) and from the Russian Far East (Amur Region) – have up to ten clear-cut secondary constrictions on metacentric chromosomes. By contrast, in the other two analyzed populations from Eastern Siberia (Krasnoyarsk Territory) and from the Russian Far East (Kamchatka Territory), we found up to four secondary constrictions in metacentric chromosomes. At the same time, the specimens from Krasnoyarsk Territory also showed satellites on telocentric chromosomes, as described before for plants from Tomsk Region (Malakhova et al. 1976; Dubrova 1981). In the same plants in our study, we detected secondary constrictions in the long arms of shorter submetacentric chromosomes (pair 7). A similar localization of constrictions in the seventh submetacentric pair of chromosomes has previously been noted in *Actaea cimicifuga* L. ($\equiv$ *Cimicifuga foetida* L.) and *A. spicata* (Malakhova et al. 1976).

Secondary constrictions are associated with nucleolus organizing regions. Their presence or absence in specific chromosomes of a set is not always a stable karyotypic trait. It is believed that the presence of secondary constrictions reflects functional features of certain cells. In this context, the number and localization of nucleolus organizing regions (i.e., 45S ribosomal DNA) detected by molecular cytogenetic methods may be a more important chromosome-specific and species-specific feature than the presence/absence of secondary constrictions (Raskina et al. 2008; Roa and Guerra 2012).

Our comparative analysis of karyotypes of the three *Actaea* species revealed similarities both of their structure and of parameters of intrachromosomal and interchromosomal asymmetry (Peruzzi and Eroğlu 2013). According to the literature, two types of karyotype formula $2n = 16 = 10m + 4sm + 2T/t$ and $2n = 16 = 10m + 2sm + 2st + 2T/t$ are characteristic of almost all *Actaea* species that have been analyzed in this regard. The first formula is much more common. Mainly number and position of the identified secondary constrictions/satellites vary (Kurita 1956; Kawano et al. 1966; Blair et al. 1975; Lee and Park 1998; Yang 1999; Yang 2002; Yuan and Yang 2006; Luo et al. 2016). Differences (from these two formulas) presented by some researchers can be usually explained by an incorrect description of karyotype structure.

Regarding other members of the tribe Cimicifugeae, more pronounced intragenetic karyotype polymorphism has been identified in the very small genus *Beesia* (Shang 1985; Yang 1999; Yang 2002; Yuan and Yang 2006) and in the genus *Eranthis*

(Mitrenina et al. 2021; Mitrenina et al. 2023). The polymorphism of chromosome sets of *Eranthis* manifests itself in the existence of two basic chromosome numbers $x = 7$ and $x = 8$ (in contrast to other genera of the tribe exclusively having $x = 8$), in the presence of polyploid taxa, and in interspecies differences of karyotype structure. It should be pointed out that *E. sibirica* DC. and the recently described species *E. tanhoensis* Erst (Erst et al., 2020) evidently possess the most symmetrical karyotypes within the tribe (Mitrenina et al. 2021). Their chromosome sets contain only metacentric and submetacentric chromosomes, in contrast to other examined representatives of the tribe. Nevertheless, this symmetry is a secondary trait (Weiss-Schneeweiss and Schneeweiss, 2013) because these species have arisen later in evolution (Xiang et al., 2021) as compared to species having more asymmetric karyotypes (e.g., *E. stellata* Maxim. and *E. lobulata* W.T. Wang) (Mitrenina et al. 2021). Thus, the symmetry of *E. tanhoensis* and *E. sibirica* karyotypes is not related karyotype primitivity, in contrast to assumption made about *Actaea asiatica* (Tamura 1995).

Conclusions

The karyotype of *A. acuminata* was re-examined. For *A. asiatica* and *A. erythrocarpa*, karyotypes of plants from previously unstudied populations growing in China and Russia, respectively, were investigated here. It was shown that the karyotype of *A. acuminata* differs from the karyotype of *A. spicata*, probably because of the independent species status of the former, in contrast to regarding it as the subspecies *A. spicata* var. *acuminata*. Chromosome sets of *A. asiatica* and *A. erythrocarpa* from the locations analyzed by us are similar to chromosome sets of plant specimens previously examined by other researchers. Our results and literature data indicate high conservatism of karyotype structure within *Actaea*. Their evolution may have proceeded mostly avoiding substantial chromosomal rearrangements in contrast to *Eranthis*.

Acknowledgments

The work was supported by the Russian Science Foundation, grant No. 23-14-00230, <https://rscf.ru/project/23-14-00230/> (karyotype analysis); government-funded projects of the Central Siberian Botanical Garden SB RAS (AAAA-A21-121011290024-5) (collection of materials in Novosibirsk Oblast’); world-class scientific and educational centers Yenisei Siberia (collection of materials in Khakass Republic); the National Natural Science Foundation of China, grant No. 32361133549, 32400182 and the Beijing Natural Science Foundation, 5244047 (data curation).

The authors are grateful to Roman V. Annenkov for compiling Figures 2 and 3.

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