Botanical Journal of the Linnean Society, 2005, **147**, 275–290. With 2 figures

The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and *atp*B-*rbc*L intergenic spacer (IGS) versus conventional taxonomy

VOLKER WISSEMANN FLS* and CHRISTIANE M. RITZ

Friedrich-Schiller University Jena, Department of Systematic Botany, Philosophenweg 16, D-07743 Jena, Germany

Received March 2004; accepted for publication September 2004

Sequences of the nrDNA internal transcribed spacer 1 (nrITS-1) and *atp*B-*rbc*L intergenic spacer (IGS) of the cpDNA were analysed for all sections of the genus *Rosa* L. (Rosoideae, Rosaceae) to study molecular infrageneric taxonomy and relationships of *Rosa* with respect to conventional taxonomy based upon morphological and anatomical data as well as phytochemical characters. The results suggest that *Rosa* in its traditional infrageneric circumscription is not reflected by molecular data. *Cinnamomeae*, *Carolinae* and *Pimpinellifoliae* are not monophyletic based on the molecular data and this is mirrored in conventional taxonomy that separates these sections by weak morphological characters such as sepal performance, existence of bracts, and number of flowers per inflorescence. Section *Pimpinellifoliae* is split by the monotypic sections *Laevigatae*, *Platyrhodon*, *Bracteatae* and *Hesperhodos*. Section *Caninae* is a natural allopolyploid group characterized by its autapomorphic ITS C-type and Canina-meiosis. CpDNA subdivides sect. *Caninae* into two natural clusters of eglandular and glandular species. NrITS shows sect. *Synstylae*/*Indicae* to be the direct sister group to sect. *Caninae*, not *Rosa* (*Gallicanae*) although both groups are morphologically characterized by pinnate sepals. From our molecular data sect. *Indicae* and sect. *Synstylae* are consectional. The highest taxonomic rank below the generic level should be the sectional status.

ADDITIONAL KEYWORDS: evolution – *Hulthemia* – hybridization – molecular phylogeny – wild roses.

INTRODUCTION

Rosa L. is distributed throughout the temperate and subtropical regions of the Northern hemisphere (Rehder, 1949). The genus comprises about 200 species and the taxonomic treatment of the highly diverse group is complicated due to biological phenomena in reproductive biology, insufficient morphological and anatomical characters to adequately discriminate between species and the human impact by rose breeding (Wissemann, 2003a). Conventional taxonomy (Rehder, 1949; Wissemann, 2003a) divides the genus into four subgenera, three of which are monotypic or contain two species: *Hulthemia* (Dumort.) Focke, 1888, *Platyrhodon* (Hurst) Rehder, 1940, *Hesperhodos* Cockerell, 1913 and *Rosa*. A fourth subgenus *Rosa* harbours about 95% of all species and is subdivided into ten sections: *Pimpinellifoliae* (DC.) Ser. 1825; *Rosa* (= sect. *Gallicanae* (DC.) Ser. 1825); *Caninae* (DC.) Ser. 1825; *Carolinae* Crép., 1891; *Cinnamomeae* (DC.) Ser. 1825; *Synstylae* DC. 1813; *Indicae* Thory, 1820; *Banksianae* Lindl., 1820; *Laevigatae* Thory, 1820; *Bracteatae* Thory, 1820. Since the lectotypification by Britton & Brown (1913), *R. centifolia* L. from the former *Gallicanae* is the generic type. However, this typification has been disputed (e.g. de la Roche, 1978; Rowley, 1992) but the proposal to replace this typification by *R. cinnamomeae* L. as been rejected by the nomenclatural committee in Tokyo 1995 and again in Saint Louis 2000 and thus *R. centifolia* is still the valid choice.

Since roses have been of great influence on human cultural evolution, the earliest attempts to classify the genus date back to the 16th century, when roses were treated either as wild or 'gentle' species and were additionally divided based on petal colour (Wissemann,

*Corresponding author. E-mail: Volker.Wissemann@uni-jena.de

2000). This was accepted until Linnaeus (1753, 1772) classified all species of *Rosa* known at that time, mainly on the shape of the hip. Willdenow (1811) introduced the presence and form of prickles as well as the indumentum and occurrence of glands as taxonomic relevant characters. From then on, the number of described species increased rapidly into several thousands (most prominent Déséglise, 1877; Gandoger, 1892) mirroring uncertainty about the diversity of roses rather than insight into evolutionary processes (Wissemann, 2003a). Delimitation of species in nature, as indicated by natural populations, has been a problem in roses for a long time. Introduction of different gene pools by replanting of disturbed or protected areas is one of the most serious problems for conservation genetics. Herrmann (1762) claimed that horticulture had merged the species so that they would be no longer recognizable. He also pointed to the shortage of phenotypic characters that delimit determination. This lack of characters has been the source of several attempts based on different markers to get insight into phylogenetic relationships within the genus. Debener, Bartels & Mattiesch (1996), Matsumoto & Fukui (1996) and Millan *et al*. (1996) used RAPD markers or RFLP studies (Matsumoto, Wakita & Fukui, 1997) to elaborate the phylogeny of *Rosa*. Sequence data from *mat*K and nrITS have been investigated for their ability to resolve the phylogeny (Matsumoto *et al*., 1998, 2000; Wu *et al*., 2000, 2001) and Grossi, Raymond & Jay 1998 and Grossi *et al.* 1999 investigated biochemical data (flavonoids and isoenzyme polymorphisms). However, results of these investigations remain contradictory due to the small sample of investigated taxa and the inadequate resolution of the markers, which in most cases were also not discussed in the context of morphology, distribution and other sources of evidence. We applied sequences of the nrDNA internal transcribed spacer 1 (nrITS-1) and the cpDNA marker: *atp*B-*rbc*L intergenic spacer (IGS) to study molecular infrageneric taxonomy and phylogenetic relationships among *Rosa* with respect to conventional taxonomy. The results are interpreted in a broader context of evolutionary biology in *Rosa*.

MATERIAL AND METHODS

TAXON SAMPLING

We sequenced four *Rubus*-species (*R. caesius* L., *R. idaeus* L., *R. saxatilis* L. and *R. ulmifolius* Schott) as outgroup taxa according to the results of the molecular analyses in Rosoideae by Eriksson *et al*. (1998, 2003). Sampling of the ingroup taxa represent all sections of the genus *Rosa* (Table 1). Table 2 presents the

Table 1. List of taxa and sources of plant material analysed. Subgeneric classification and nomenclature follows Wissemann (2003a). Accession numbers are for the EMBL data base. Abbreviations: SGH = Europa-Rosarium Sangerhausen, Germany, Aeuble = Wildrose collection of the Schwäbische Albverein Rottenburg/Neckar, Germany; Kassel: Rose collection Kassel-Wilhelmshöhe, Germany; TAMU: Collection at Texas A.M. University, Department of Horticultural Sciences, USA

Taxon	Accession	nrITS1	*atp*B-*rbc*L IGS
Subgen. *Hulthemia* (Dumort.) Focke (1888)			
R. persica Michx. ex Juss.	D-Lower Saxony Göttingen, Bot. Garden, Sect. Ecology, leg. VW	AJ631841	AJ628770
Subgen. *Platyrhodon* (Hurst) Rehder 1940			
R. roxburghii Tratt.	Kassel, leg. VW	AJ631843	AJ628823
Subgen. *Hesperhodos* Cockerell 1913			
R. stellata Wooton	Kassel, leg. VW	AJ631842	AJ628824
Subgen. *Rosa*			
Sect. *Pimpinellifoliae* (DC.) Ser. 1825			
R. altaica Willd.	Altai, Ortsausgang Aktasch, Richtung Ust-Ulangom, leg. F. Schlütz 02.09. 2000	AJ631849	AJ628774
R. ecae Aitch.	SGH, leg. V.W.	AJ631878	AJ628781
R. foetida J. Herrm.	SGH, leg. V.W.	AJ631879	AJ628785
R. hugonis Hemsl.	SGH, leg. V.W.	AJ631882	AJ628780
R. primula Boul.	SGH, leg. V.W.	AJ631876	AJ628822
R. sericea Lindl.	SGH, leg. V.W.	AJ631874	AJ628784

