Species of the genus *Rosa* have always been known for their beauty, healing properties and nutritional value. Since only a small number of properties had been studied, attempts to classify and systematize roses until the 16th century did not give any results. Botanists of the 17th and 18th century paved the way for natural classifications. At the beginning of the 19th century, de Candolle and Lindley considered a larger number of morphological characters. Since the number of described species became larger, division into sections and subsections was introduced in the genus *Rosa*. Small differences between species and the number of transitional forms lead to taxonomic confusion and created many different classifications. This problem was not solved in the 20th century either. In addition to the absence of clear differences between species, the complexity of the genus is influenced by extensive hybridization and incomplete sorting by origin, as well as polyploidy. Different analytical methods used along with traditional, morphological methods help us clarify the phylogenetic relations within the genus and give a clearer picture of the botanical classification of the genus *Rosa*. Molecular markers are used the most, especially AFLPs and SSRs. Nevertheless, phylogenetic relationships within the genus *Rosa* have not been fully clarified. The diversity of the genus *Rosa* has not been specifically analyzed in Croatia until now.

**Key words**

*Rosa* sp., taxonomy, molecular markers, classification, phylogeny
Introduction

The Rosaceae family offers plenty of systematic challenges that are beyond morphological determination. The Latin name Rosaceae, as the name of the rose family, was first published by Adanson (1763). According to size, it is the 19th biggest plant family (Hummer and Janick, 2009), and includes between 95 and more than 100 genera, as well as between 2830 and 3100 species (Judd et al., 1999).

According to the Croatian flora database (Flora Croatica database), there are 37 species in Croatia that belong to the genus Rosa. The genus Rosa belongs to the group of plants that are taxonomically complex. There are many synonyms for each species in the Croatian and Latin terminology, which significantly complicates their mutual and individual sorting. Twenty-seven different Latin and 19 Croatian names are mentioned in this database for Rosa canina species alone (Anonymous 1, 2017).

Natural populations of the wild rose (R. canina L.) are present throughout Croatia. Independent studies conducted among populations of wild roses in Croatia indicate the existence of variability. Genotypes from different biogeographical regions were examined, and differences in size, shape of the fruit, prickliness, the amount of dry matter, yield and other agronomically important traits were established. The most important causes of polymorphism observed in this genus are: hybridization, polyploidy and, particularly in the Caninae section, the mechanism of cytotype stabilization of odd chromosome numbers resulting from the specific course of the so-called canina type meiosis (Wrońska-Pilaruk, 2010).

Origin of roses

According to fossil remains, it is assumed that roses are 35 million years old. The cultivation of roses began approximately 5,000 years ago, probably in China. According to research of macroscopic remains of the British flora, fruits of various species of wild rose were used for food in the late Neolithic Age, the Iron Age, the Ancient World and the Middle Ages (Godwin, 1975).

The beauty and the scent of roses made them popular in the Ancient World. The frescoes painted at the height of the Minoan civilization on the island of Crete depict roses. The Greeks associated the rose with Aphrodite, the Muses and grace. Theophrastus (1616) knew of wild and cultivated roses. Carbonized remains of wood and paintings were found on the walls in Pompeii. Pliny (1916) knew of wild and cultivated roses. Carbonized remains of the British flora, fruits of various species of wild rose were used for food in the late Neolithic Age, the Iron Age, the Ancient World and the Middle Ages (Godwin, 1975).

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The history of research on the genus Rosa - artificial classifications

The first attempts to classify and systematize plants were connected to Theophrastus (1616), the “father of botany”. Theophrastus (c. 371 - c. 287 BC), considering their quality, divides plants into seven classes: according to generation, origin, size of trees, shrubs, use value, edibility of seeds and the ones that give juice. He faces difficulties in classifying plants when defining the essential plant “parts”. According to his classification, the wild rose belongs to thorny plants category. He writes the following about it: “Dog rose (wild rose) has a reddish fruit like that of pomegranate and, as well as pomegranate, it represents the transition between bushes and trees; its leaf is thorny.” Theophrastus’ basic division of trees, plants and grass would be maintained until the 17th century.