Table 1. *Continued*

Taxon	Accession	nrITS1	*atp*B-*rbc*L IGS
R. spinosissima L.	A-Senftenberg, Krems, Austria leg. M. Koch V14	AJ631880	AJ628787
Sect. *Rosa* (*Gallicanae* (DC.) Ser. 1825)			
R. gallica L.	D-Rottenburg/Neckar, Seebronn. leg. G. Timmermann	AJ631922	AJ628788
Sect. *Caninae* (DC.) Ser. 1825			
R. abietina Gren. ex Christ	H-Kanton Glarus, Braunwald, leg. G. Timmermann	AJ631940 (C9–1) AJ631941 (C9–2) AJ631942 (C9–3)	AJ628797
R. agrestis Savi	D-Niedersachsen, Banenrode, leg. E. Garve & H. Henker Ro 12/92	–	AJ628816
R. *caesia* Sm.	D-Schleswig-Holstein, Fehmarn, leg. V.W.	–	AJ628791
R. jundzillii L.	D-Niedersachsen, Bovenden, north of Göttingen, leg. V.W.	AJ628795	AJ631923
R. rubiginosa ssp. *columnifera*	D-Mecklenburg-Vorpommern, Neubrandenburg, Lindenberg leg. A. Mohr Schwertschlager	AJ631934 (C3–1)	AJ628811
R. corymbifera Borkh.	D-Niedersachsen, Gross Schneen near Göttingen, leg. V.W.	AJ628793	
R. dumalis Bechst.	D-Schleswig Holstein, Fehmarn, north of Bisdorf 1996, Wissemann 1013	AJ811537	–
R. elliptica Tausch	D-Thüringen, Schmon, leg. G. Schulze 5/90	–	AJ628814
R. glauca Pourr.	D-Niedersachsen, Göttingen, Bot. Garden, Sect. Systematics, leg. V.W.	–	AJ628808
R. inodora Fries	D-Mecklenburg-Vorpommern, Wismar, leg. H. Henker Ro 1/92	–	AJ628815
R. jundzillii Besser	D-Rheinland-Pfalz, Mertesdorf near Trier, leg. H. Reichert 93–199	AJ631924	AJ628794
R. micrantha Borrer ex Sm.	D-Mecklenburg-Vorpommern, Neustrelitz, leg. H. Henker Ro 46/92	AJ631929 (C2–3) AJ631930 (C2–4) AJ631933 (C2–6)	AJ628806
R. mollis Sm.	D-Schleswig-Holstein, Geltinger Birk, Flensburg, leg. V.W.	AJ631949 (VW152–7)	AJ628812
R. montana Chaix	I-Südtirol, Vinschgau, Sonnenberg near of Schlanders, leg. V.W.	AJ631947 (C8–1) AJ631948 (C8–2)	AJ628796
'*R. mosqueta*' = *R. rubiginosa* L. from South-America	Argentinia, Provincia del Chubut, Comarca Andino Parallelo 42, Warton, leg. C. Ritz	–	AJ628809
R. pseudoscabriuscula (R. Keller) Henker & G. Schulze	D-Mecklenburg-Vorpommern, Burg Stargard, leg. H. Henker Ro6/91	AJ631927 (C1–1) AJ631928 (C1–2) AJ631932 (C1–3)	AJ628810,
R. rubiginosa L.	D-Schleswig-Holstein, Helgoland, leg. V.W.	AJ631885	AJ628819
R. sherardii Davies	D-Mecklenburg-Vorpommern, Neukloster, leg. H. Henker 23/87	AJ631925	AJ628813
R. sicula Tratt.	SGH, leg. V.W.	AJ631937 (VW161–1) AJ631938 (VW161–2) AJ631939 (VW161–3)	AJ628817
R. stylosa Desvaux	D-Baden-Württemberg, Badenweiler, leg. G. Timmermann	AJ631926	AJ628798
R. subcanina (H. Christ) R. Keller	D-Mecklenburg-Vorpommern, Warin, leg. H. Henker 24/87	AJ631935 (VW141–1) AJ631936 (VW141–2)	AJ628790
R. subcollina (H. Christ) R. Keller	D-Niedersachsen, Westharz, Hohegeiss, leg. H. Henker Ro 10/92	–	AJ628792
R. tomentella Léman	D-Mecklenburg-Vorpommern, Poischendorf, leg. H. Henker 20/87	AJ631945 (VW146–1)	AJ628789

Table 1. *Continued*

Taxon	Accession	nrITS1	*atp*B-*rbc*L IGS
R. tomentosa Sm.	D-Mecklenburg-Vorpommern, Züsow, leg. H. Henker 18/87	AJ631943 (VW142–1) AJ631944 (VW142–2)	AJ628805
R. villosa L.	D-Mecklenburg-Vorpommern, Lübz, leg. H. Henker 34/88	–	AJ628807
Sect. *Carolinae* Crép. 1891			
R. carolina Willd.	SGH, leg. V.W. (C29)	AJ631855	AJ628771
R. nitida Willd.	D-Göttingen, Leonard Nelsonstrasse, leg. V.W.	AJ631860	AJ628828
R. palustris Marsh.	D-Niedersachsen, Göttingen, Bot. Garden, Sect. Systematics, leg. VW	AJ631864	AJ628830
R. virginiana Herrm.	SGH, leg. V.W.	AJ631857	AJ628776
Sect. *Cinnamomeae* (DC.) Ser. 1825			
R. arkansana I Porter ex. I.M. Coult	SGH, leg. V.W. (C30)	AJ631858	AJ628778
R. arkansana II Porter ex. I.M. Coult	SGH, leg. V.W. (C35)	AJ631862	AJ628779
R. beggeriana Schrenk	SGH, leg. V.W.	AJ631866	AJ628829
R. blanda Ait.	SGH, leg. V.W.	AJ631859	AJ628772
R. laxa Retz	China, Xinjiang, Kongur, Atoinak, 2750 m, leg. M. Richter 1996-07-04	AJ631881	AJ628775
R. majalis Herrm.	Rottenburg/Neckar, Äuble, leg. C. Ritz	AJ631867	AJ628777
R. multibracteata Hemsl. et E.H. Wilson	SGH, leg. V.W.	AJ631872	AJ628821
R. rugosa Thunb.	D-Schleswig-Holstein, Sylt, leg. D. Loessner	AJ631865	AJ628782
R. sertata Rolfe	SGH, leg. V.W.	AJ631856	AJ628773
R. suffulta Greene	SGH. Leg. V.W.	AJ631851	AJ628820
R. willmottiae Hemsl.	D-Niedersachsen, Göttingen, Bot. Garden, Sect. Systematics, leg. V.W.	AJ631871	AJ628783
R. woodsii Lindl.	Kassel, leg. V.W.	AJ631852	AJ628826
Sect. *Synstylae* DC. 1813			
R. arvensis Huds.	I-Südtiro, Kastel Feder, leg. V. W.	–	AJ628804
R. helenae Rehd. & Wils.	Kassel, leg. V.W.	AJ631877	AJ628802
R. multiflora Thunb. ex. Murr.	D-Niedersachsen, Göttingen, Bot. Garden, Sect. Systematics, leg. V.W.	AJ631845	AJ628799
R. wichurana Crép.	D-Niedersachsen, Göttingen, Bot. Garden, Sect. Systematics, leg. V.W.	AJ631846	AJ628827
Sect. *Indicae* Thory, 1820			
R. chinensis Jacq. (C38)	SGH, leg. V.W.	AJ631847	AJ628800
R. chinensis Jacq. (C20)	SGH, leg. V.W.	–	AJ628802
R. odorata (Andrews) Sweet	SGH, leg. V.W.	AJ631848	AJ628801
Sect. *Banksianae* Lindl., 1820			
R. banksiae Ait.	TAMU, leg. D. Byrne	AJ631853	AJ628825
Sect. *Laevigatae* Thory, 1820			
R. laevigata Michx.	TAMU, leg. D. Byrne	AJ631873	AJ628786
Sect. *Bracteatae* Thory, 1820			
R. bracteata Wendl.	TAMU, leg. D. Byrne	AJ631863	AJ628818
Outgroup taxa			
Rubus caesius L.	I-Südtirol, Andrian, leg. V.W.	AJ631965	–
Rubus idaeus L.	I-Südtirol, Nals, leg. V.W.	AJ631962	–
Rubus saxatilis L.	I-Südtirol, Felixer Weiher, leg. V.W.	AJ631963	AJ628832
Rubus ulmifolius Schott	I-Südtirol, Andrianer Wald, leg. V.W.	AJ631964	AJ628831

Table 2. List of taxa analysed. Subgeneric classification and nomenclature follows Wissemann (2003a). The first number indicates the estimated total number of species in the section, the second number represents the number of species included in this study

Taxon	
Subgen. *Hulthemia* (Dumort.) Focke, 1888	1/1
Subgen. *Platyrhodon* (Hurst) Rehder, 1940	1/1
Subgen. *Hesperhodos* Cockerell, 1913	2/1
Subgen. *Rosa*	184?/58
Sect. *Pimpinellifoliae* (DC.) Ser. 1825	15/7
Sect. *Rosa* (*Gallicanae* (DC.) Ser. 1825)	1/1
Sect. *Caninae* (DC.) Ser. 1825	50/25
Sect. *Carolinae* Crép., 1891	5/4
Sect. *Cinnamomeae* (DC.) Ser. 1825	80?/12
Sect. *Synstylae* DC., 1813	25/4
Sect. *Indicae* Thory, 1820	3/2
Sect. *Banksianae* Lindl., 1820	2?/1
Sect. *Laevigatae* Thory, 1820	1/1
Sect. *Bracteatae* Thory, 1820	2?/1

distribution of the sampling within the genus. All specimens are deposited at the author's herbarium (Herbarium Wissemann).

DNA ISOLATION, PCR AMPLIFICATION, SEQUENCING, CLONING

Total DNA was extracted from silica gel-dried material of living plants or herbarium specimen using E.Z.N.A. Plant DNA Mini Kit (Peqlab Biotechnologie GmbH) following the users protocol. Amplification of double stranded DNA was performed on 25 µl containing 2.5 µl 10-fold polymerase buffer, 2.5 µl 2 mM dNTP, 10 pmol μl^{-1} of each primer, 1 unit of Taq polymerase (Appligene), 1 µl DNA template. Primers for ITS-1 regions were taken from White *et al*. (1990): 'ITS5' 5′-GGAAGTAAAAGTCGTAACAAGG-3′ and from Ochsmann (2000): 'P2' 5′-CTCGATGGAACACGGGATT CTGC-3′. Primers for the amplification of the 5′ end of the *atp*B-*rbc*L intergenic spacer ('2' 5′-GAAGTAGTAG GATTGATTCT-3′ and '10' 5′-CATCATTATTGTATAC TCTTTC3′)-were taken from Savolainen *et al*. (1994). The standard PCR conditions consist in an initial denaturation of 180 s at 95 °C, 28 cycles of 30 s at 95 °C, 60 s at 48 °C and 120 s at 72 °C with a final extension of 180 s at 72 °C. PCR products except for samples of ITS-1 PCR products of section *Caninae* and *Gallicanae* were directly sequenced in both directions with the same primers as for amplification with Amersham Bioscience Thermo Sequenase labelled Primer Cycle Sequencing kit with 7-deaza-dGTP. Samples of section *Caninae* and *Rosa* were subcloned before sequencing. PCR products were purified using Qiaquick PCR purification kit according to the manufacturer's instructions and subcloned with a t-tailed pBluescript II SK (+) cloning vector into the *E. coli* strain JM13 via electroporation. Transformed *E. coli* cells were plated on LB agar with ampicillin (100 µg ml^{-1}), IPTG (0.2 mM) and X-Gal (40 µg ml^{-1}). White colonies were selected for growth and these clones were picked and directly added to the amplification mix for ITS-1 and afterwards sequenced (protocols and cycling profiles are identical to the ones described above).

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

DNA sequences were aligned using ClustalX, version 1.83 (Thompson *et al*., 1997) and apparent misalignments were corrected manually. The final alignment has been deposited in TreeBase http://www.herbaria.harvard.edu/treebase/, accession numbers are given in Table 1. Phylogenetic relationships were analysed via Bayesian inference using Monte Carlo Markov chains (MCMC) was conducted with MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001). Four incrementally heated simultaneous Monte Carlo Markov chains were run over 2 000 000 generations, using the general time reversible model of DNA substitution with gamma distributed substitution rates, random starting trees and default starting values of the DNA substitution model. Trees were sampled every 100 generations resulting in an overall sampling of 20 001 trees. The first 1000 trees were discarded as 'burnin'. From the remaining trees a 50% majority rule consensus tree was computed to obtain estimates for the a posteriori probabilities. Branch lengths were estimated as mean values over the sampled trees. This Bayesian approach of phylogenetic analysis was repeated four times, always using random starting trees and random starting values.

RESULTS

Description of sequence data: nrITS-1: 264 bp in total, from all positions including outgroup 75 were variable, 189 constant positions. Only ingroup: 54 variable. cpDNA: 616 bp in total, from all positions including outgroup 59 were variable, 557 constant positions. Only ingroup: 39 variable.

ITS sequences of species of sect. *Caninae* and *Rosa* evolve non-concerted (Wissemann, 1999, 2000, 2002, 2003b), polymorphisms can be easily detected by direct sequencing but required a cloning step to obtain sequences suitable for phylogenetic reconstruction. No polymorphisms (double bands) were detected when the ITS sequences of non-dog roses and non-gallicaroses were sequenced directly after PCR

Table 3. Synopsis of the main characters which (a) support or (b) conflict with the current taxonomy. For details see the Discussion

Taxon	Support	Conflict
Subgen. *Hulthemia* (Dumort.) Focke, 1888	Molecular: nrITS data support inclusion in genus *Rosa*. Morphology: leaf, pollen exine patterns, hips.	Molecular: cpDNA support sister relationship of *Hulthemia* to the remainder of the genus.
Subgen. *Platyrhodon* (Hurst) Rehder, 1940	Morphology: peeling bark, hip, number of leaflets. Reproductive biology: reduced interfertility with other species.	Molecular: nrITS and cpDNA are ambiguous.
Subgen. *Hesperhodos* Cockerell, 1913	Molecular: nrITS data and cpDNA support inclusion in genus *Rosa*. Morphology: leaf, autapomorphic pollen exine pattern, hips. Absence of disc. Cytology: few metacentric chromosomes.	Molecular: nrITS data and cpDNA support inclusion in genus *Rosa*, but uncertain placement.
Subgen. *Rosa*		
Sect. *Pimpinellifoliae* (DC.) Ser. 1825	Phytochemistry: kaempferol, quercitin 4′-glucosides). Morphology: high number of leaflets, single flowers without bracts.	Molecular: nrITS and cpDNA data do not show monophyly of the section. RFLP combines it with *Cinnamomeae*. Morphology: black hips occur also in *Cinnamomeae*.
Sect. *Rosa* (*Gallicanae* (DC.) Ser. 1825)	Molecular: Distinct ITS-sequence, but ambiguous position. Morphology: hip with long glandular stalk.	Morphology: pinnate sepals occur also in sect. *Caninae*.
Sect. *Caninae* (DC.) Ser. 1825	Molecular: nrITS data show monophyly of the allopolyploid section by existence of an autapomorphic ITS-type. CpDNA separates the section from other sections. Reproductive biology: heterogamy. Cytology: Caninae-meiosis.	Morphology: characterization is only possible by a combination of characters, no autapomorphic character state exists.
Sect. *Carolinae* Crép., 1891	Morphology: deciduous sepals. Phytochemistry: specific anthocyanin.	Molecular: *mat*K, nrITS and cpDNA data do not show monophyly of the section, species are intermixed with *Cinnamomeae*. Morphology: deciduous sepals occur also in sect. *Caninae* and are used to separate species within a section. Phytochemistry: flavonoid, enzyme polymorphisms are the same as within *Cinnamomeae*.
Sect. *Cinnamomeae* (DC.) Ser. 1825	Morphology: entire sepals, corymbose flowers. Single flowers with bracts.	Molecular: nrITS and cpDNA data do not show monophyly of the section and intermixes with *Pimpinellifoliae*. RFLP combines it with *Pimpinellifoliae*. Phytochemistry: close affinity and intermixing of biochemical patterns.
Sect. *Synstylae* DC., 1813	Morphology: agglutinated styles. Phytochemistry: biochemical data, carotene.	Molecular: nrITS and cpDNA data do not show monophyly of the section, content but consectionality with sect. *Indicae*. Phytochemistry: similar flower flavonoid composition with *Indicae*.
Sect. *Indicae* Thory, 1820	Morphology: exserted styles, but not agglutinated.	Molecular: nrITS and cpDNA data do not show monophyly of the section, but consectionality with sect. *Synstylae*. Phytochemistry: similar flower flavonoid composition with *Synstylae*.
Sect. *Banksianae* Lindl., 1820	Morphology: see Discussion.	Molecular: nrITS and cpDNA are ambiguous.
Sect. *Laevigatae* Thory, 1820	Morphology: see Discussion.	Molecular: nrITS and cpDNA are ambiguous.
Sect. *Bracteatae* Thory, 1820	Morphology: see Discussion.	Molecular: nrITS and cpDNA are ambiguous.

amplification and thus gave no hint for non-concerted ITS evolution in these sections so far, although they include a range of ploidy levels up to the tetraploid status. Analysis of cpDNA and nrDNA-1 sequences resulted in partly incongruent topologies of the trees. Since cpDNA is maternally inherited and nuclear sequences originate by biparental inheritance, we do not expect congruent topologies. Extensive reticulation and non-concerted evolution of the ribosomal repeat restrict the assumption of congruent trees. However, complementary information with respect to hybridization events are expected and are discussed below by the description of the different sections. The lack of resolution in certain parts is in accordance with findings by other authors employing different molecular markers (see Discussion for further possible explanation of this phenomenon). Both data sets showed *Rosa* to be monophyletic with respect to the outgroup taxa. By cp-data *Rosa persica* Michx. (section *Hulthemia*) is nested within *Rosa* and not a separate genus *Hulthemia*, supporting the view of Wissemann (2003c), but nrITS sequences place *Hulthemia* in a sister relationship to the remainder of the genus. NrITS-1 data reveal *Rosa* sect. *Caninae* to be a monophyletic group by the existence of the autapomorphic C-type ITS (Wissemann, 2000, 2002, 2003b). Within the *Caninae* nrITS does not allow any conclusion for intrasectional differentiation. However, cpDNA divides the *Caninae* species into two clades, one with odorant glands (but not discriminating between the terpentine- and the wine-scented roses) and one with either eglandular or non-odorant glanded species. By nrITS sequences, the monotypic section *Rosa* with *R. gallica* appears not to be direct sister to section *Caninae. R. gallica* is characterized by its pinnate sepals, a character to be universally expressed in the *Caninae*. *R. gallica* is completely nested with its areal and its distribution amongst the *Caninae*. However, Bayesian analysis does not mirror this relationship, here section *Synstylae* is next to section *Caninae*. Chloroplast data places *R. gallica* in an uncertain position within the *Caninae*, interestingly next to *R. tomentella*, a species from subsection *Tomentellae* but also of uncertain relationship to other *Caninae* based on morphological data. Sister to the clade of *Caninae-Rosa* (*Gallicanae*) species are members of sect. *Synstylae/Indicae* according to the cpDNA data, the close relationships result in *R. arvensis* being placed within the *Caninae-Rosa* (*Gallicanae*)-clade. Morphologically the character of agglutinated styles of the *Synstylae* is not realized in the sister clade. The sister relationship to the *Caninae* is not resolved in the trees, but is focused on the two sections *Rosa* and *Synstylae*. Based on morphological data, *R. gallica*, with its distinct pinnate sepals, more resembles the *Canina*-roses. However, pinnate sepals are also known from section *Synstylae*, for example *R. longicuspis* Bertol. NrITS and *atp*B-*rbc*L IGS sequences unify the Asian sections *Indicae* and *Synstylae* into a consectional group. The positions of the monotypic sections *Laevigatae*, *Banksianae* and *Bracteatae* and the monotypic subgenus *Platyrhodon* and *Hesperhodos* are not resolved. There is clear evidence via both genetic sources that the two sections *Cinnamomeae* and *Carolinae*, are consectional. The North American *Carolinae*-roses are morphologically only distinguished from the *Cinnamomeae* by their reflexed, spreading and deciduous sepals after anthesis, whereas the *Cinnamomeae* have erect and persistent sepals, a character state used in the *Caninae* to separate next related species. The weak and complicated morphological separation of sect. *Pimpinellifoliae* from sect. *Cinnamomeae* by mostly solitary flowers without bracts, is mirrored in both data sets, which include sequences from the *Pimpinellifoliae* into the *Cinnamomeae-Carolinae*-clade. As a future-orientated proposal, the highest subgeneric rank should be the sectional status. However, more data need to be evaluated and we discuss here the pro and cons of a new taxonomy using the backbone of conventional taxonomy.