Pliny the Elder (23-79 AD) mentions the healing properties of root of dog roses using the example of a soldier who was bitten by a rabid dog. Moreover, he mentions other beneficial effects, such as the use of “spongy excrescences” in the midst of prickles which, reduced to ashes and mixed with honey, cure baldness.

Dioscorides (2000) classifies in 1st century AD plants according to their therapeutic properties and divides them into four groups: aromatic, for food, for healing and for wine (Adanson, 1763). In the book De materia medica, he describes the appearance of the whole plant, habitats, and ways of collecting plant parts, giving information on the therapeutic effect of plants and processes of making medicinal preparations from them (Paroječić et al., 2006). He mentions the process of making rose wine, rose oil and pomander (pastilles), as well as the species of Rosa centifolia and Rosa gallica.

Until the 16th century, works of Dioscorides and Theophrastus were mostly commented, which included discussions about medical or economic properties, but not the actual qualities of plants and their classification. Doctors who used plants for medical and therapeutic purposes made first attempts to classify plants after the ancient authors.

Hieronymus Bock (1498-1554) develops his own artificial system of plant classification. He describes plants in Germany, lists their names, properties and medical use, and divides them into three groups: (1) wild plants with fragrant flowers; (2) clover, grass, vegetables and creepers; and (3) trees and shrubs.

Leonhart Fuchs (1501-1566) classifies plants alphabetically by Greek names, relying on illustrations that he used as the main means of identification. His descriptions are short and sometimes inaccurate (Gardham, 2002).

Adam Lonitzer (according to Adanson, 1763) divides plants into two classes: (1) trees and shrubs; and (2) herbs. In his Naturalis historiae opus novum (1551), he mentions Rosa sylvestris and its medicinal properties.

Rembert Dodoens (1517-1585), Flemish physician and botanist, divides plant kingdom into six groups, while particularly elaborating on medicinal plants.

Matthias de l’Obel (1538-1616), also a Flemish physician and botanist, in his herbal entitled Icones stirpium from 1591, attempts to be the first person to classify plants according to their natural inclinations, rather than their medical purpose. L’Obel (1591) divides plants into six groups: (1) grass, (2) orchids, (3) vegetables, (4) trees and bushes, (5) palms and (6) moss.

John Gerard (circa 1545-1612) in his The Herball or Generall Historie of Plantes (1597) devotes 13 pages to roses. He divides them into musk roses (Rosa moschata simplici flore, Rosa moschata multiplex, Rosa holosericea, Rosa lutea and Rosa cinamomome pleno flore) and wild roses (Rosa sylvestris odora, Rosa canina inodora and Rosa pimpinella). He also lists the following species: Rosa alba, Rosa rubra, Rosa provincialis sine Damascena, Rosa provincialis minor, Rosa sine spinis and Rosa hollandica. Descriptions of roses are generalized (shrub height, prickliness, number, color and hairiness of leaflets, pedicel length, color and scent of flowers, number of petals, sepals, seed hardness, color and shape of the fruit, root length). The structure of the pistil is not mentioned, while descriptions of stamens are very sketchy, listing them as “threads”.

Andrea Cesalpino (1519-1603) classifies plants according to their fruits and seeds.
Jacques Daléchamps (1513-1588) has no natural classification system either; he divides plants into 18 classes.

William Turner (1509/10-1568), the "father of English botany", lists only two species of roses with their Greek names, cyanorrhodos (Rosa rubiginosa) and cynosbatus (Rosa canina). He describes them very briefly as bushes, differentiating the two species by stating that the leaves of the first species have no smell.

Adam Zaluszký (1555-1613) divides plants into 22 classes, of which only three are natural. He is the first who completely separates botany from medicine.

Caspar Bauhin (1560-1624) has both natural and artificial system of plant classification. In his description of the Rosa species, he lists many synonyms and uses descriptive and binomial labeling, which makes him Linne's predecessor in introducing the binomial nomenclature. Descriptions of species are generalized and mainly refer to the color, smell, number of leaves and origin. He lists the total of 39 species, dividing them into three groups: Rosa sativa, Rosa sylvestris and Rosa Hierichuntica dicta.