DISCUSSION

The low resolution in *Rosa* of the molecular data in this extensive study is new for these markers, but is in accordance with low resolution obtained by the use of other markers (*mat*K; *trn*L/*trn*F: Starr & Bruneau, 2002; *mat*K: Matsumoto *et al*., 1998). The reconstruction of the evolutionary history of *Rosa* is further complicated by insufficient morphological, anatomical and phytochemical data (see Table 3). This deficiency is not due to a lack of data, but rather the non-existence of informative character states between species. From the point of cultural history, *Rosa* is a typical genus being 'oversystematized', where numerous scientists have put more opinion than knowledge into monographic studies. There is no revision available for the genus, nomenclature of the thousands of names (species and cultivars) is in its infancy, and the complicated open breeding system with interfertility of many species reticulates all taxa in the genus. The fossil record of roses dates back to the Middle Oligocene of the Cenozoic (Mai & Walther, 1978, 1988). For the uniformity of characters in *Rosa* the principal scenario is likely that the explosive radiation of the Tertiary genus happened in the Holocene of the Quaternary. The still observable close relationship of all character states is the product of a lack of time since radiation and interfertility because there was no time since the spread to develop sufficient reproductive barriers. Secondary evidence for this assumption is that neither

cpDNA nor nrITS sequence positions in the trees follow a geographical distribution of the taxa.

Ecological, geographic or genetic separation triggers speciation by genetic isolation and evolution of sterility barriers, but is rarely seen in the genus since differentiation is rare, and it has not resulted in isolation within the genus. Examples are *R. persica* from the monotypic section *Hulthemia*, which is geographically separated by its distribution in the Afghanistan region and *R. palustris* from sect. *Carolinae*, which is ecologically niched into the swampy regions in North America. Recently Rieseberg *et al*. (2003) demonstrated, that ecological transitions, and thus subsequent speciation by genetic isolation, are facilitated by hybridization. This also happened in dog roses: genetically separated (but not isolated) is the sect. *Caninae*, which is of allopolyploid origin (Wissemann, 2000, 2002) and characterized by the unique heterogamous reproduction via *Caninae*-meiosis (Täckholm, 1920; 1922) and the existence of an autapomorphic nrITS type (Ritz & Wissemann, 2003a).

Evolution of and within the subgeneric taxonomical units (nomenclature according to Wissemann, 2003a)

Subgenus *Hulthemia* (Dumort.) Focke (1888)

Our nuclear molecular data clearly demonstrate that *Hulthemia* is a member of the genus *Rosa* and is not a separate genus. This monotypic subgenus has been disputed since its description by Dumortier (1824). The outstanding character of the central Asian and east Asian (Siberian) *Rosa persica* Michx. ex Juss., 1789 is the reduction of the leaf to a single leaflet without stipules. The position of *R. persica* within the genus *Rosa* in our phylogenies (Figs 1, 2) does not indicate this simplicity to be either an archetypal or an advanced character. Within the genus *Rosa*, variability in the number of leaflets is high from 11 to 9–7 leaflets in sect. *Pimpinellifoliae*, 9–7 in *Bracteatae*, 7 (–5) in most sections, e.g. *Caninae*, 5 in *Indicae* (5)–3 in *Laevigatae* and subgenus *Hesperhodos*, and 1 in *Hulthemia*. In agreement with Parmentier (1897) we interpret the reduction to be a result of ecological adaptation to the hot summer season in the areas where they are found in central Asia (Afghanistan, Usbekistan, Iran). Two morphotypes are reported for *R. persica*, a more southern distributed form with hairy branches and leaves (Boissier, 1872; Meikle, 1966; Zielinski, 1982) and a second glabrous taxon (used in this study here) in the northern range of distribution (Bean, 1980), but the taxonomic relevance of this character state is not clear. Regel (1877) combined both types into one single species (*R. berberifolia* Pall.). Our chloroplast data of *atp*B-*rbc*L-IGS do not support the finding of Starr & Bruneau (2002) that based on chloroplast *trn*L/*trn*L-F, *Hulthemia* is nested within the subgenus *Rosa*. In our cpDNA tree *R. persica* is sister to the remainder of the genus, however, in contrast to Wu *et al*. (2001) whose nrITS data place the species with *R. stellata* (subgenus *Hesperhodos*) in a sister relationship to some species of the *Pimpinellifoliae* and *Laevigatae*. Interestingly, Ueda & Tomita (1989) claimed close phylogenetic relationships between *Pimpinellifoliae* and *Hulthemia* based on pollen exine patterns. With its chestnut-like hips and basal insertion of the seeds, the fruit characters resemble subgenus *Hesperhodos* (*R. stellata* Wooton, 1898) and the subgenus *Platyrhodon* (*R. roxburghii* Tratt., 1823). *R. persica* is able to hybridize with other species from the genus *Rosa*. This has been reported for naturally occurring plants by de la Roche (1978) and Bean (1980), as well as for ornamental breeding (Harkness, 2003). Based on similar insertion patterns of the seeds in the hip, Parmentier (1897) assumed *Hulthemia*, *Hesperhodos* and *Platyrhodon* to be closely related. Unfortunately he interpreted the insertion as a basiparietal insertion and correlated it to the similar situation in *Cinnamomeae*, by which *Cinnamomeae* became an archetype of roses in the genus. In fact, seed insertion of *Hulthemia*, *Hesperhodos* and *Platyrhodon* is only basal, thus supporting the relationship between the three subgenera. Seeds do not grow on the side walls of the hip and thus seed insertion in these three subgenera is fundamentally different from the situation in *Cinnamomeae* (see Crépin, 1898). Molecular, anatomical, as well as reproductive, data (natural hybridization with *Rosa*-species) support the view of *R. persica* being a member of the genus *Rosa*, thus *Hulthemia* is best treated as a section, but not as a separate genus.

Subgenus *Hesperhodos* Cockerell, 1913

Again the molecular data do not support the treatment of *Hesperhodos* at generic rank. At first glance the North American subgenus *Hesperhodos* seems to be most closely related to the central Asian subgenus *Hulthemia*. Both subgenera have prickly, chestnut-like hips and basal insertion of seeds. *R. stellata* has few leaflets (3–5), but which again are interpreted as the result of ecological adaptation to its dry habitat. However, our nrITS data also indicate a close relationship of the two subgenera. Morphologically, the two subgenera are separated by the existence of adnate stipules with divergent, rounded or broadened auricles in *Hesperhodos*. The most prominent morphological character of subgenus *Hesperhodos* is the complete absence of the disc. Since in both trees *R. stellata* is nested within species from subgenus *Rosa*, the elevation of *Hesperhodos* to generic rank

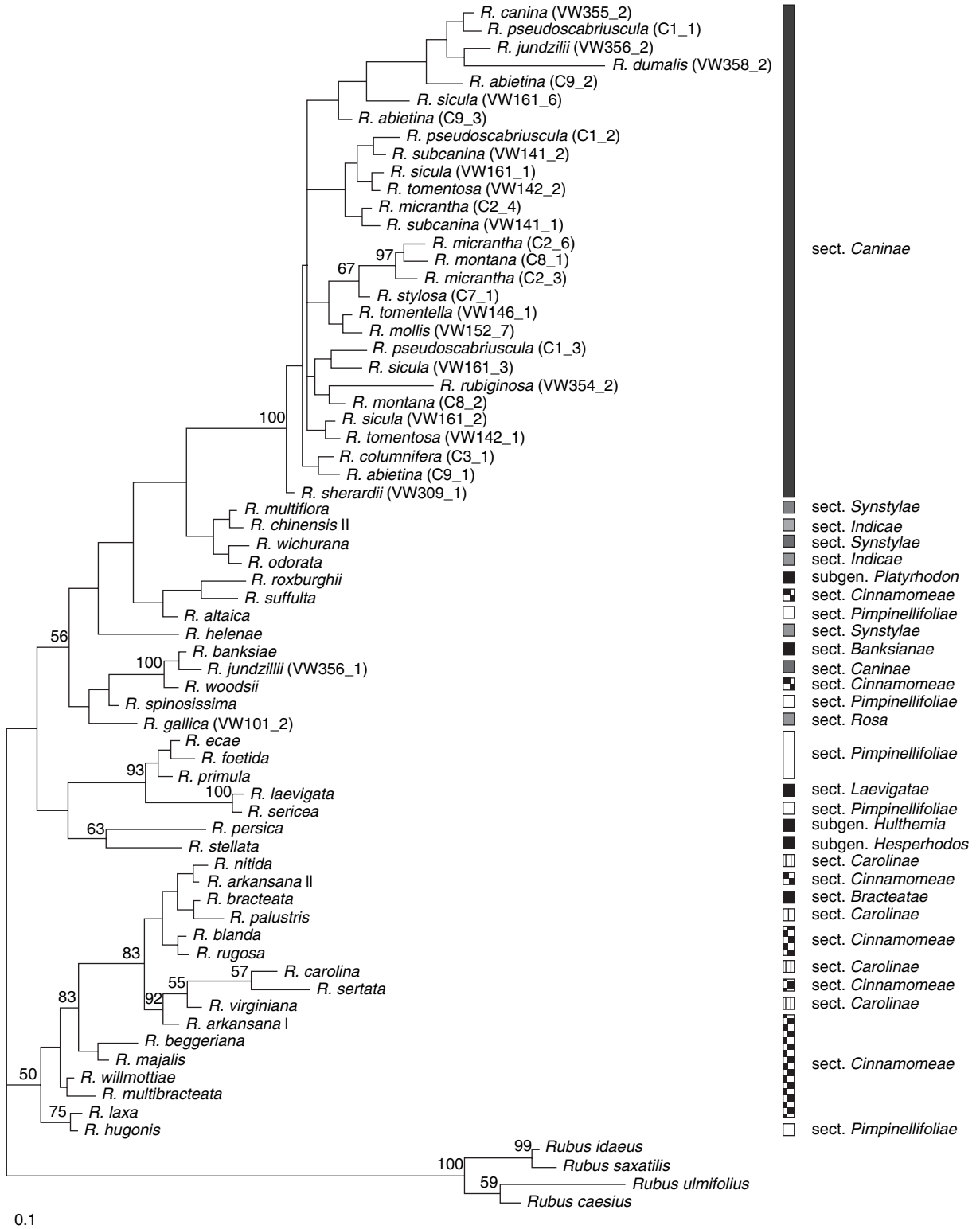

Figure 1. Bayesian inference of phylogenetic relationships of representatives of the genus *Rosa*: Monte Carlo Markov chain analysis based on nrITS-1 sequence data (using the general time reversible model of DNA substitution with gamma distributed substitution rates, 2 000 000 generations). The 50%-majority rule consensus tree was computed from 19 001 trees that were sampled after the process had reached stationarity. The topology was rooted with *Rubus idaeus*, *R. saxatilis*, *R. ulmifolius* and *R. caesius*. Numbers on branches are estimates for a posteriori probabilities.

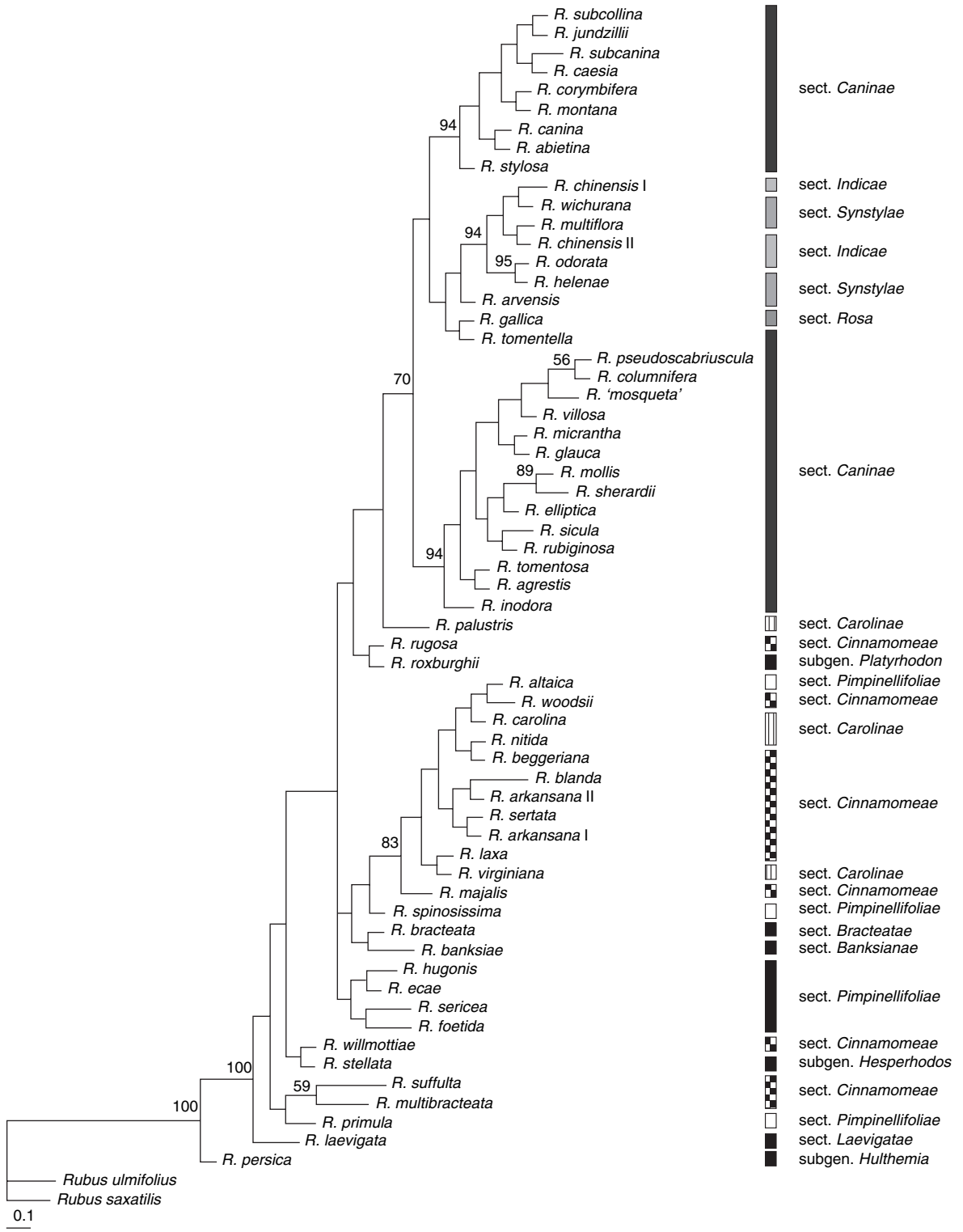

Figure 2. Bayesian inference of phylogenetic relationships of representatives of the genus *Rosa*: Monte Carlo Markov chain analysis based on sequence data of the 5′-region of the cpDNA marker 'atpB-rbcL IGS' (using the general time reversible model of DNA substitution with gamma distributed substitution rates, 2 000 000 generations). The 50%-majority rule consensus tree was computed from 19 001 trees that were sampled after the process had reached stationarity. The topology was rooted with *Rubus saxatilis* and *R. ulmifolius*. Numbers on branches are estimates for a posteriori probabilities.

(e.g. by Boulenger or Hurst, see Bean, 1980: 121) is not justifiable. The morphological examinations by Lewis (1965) indicated that *Hesperhodos* is the only group with finely reticulate pollen surface, whereas the pollen sculpturing of all other species of *Rosa* is striate. Parmentier (1897: 110) analysed the pericycle of *R. minutifolia* Engelmann. He found shorter and oval cells differing from those in all other members of *Rosa* where the pericycle consists of elongate and fusiform cells. Ma *et al*. (1997) detected significantly fewer metacentric chromosomes in *R. minutifolia* than in the other sections. Taken together the morphological differences elucidated by Parmentier (1897) and Lewis (1965) and the genetic separation detected in this study, *Hesperhodos* is best treated at sectional level and not at subgeneric rank.

Subgenus *Platyrhodon* (Hurst) Rehder, 1940

The third subgenus with prickly, chestnut-like hips with basal insertion of seeds is the monotypic subgenus *Platyrhodon* (*R. roxburghii* Tratt., 1823) but differs in a number of autapomorphic character states, which do not allow a satisfying placement based on morphology. Zielinski (1985) interprets the restricted distribution, the non-juicyness of the fruit and the disc structure as the most primitive characters in the whole genus. The number of leaflets per leaf is high (> 7–15) and it is the only species in the genus with peeling bark. Subulate auricles at the tip of the adnate stipules is a further character state which discriminates *Platyrhodon* from *Hesperhodos*. The molecular data are ambiguous. NrITS indicates a close relationship to *R. gallica*, which is unsupported by morphology. CpDNA places the species next to *R. rugosa* from the *Cinnamomeae*, again not supported by morphology, but supporting the view held by Ma, Crane & Byrne (1996, 1997) based on karyotypic relationships, Lewis & Basye (1961) based on cross compatibility and Kim (1994) from isoenzyme analysis (the latter two references are cited in Ma *et al*., 1997). The phylogenetic relationships of *R. roxburghii* remain uncertain. Hip morphology indicates the affinity to *Hesperhodos* and *Hulthemia*, although the basal insertion of the seeds is not as flat as in the other subgenera, but on a placenta-like structure, already mentioned by Crépin (1891). The functional most prominent character of the peeling bark, not known in any other species of the genus, has not been discussed in the literature. By cpRFLP, Takeuchi *et al*. (2000) proposed a separate placement of *Platyrhodon* in *Rosa*. Furthermore, crossability of *R. roxburghii* is difficult, again pointing towards the isolated position of *R. roxburghii* in the genus. Naturally, only *R.* × *micrugosa* originated by hybridization between *R. rugosa* × *R. roxburghii*, but pollen-, seed-parent direction is unknown. Wulff (1954) and Bean (1980) report rare cases from the ornamental breeding in which *R. roxburghii* served as pollen donor. Schum, Hofmann & Felten (2002) established somatic hybrids with *R. roxburghii*. From the overall view of the data, *Platyrhodon* seems to be a morphologically isolated member of the subgenus *Rosa*, thus not deserving subgeneric rank, but should presumably be treated in a monotypic sectional status within subgenus *Rosa*. The data available at present contradict the view of Zielinski (1985) that *R. roxburghii* is the most basal, ancient rose species.

Subgenus *Rosa*

Section *Pimpinellifoliae* (DC.) Ser. 1825

Based on morphology, *Pimpinellifoliae* seems to be a rather loosely-defined group by their mostly single flowers without bracts, a high number of small, round leaflets per leaf, and intensive coloured, often black, hips (although *R. gymnocarpa* Nutt. from *Cinnamomeae* also has black hips). Currently the group of *Pimpinellifoliae* is still widely accepted in practice and literature. Mikanagi *et al*. (1995) recognized the occurrence of unique kaempferol and quercetin 4′-glucosides in *Pimpinellifoliae*. However, recently more data have emerged, which raise doubts about the monophyletic status of the section *Pimpinellifoliae* (Matsumoto *et al*., 2000, 2001). *R. sericea* is morphologically distinct by flowers with mainly four petals and wedge-shaped prickles and extremely high numbers of leaflets (–17), but also has individuals with five petals. Its position is uncertain since both markers, cp- and nrDNA, place *R. sericea* in different positions. Whereas ITS combines it with *R. laevigata* (sect. *Laevigatae*) in a sister group relationship to members of the yellow-flowering Lutea-group of *Pimpinellifoliae*, cpDNA indicates a position of *R. sericea* within these Lutea-species. The Lutea group itself with *R. ecae*, *foetida*, *hugonis*, *primula* is clearly paraphyletic, but relationships are not resolved, although we can reject the view of Rowley (1961), that the bright yellow Austrian briar, *Rosa foetida*, is the nearest ally of *R. spinosissima* as was also shown by the extensive morphological study on *Pimpinellifoliae* by Roberts (1977). It is noteworthy that the proposal of Roberts (1977), based on morphology to transfer *R. farreri* and *R. forrestiana* from *Pimpinellifoliae* into *Cinnamomeae*, is supported by *mat*K-analysis (Matsumoto *et al*., 2001). The most divergent group within the *Pimpinellifoliae* are the Scots roses themselves. *R. spinosissima* L. and its morphological twin *R. altaica* Willd., which can currently be separated morphologically only by size, are genetically completely distinct (both species are tetraploid, at least *R. spinosissima* seems to be allotetraploid: V. Wissemann, isozyme-analysis, unpubl. data). The unresolved position of *R. altaica* in the nrITS tree next to

R. suffulta as well as the placement of *R. altaica* in the *atp*B-*rbc*L IGS tree within the *Carolinae–Cinnamomeae* clade might point to a cryptic hybridization event between a *R. spinosissima*-derivate and a member of *Cinnamomeae*. On this assumption the occurrence of black hips on some morphotypes of *R. altaica* completely resembling the receptacles from *R. rugosa* Thunb. (*Cinnamomeae*) can be explained, as well as the biochemical relationships detected by Grossi *et al*. (1998). Interestingly, RFLP studies by Matsumoto *et al*. (1997) also combined *R. rugosa* (*Cinnamomeae*) with *R. spinosissima* (*Pimpinellifoliae*). De la Roche (1978) mentioned that all natural hybrids known so far from *Pimpinellifoliae* are by *R. spinosissima*, and always with *R. spinosissima* as the pollen parent. However, molecular evidence for this assumption is currently lacking.

Section *Caninae* (DC.) Ser. 1825

In Europe, after the retreat of the last glaciation, dog roses (sect. *Caninae*) spread over the landscape. Because of their vigorous allopolyploid constitution (Wissemann, 2002) they were able to take the area by force and started to establish ecological types in different niches. This ecological differentiation within *Caninae* can be seen for example in the differentiation of the ecological 'L-' and 'D-type' roses (Christ, 1873, 1884; Reichert, 1998; Wissemann, 2000). Dog roses have been identified as allopolyploids (Wissemann, 2000, 2002) but are characterized as a natural evolutionary unit by the autapomorphic characters of a specific nrITS-type and the heterogamous mode of reproduction via Canina-meiosis (Täckholm, 1920, 1922; Blackburn & Harrison, 1921; Lim *et al*., 2000). Given the specific nrITS-type, this type is distinct, but closely related to sequences from members of the *Synstylae* and *Indicae*. Currently we cannot draw the conclusion that this is a hint for the geographical or seed paternal origin of the *Caninae*, since characters of these two sections, e.g. agglutinated styles and entire sepals, are not represented in the dog roses. Further insight from biochemical and molecular data is needed here. However, Zielinski (1985) claimed that members of *Indicae* and *Synstylae* are the closest relatives to *R. canina* from sect. *Caninae* based on morphology. From the cpDNA data, *Caninae* is split into two major clades, one with eglandular or with non-odorant glands and one with odorant (wine and terpentine-scented) glands. Interestingly, these clades are split by a clade including *R. gallica* from sect. *Rosa* and members of the *Synstylae-Indicae*-group, which again supports a close relationship of *Caninae* with *Synstylae*. The unique heterogamous meiosis has led to numerous opinions about the mode of reproduction in this section including apomixis. However, Wissemann & Hellwig (1997) were able to show, that sexual reproduction via heterogamy is the predominant way of reproduction in this section, although apogamy cannot be excluded in certain cases (e.g. Fagerlind, 1940; Flory, 1950; Wissemann & Hellwig, 1997; Werlemark *et al*., 2000).

Section *Rosa* (= sect. *Gallicanae* (DC.)) Ser. 1825

Section *Rosa* is a monotypic section with the European and west Asian species *R. gallica* L., 1759 (Wissemann, 2003a). All other OTUs given species rank from this section are long cultivated hybridogenic, synanthropic species of which natural populations are not known (de la Roche, 1978). From molecular data the position of *R. gallica* is uncertain. The close relationship of *R. gallica* to the upper *Caninae*-clade in the cpDNA-dataset is morphologically supported by lobed or pinnate sepals and the occurrence of non-odorant glands. However, *R. gallica* is a homogamous species with regular meiosis and does not harbour the specific *Caninae*-nrITS (Wissemann, 1999). We still believe this species, or an unknown and extinct close relative, to be one partner during the process of allopolyploidization of the *Caninae*, which has introduced the morphological character of pinnate sepals. The possibility of this has been shown for the origin of *R. jundzillii* (Wissemann, 1999).

Section *Carolinae* Crép. 1891

Section *Carolinae* is completely dispersed within the clade of roses from sect. *Cinnamomeae*, supporting the view of Wylie (1954) who treated both groups as consectional. Morphologically only the non-persistence of sepals separates *Carolinae* from the *Cinnamomeae*, a character used in the *Caninae* to separate closely related species and subject to dominant inheritance (Ritz & Wissemann, 2003b). Based on anatomical data, Parmentier (1897) had already claimed consectional status for *Carolinae* and *Cinnamomeae*. Grossi *et al.* (1998) analysed flavonoid and enzyme polymorphisms of *Carolinae* and *Cinnamomeae* and were able to show that again *Carolinae* grouped with the *Cinnamomeae*. However, Grossi *et al*. (1999) detected a specific anthocyanin (pelargonidin-substituted) present in *Carolinae* but not in *Cinnamomeae*. *Mat*K-analysis by Matsumoto *et al*. (1998) also combined the two sections. From the knowledge of character inheritance in *Caninae* (Wissemann, 2000; Ritz & Wissemann, 2003b), we do not expect that the species in *Carolinae,* if included into the *Cinnamomeae*, represent one clade of closely related species based on presence of deciduous sepals. Species with deciduous sepals in the *Caninae* are completely mixed in the section. Our analysis of the cpDNA (as well as nrITS sequences) support this view; species with deciduous sepals do not form a monophyletic group in the trees (Figs 1, 2). Section *Carolinae* is here included in section *Cinnamomeae*.

Section *Cinnamomeae* (DC.) Ser. 1825 (incl. section *Carolinae* Crép., 1891)

Section *Cinnamomeae* is by far the largest section within the genus with *c*. 80 species. After inclusion of the *Carolinae* it harbours about 50% of all species in the genus. Differentiation within this section is high and there is much variability between the described species. Ecological and subsequent genetic differentiation occurred within *R. palustris* Marsh., 1785, that is clearly separated by the cp-sequence but nested within *Cinnamomeae* by nrITS sequences. Our data suggest from the nrITS sequence a closer relationship of *Cinnamomeae-Carolinae* to the subgenera *Hulthemia*, *Platyrhodon* and *Hesperhodos*, which Parmentier (1897) already had assumed by the 'basi-parietal insertion of seeds'. However, the interpretation of Parmentier (1897), that these three subgenera have basiparietal insertion of seeds is wrong, as already pointed out by Crépin (1898). *Hulthemia*, *Platyrhodon* and *Hesperhodos* have a pure basal insertion of the seeds at the bottom of the hip, not on the walls (Herring, 1925). Affinity of parts of *Pimpinellifoliae* to the *Cinnamomeae* was shown by Grossi *et al*. (1998) from the chemical and biochemical pattern that can be observed in the nrITS analysis and especially the cpDNA analysis. It is noteworthy that Grossi *et al*. (1998) found a close relationship between the *Pimpinellifoliae*-species, *R. altaica* Willd., and the *Cinnamomeae-Carolinae*-clade. We found the same connection in the *atp*B-*rbc*L IGS-tree, which indicates that conspecificity of *R. spinosissima* L. and *R. altaica* is doubtful (see further remarks under sect. *Pimpinellifoliae*).

Section *Synstylae* DC., 1813

Grossi *et al*. (1998) found the *Synstylae* to be one of the best circumscribed groups with respect to biochemical data. Unfortunately, they did not integrate members of the *Indicae* into their study. Our data, both cpDNA and nuclear DNA, show consectionality of *Synstylae* and *Indicae*. In the analysis of Japanese wild roses by Wu *et al*. (2000) *mat*K-DNA again merged both sections together. Mikanagi *et al*. (1995) showed similar flower flavonoid composition for *Synstylae* and *Indicae*, but Cao, He & Li (1996) pointed to the extreme differences in carotene content between the two sections (*Synstylae* on average > 6 mg/100 g; *Indicae* on average < 0.4 mg/100 g). Based on morphology the only taxonomically useful difference is the agglutinated style of the *Synstylae* but with respect to all other characters, the morphological character of columnated styles becomes doubtful as an autapomorphic character state. From the point of history of science it is noteworthy, that this character was the first and oldest morphological character proposed in the classification of the whole genus (Seringe, 1818). The only European species of *Synstylae*, *R. arvensis* Huds. appears to be sister to the *Synstylae-Indicae*-clade in the cp-tree.

Section *Indicae* Thory, 1820

The Chinese section *Indicae*, with only three species (*R. odorata* (Andrews) Sweet, 1818, *R. gigantea* Collet ex Crép. 1888 and *R. chinensis* Jacq. 1768), is clustered with *Synstylae* roses in both data sets. Shishkin & Yuzepchuk (1971: 331) already pointed to the close relationship of *Indicae* to *Synstylae*, which both have exserted styles, the first group only lacking the connection of the styles. For further remarks, see above (*Synstylae*).

Section *Banksianae* Lindl. 1820

This section harbours two species (*R. banksiae* Ait., 1811, *R. cymosa* Tratt., 1823), but the taxonomic status of the latter is disputed. By RAPD-analysis Millan *et al*. (1996) assigned *R. banksiae* as a member of subgenus *Rosa*. Morphologically the section is characterized by free and deciduous stipules, nonpubescent receptacles and branchlets (difference to sect. *Bracteatae*) and reflexed and deciduous sepals (difference to sect. *Laevigatae*). The receptacle is smooth and not bristly as in sect. *Laevigatae*. The taxonomic position is completely unresolved. In all phylogenetic trees, *R. banksiae* is placed within or next to the *Cinnamomeae-Carolinae*-clade, in the *atp*B-*rbc*L Bayesian tree next to *Bracteatae* (also by Wu *et al*., 2000, 2001 using *mat*K sequences and nrITS, respectively), but not with *Laevigatae*. Thus the morphological characterization by deciduous stipules indicates not synapomorphy but convergence or plesiomorphy. As in subgenus *Hesperhodos*, Ma *et al*. (1997) found fewer metacentric chromosomes than in other sections.

Section *Laevigatae* Thory, 1820

There is only one species in this monotypic Chinese section, *R. laevigata* Michx., 1803. The bristly hip discriminates the species from sect. *Banksianae*, the difference from *Bracteatae* is by non-pubescent branchlets. Morphologically this section is united in a larger group with *Banksianae* (e.g. already included into this section by Déséglise, 1877: 65 '*R. sinica* Murray') and *Bracteatae* by the free and deciduous stipules. However, as in *Banksianae*, molecular classification does not support a coherence based on this morphological character. In the nrITS tree, *R. laevigata* is nested within members of section *Pimpinellifoliae*, contradicting the view of de la Roche (1978) of *Laevigatae* being the closest relative to *Banksianae*.

Section *Bracteatae* Thory, 1820

Again this presumable monotypic south-east Asian section (*R. bracteata* Wendl., 1798, uncertain taxonomic status of *R. clinophylla* Thory) is morphologi-

cally characterized by free and deciduous stipules (as *Laevigatae* and *Banksianae*), but differs in pubescent or tomentose young branchlets and receptacles from these two sections. The phylogenetic position is completely uncertain based on cpDNA and nrDNA data. Whereas the ITS data indicate a position within the *Cinnamomeae-Carolinae* clade, cpDNA places it unsupported as sister to *R. banksiae* (also by Wu *et al*., 2000), nested within the *Pimpinellifoliae* clade.

PERSPECTIVES

As presented above, our understanding of evolution and phylogenetic relationships within the genus *Rosa* is on the one hand contradictory, and on the other in its infancy. The enormous phenotypic, genotypic and ecological variability and plasticity, influenced by evolutionary processes such as hybridization, currently restrict a taxonomic revision of the genus. From the classical taxonomic view, knowledge of the genus *Rosa* suffers from two problems. First, the European section *Caninae* is such a problematic taxonomic and evolutionary group, that rhodology is a eurocentric field of science. The intensive work on dogrose species since Linnaean times made *Rosa* into a genus completely 'oversystematized' for European species, but neglecting the bewildering diversity outside Europe. Second, we lack extensive knowledge of rose taxa from the centres of diversity in central Asia, necessary to understand the intrageneric relationships. Additionally, we do not only need more data, but a deeper understanding of the processes underlying the evolutionary history of the markers used for classification. This must include more detailed studies on the specific marker systems (e.g. Álvarez & Wendel, 2003; Wissemann, 2003b) and their performance under selective forces, as well as breeding experiments (e.g. Ritz & Wissemann, 2003b) to understand character inheritance and impact of ecological factors on character expression. For the reconstruction of phylogenetic relationships at species level we need a better resolving molecular marker. Nr-ITS as well as the cp-DNA currently used in *Rosa*-studies give insight into questions of consectionality of sections, but do not resolve deep species relationships.

ACKNOWLEDGEMENTS

The work was funded by a grant from the Deutsche Forschungsgemeinschaft (DFG) to V.W. (Wi 2028-1). We thank the Europarosarium Sangerhausen and the Rosensammlung Kassel-Wilhelmshöhe for providing plant material. Support by the VDR Stiftung Europa Rosarium Sangerhausen is gratefully acknowledged. We thank F. H. Hellwig (Jena) for allowing work in a molecular laboratory. We like to thank the two anonymous referees which helped to improve significantly the quality of the paper by raising questions which are lost if one is too much in the subject.

REFERENCES

Álvarez I, Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29:** 730–743.

Bean WJ. 1980. *Trees and shrubs hardy in the British Isles.* London: Murray, Vol. 4, 1–73.

Blackburn KB, Harrison JWH. 1921. The status of the British rose forms as determined by their cytological behaviour. *Annals of Botany* **35:** 159–188.

Boissier E. 1872. *Rosa*. In: *Flora Orientalis Sive Enumeratio Plantarum in Oriente a Graecia et Aegypto ad Indiae Finis Hucusque Observatarum*, Vol. 2: *Calyciflorae, Polypetalae.* Genf, Basel: H. Georg, 669–689.

Britton NL, Brown A. 1913. *An illustrated flora of the northern United States* 2nd edn. New York: Scribner, 1–3.

Cao YL, He YH, Li CL. 1996. Vitamin contents in the hips of 38 species of *Rosa* and their relation to division of sections. *Acta Botanica Sinica* **38:** 822–827.

Christ H. 1873. *Die Rosen der Schweiz mit Berücksichtigung der unliegenden Gebiete Mittel- und Südeuropas*. Basel, Genf, Lyon: H. Georg.

Christ H. 1884. Allgemeine Ergebnisse aus der systematischen Arbeit am Genus *Rosa*. V. Locale Scheidung der Formen. *Botanisches Centralblatt* **18.11:** 343–350.

Crépin F. 1891. Nouvelle classification des roses. Extrait du. *Journal des Roses* 3–5. Drosne: Melun, E., 3–30.

Crépin F. 1898. Les idées d'un anatomiste sur les espèces du genre *Rosa* et sur leur classification. *Bulletin Société Royal Botanique de Belgique* **37:** 151–201.

Debener Th, Bartels Ch, Mattiesch L. 1996. RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. *Molecular Breeding* **2:** 321–327.

Déséglise A. 1877. *Catalogue raisonné ou énumeration méthodique des espèces du genre rosier pour l'Europe, l'Asie et l'Afrique spécialement les rosiers de la France et de l'Angleterre*. Genéve: Mentz, 1–348.

Dumortier BC. 1824. *Notice sur un nouveau genre de plantes: Hulthemia; précédée d'un aperçu sur la classification des roses.* Ainé: Tournay: Impr. de J. Casterman, 13–14.

Eriksson T, Donoghue MJ, Hibbs MS. 1998. Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal internal transcribed spacers (IST), and implications for the classification of *Rosoideae* (Rosaceae). *Plant Systematics and Evolution* **211:** 155–179.

Eriksson T, Hibbs MS, Yoder AD, Delwiche CF, Donoghue MJ. 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the TRNL/F region of chloroplast DNA. *International Journal of Plant Science* **164:** 197–211.

Fagerlind F. 1940. Sind die Canina-Rosen agamospermische Bastarde? *Svensk Botanisk Tidskrift* **34:** 334–354.

Flory WS. 1950. Pollen condition in some species and hybrids of Rosa with a consideration of assosiated phylogenetic factors. *The Virginia Journal of Science New Series* **1:** 11–59.

Gandoger M. 1892. *Monographia Rosarum Europae et Orientis terrarumque adjacentium.* Tom. 2, Paris: Baillière et fil. Bibliopolae.

Grossi C, Raymond O, Jay M. 1998. Flavonoid and enzyme polymorphisms and taxonomic organisation of *Rosa* sections: Carolinae, Cinnamomeae, Pimpinellifoliae and Synstylae. *Biochemical Systematics and Ecology* **26:** 857–871.

Grossi C, Raymond O, Sanlaville-Boisson C, Jay M. 1999. *Rosa* taxonomy and hierarchy of markers defined by ACT STATIS. *Zeitschrift Fuer Naturforschung* **54c:** 25–34.

Harkness P. 2003. 16. Kasseler Rundgespräch in Sangerhausen: Unsere besten Rosen des 20. Jahrhunderts. *VDR Rosenjahrbuch*. Verein Deutscher Rosenfreunde Baden-Baden, 60–70.

Herring P. 1925. Classifications of *Rosa*. *Dansk Botanisk Arkiv* **4:** 1–24.

Herrmann J. 1762. *Dissertatio Inauguralis Botanico-Medica de Rosa*. Straßburg: Officina J.H. Heitz.

Huelsenbeck JP, Ronquist F. 2001. MrBayes – Bayesian inference of phylogeny, Version 3.0b4. *Bioinformatics* **17:** 754–755.

Kim Y-J. 1994. A study of selected species of *Rosa* using isozyme polymorphisms. MSc Thesis, College Station, Texas A & M University.

Lewis WH. 1965. Monograph of *Rosa*. North America. V. Subgenus *Hesperhodos*. *Annals of Missouri Botanical Garden* **52. 2:** 99–113.

Lewis WH, Basye RE. 1961. Analysis of nine crosses between diploid *Rosa* species. *Proceedings of the American Society of Horticultural Sciences* **78:** 572–579.

Lim KY, Bringloe JB, Werlemark G, Meynet J, Roberts AV, Leitch AR. 2000. Molecular cytogenetic investigation in *Rosa* section *Caninae*. In: Werlemark G, ed. Genetic variability and reproductive strategies in Nordic dogroses, *Rosa* section *Caninae*. *Acta Universitatis Agriculturae Sueciae, Agraria 257. Paper* **VIII:** 1–15.

Linnaeus C. 1753. *Species plantarum.* Holmiae, Tomus **1:** 491–492.

Linnaeus C. 1772. *Systema naturae ex editione duodecima in epitomen redactum et praelectionibus academicis accomodatum at Iohanne Beckmanno.* Tom 2: Regnum Vegetabile. Göttingen: Vandenhoeck.

Ma Y, Crane CF, Byrne DH. 1996. Meiotic behavior of hybrids involving *Rosa laevigata*. *Cytologia* **61:** 457–463.

Ma Y, Crane CF, Byrne DH. 1997. Karyotypic relationships among some *Rosa* species. *Caryologia* **50. 3–4:** 317–326.

Mai DH, Walther H. 1978. Die Floren der Haselbacher Serie im Weisselster-Becken. *Abhandlungen des Staatlichen Museums für Mineralogie und Geologie* **28:** 101–102.

Mai DH, Walther H. 1988. Die pliozänen Floren von Thüringen, Deutsche Demokratische Republik. *Quatärpaläontologie* **7:** 5–297.

Matsumoto S, Fukui H. 1996. Identification of rose cultivars and clonal plants by random amplified polymorphic DNA. *Scientia Horticulturae* **67:** 49–54.

Matsumoto S, Kouchi M, Fukui H, Ueda Y. 2000. Phylogenetic analyses of the subgenus *Eurosa* using the ITS nrDNA sequence. *Acta Horticulturae (ISHS)* **521:** 193–202.

Matsumoto S, Kouchi M, Yabuki J, Kusunoki M, Ueda Y, Fukui H. 1998. Phylogenetic analyses of the genus *Rosa* using the *mat*K sequence: molecular evidence for the narrow genetic background of modern roses. *Scientia Horticulturae* **77:** 73–82.

Matsumoto S, Nishio H, Ueda Y, Fukui H. 2001. Phylogenetic analyses of genus *Rosa*: polyphyly of section *Pimpinellifoliae* and origin of *Rosa* × *fortuniana* Lindl. *Acta Horticulturae (ISHS)* **547:** 357–363.

Matsumoto S, Wakita H, Fukui H. 1997. Molecular classification of wild roses using organelle DNA probes. *Scientia Horticulturae* **68:** 191–196.

Meikle RD. 1966. *Rosa*. In: Townsend CC, Guest E, Al-Rawi A, eds. *Flora of Iraq*. Glasgow: University Press, 141–151.

Mikanagi Y, Yokoi M, Ueda Y, Saito N. 1995. Flower flavonol and anthocyanin distribution in subgenus *Rosa*. *Biochemical Systematics and Ecology* **23:** 183–200.

Millan T, Osuna F, Cobos S, Torres AM, Cubero JI. 1996. Using RAPDs to study phylogenetic relationships in *Rosa*. *Theoretical and Applied Genetics* **92:** 273–277.

Ochsmann J. 2000. Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-*Cardueae*) in Europa. *Dissertationes Botanicae* 324. Berlin, Stuttgart: J. Cramer.

Parmentier P. 1897. Recherches anatomiques et taxinomiques sur les rosiers. *Annales de Sciences Naturelles, Series VIII (Botanique)*, **6.1–3:** 1–175 + 8 pl. (published 1898).

Regel E. 1877. *Tentamen Rosarum Monographiae.* St. Petersburg, 1–114.

Rehder A. 1949. *Bibliography of cultivated trees and shrubs hardy in the cooler temperate regions of the northern hemisphere*. Jamaica Plain Massachusetts: Arnold Arboretum. Reprint 1978 by O. Koeltz Koenigstein. *Collectanea Bibliographica*. Koeltz S, ed. Vol. 10, 296–317.

Reichert H. 1998. Die zwei Wuchstypen bei Rosen der Sektion *Caninae* und ein Vorschlag für eine Kurzbezeichnung derselben. *Acta Rhodologica* **1:** 29–35.

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301:** 1211–1216.

Ritz CM, Wissemann V. 2003a. The evolution of diversity in *Rosa* L., section *Caninae* (DC) Ser., Rosaceae. *16th Symposium Biodiversität und Evolutionsbiologie, 17th Senckenbergkolloquium, Staadt Frankfurt/Main*, 89.

Ritz CM, Wissemann V. 2003b. Male correlated non-matroclinal character inheritance in reciprocal hybrids of *Rosa*, section *Caninae* (DC) Ser., Rosaceae. *Plant Systematics and Evolution* **241:** 213–221.

Roberts AV. 1977. Relationship between species in the genus *Rosa*, section *Pimpinellifoliae*. *Botanical Journal of the Linnean Society* **74:** 309–328.

de la Roche G. 1978. Commentary on 'Les Roses'. In: Roche G De la, Rowley GD, Lawalrée A, Stearn WT, eds. *Commentar-*

ies to Les Roses by P.J. Redouté. A contribution to the history of the genus Rosa. Antwerpen: De Schutter, 148–341.

Rowley G. 1961. The scotch rose and its garden descendants. *Journal of the Royal Horticultural Society of London* **86:** 433–437 + 5 plates.

Rowley GD. 1992. Typ. Cons. Prop: *R. cinnamomeae* L. *Taxon* **41:** 568–569.

Savolainen V, Manen JF, Douzery E, Spichiger R. 1994. Molecular phylogeny of families related to Celastrales based on rbcL 5′-flanking sequences. *Molecular Phylogenetics and Evolution* **3:** 27–37.

Schum A, Hofmann K, Felten R. 2002. Fundamentals for integration of somatic hybridization in rose breeding. *Acta Horticulturae (ISHS)* **572:** 29–36.

Seringe NC. 1818. Allgemeine Betrachtungen über die Rosen. *Museum der Naturgeschichte Helvetiens, Botanische Abtheilung* **I:** 1–8.

Shishkin BK, Yuzepchuk SV. 1971. *Flora of the USSR: Rosaceae-Rosoideae, Prunoideae*. Reprint: Koenigstein: Koeltz Scientific Books, Vol. 10, 322–377.

Starr J, Bruneau A. 2002. Phylogeny of *Rosa* L. (Rosaceae) based on *trn*L-F intron and spacer sequences. Abstract Botany 2002, 1.ASPT Colloquium: *Rosaceae phylogeny: current knowledge, problems, and prospects*. [http://www.2002.botanyconference.org/cgi–bin/new–view02.pl].

Täckholm G. 1920. On the cytology of the genus *Rosa*. A preliminary note. *Svensk Botanisk Tidskrift 14* **2–3:** 300–311.

Täckholm G. 1922. Zytologische Studien über die Gattung *Rosa*. *Acta Horti Bergiani* **7:** 97–381.

Takeuchi S, Nomura K, Uchiyama H, Yoneda K. 2000. Phylogenetic relationship in the genus *Rosa* based on the restriction enzyme analysis of the chloroplast DNA. *Journal of the Japanese Society for Horticultural Science* **69:** 598–604.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876–4882.

Ueda Y, Tomita H. 1989. Morphometric analysis of pollen exine patterns in roses. *Journal of the Japanese Society for Horticultural Science* **58:** 211–220.

Werlemark G, Nybom H, Olsson A, Uggla M. 2000. Variation and inheritance in hemisexual dogroses, *Rosa* section *Caninae*. *Biotechnology & Biotechnological Equipment* Spec. issue **14:** 28–31.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White JW, eds. *PCR protocols. A guide to methods and applications*. New York: Academic Press, 315–322.

Willdenow CL. 1811. *Berlinische Baumzucht, oder Beschreibung der im Königlichen Botanischen Garten bei Berlin im Freien Ausdauernden Bäume und Sträucher, für Gartenliebhaber, Forstmänner und Freunde der Botanik* 2nd edn. Berlin, Nauck.

Wissemann V. 1999. Genetic constitution of *Rosa* sect. *Caninae* (*R. canina, R. jundzillii*) and sect. Gallicanae (*R. gallica*). *Journal of Applied Botany / Angewandte Botanik* **73:** 191–196.

Wissemann V. 2000. Molekulargenetische und morphologisch-anatomische Untersuchungen zur Evolution und Genomzusammensetzung von Wildrosen der Sektion *Caninae* (DC.) Series *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **122:** 357–429.

Wissemann V. 2002. Molecular evidence for allopolyploid origin of the *Rosa canina*–complex (Rosaceae, Rosoideae). *Journal of Applied Botany/Angewandte Botanik* **76:** 176–178.

Wissemann V. 2003a. Conventional taxonomy of wild roses. In: Roberts A, Debener T, Gudin S, eds. *Encyclopedia of rose science*. London: Elsevier, 111–117.

Wissemann V. 2003b. Hybridization and the evolution of the nrITS spacer region. In: Sharma AK, Sharma A, eds. *Plant genome, biodiversity and evolution*, Vol. 1 Part A. Enfield, New Hampshire: Science Publishers, Inc. 57–71.

Wissemann V. 2003c. Wildrosen I: Einleitung und Untergattung *Hulthemia*. *VDR Rosenjahrbuch* 142–145.

Wissemann V, Hellwig FH. 1997. Reproduction and hybridisation in the genus *Rosa*, section *Caninae* (Ser.) Rehd. *Botanica Acta* **110:** 251–256.

Wu S, Ueda Y, He HY, Nishihara S, Matsumoto S. 2000. Phylogenetic analysis of Japanese *Rosa* species using matK sequences. *Breeding Science* **50. 4:** 275–281.

Wu S, Ueda Y, Nishihara S, Matsumoto S. 2001. Phylogenetic analysis of Japanese *Rosa* species using DNA sequences of nuclear ribosomal internal transcribed spacers (ITS). *Journal of Horticultural Science and Biotechnology* **76:** 127–132.

Wulff HD. 1954. Notes on the breeding behavior of *Rosa roxburghii* and *Rosa multibracteata*. *American Rose Annual* 73–77.

Wylie AP. 1954. The history of garden roses. Masters Memorial Lecture 1954. *Journal of the Royal Horticultural Society* London **79:** 555–571, **80** (1955)**:** 8–24, 77–87.

Zielinski J. 1982. Rosaceae II-*Rosa*. In: Rechinger KH, ed. *Flora Des Iranischen Hochlandes und der Umrahmenden Gebirge.* Graz, Austria: Akad. Druck- u.-Verlagsanstalt **152:** 1–32.

Zielinski J. 1985. Studia nad rodzajem *Rosa* L. Systematyska sekcji *Caninae* DC. em Christ. *Arboretum Kórnickie* **30:** 3–109.