Pierre Magnol (1638-1715) creates the concept of natural plant classification with division into families, based on a combination of morphological properties. In his Prodromus historiae generalis plantarum from 1689, he mentions roses, but without giving any additional descriptions.

August Rivin (1652-1723) is the first to propose plant classification based on the flower structure, i.e. the form of corolla. Along with de Tournefort, he is the first to use the rule that names of all species in the same genus should start with the same word.

John Ray (1627-1705) publishes his work Historia plantarum and makes an important step towards modern taxonomy (Ray, 1688). He dismisses the dichotomy system of division in which species are classified according to the already established "either/or" system. Instead of that, he classifies plants according to similarities and differences that emerged from observation. He classifies plants based on observing the "essential properties" and classifies them according to the similarity of flower, calyx, seed and receptacle (Lazenby, 1995). Ray's general classification divides plants into two groups: (1) Herbæ - (a) imperfectae (cryptogamic plants) and (b) perfectæ (spermatophytes - monocots and dicots); (2) Arboræ - monocots and dicots. He classifies roses as dicots and put them into semine nudo polyspermo group ("many naked seeds"). The names of species are polynomial, and he lists 37 species of roses in the chapter entitled De arboribus quarum flos summa fructui insidi ("About trees with flower at the top of the fruit"). The descriptions are generalized and related to the color of petals, smell, prickliness of shoots, leaf color, shape color and plumpness of fruit.

In the book "Systema naturæ", Linnaeus (1735) describes plants according to the features of the sexual organs and listed, apart from the plant kingdom, only four taxonomic units: classes, orders, genera and species. The classification which was based on the number of stamens and pistil mentions the genus Rosa but does not mention any species. He classifies roses as belonging to the group with a larger number of stamens and pistils (icosandria, polygynia). In the book entitled Species plantarum, Linnaeus (1753) describes 12 species of roses (R. cinnamomea, eglanteria, villosa, canina, spinissima, centifolia, alba, gallica, indica, sempervirens, pendulina, carolina). In the 1759 edition, Linnaeus (1759) describes 13 types of roses, i.e. he adds one more type (R. pimpinellifolia). He tries to classify roses according to the shape of the fruit, which proves unsuccessful because this feature is subject to change in many species (Bulletin de la société royale de botanique de Belgique, 1867).

A. L. de Jussieu (1748-1836) divides plants into 15 classes, 100 orders and 1754 genera. Description of plants of the genus Rosa is general, without differentiating the species.

In 1815 Desvaux publishes a paper on roses in France, in which he proposes division into two groups: (1) roses with joint stylius and (2) with free stylius.

Scientific (natural) classifications of the genus Rosa in the 19th century

Most information about roses comes from papers and monographs of West European experts. The first scientific classification of the genus Rosa is created by de Candolle (1815). He divides Rosaceae into seven groups – Drupaceae, Procèèae, Spireae, Dryadeae, Agrimonyae, Rosier and Pomaceae. As opposed to Linnaeus, De Candolle takes the plurality of morphological properties into account. According to the de Candolle’s (1825) classification, plants are divided into classes (Dicotyledonae), subclasses (2nd subclass: Calyciflorae), orders (64th order: Rosaceae), tribes (7th tribe: Rosae), genera (48th genus: Rosa) and species.

At the meeting of Linnean Society in London in 1818 Wood’s paper on English roses was presented. He divides the genus Rosa into three main groups, according to hairs and prickles: a) Setigerae (aculeis sepis rectis); b) Setis nullis, aculeis rectiuscellis; and c) Setis nullis, aculeis uncinsatis. That same year in Paris, Léman presents a new method of describing the genus Rosa based on the serration of leaflets. Also, that year, de Candolle's classification divides the genus Rosa into 11 sections (Synshtuleae, Rubigineae, Gallicanae, Chinenses, Cinnamomean, Hebecladae, Pimpinellifoliae, Villosae, Centifoliae, Caninae and Eglanteriae). This classification is the first step towards natural classification of rose species (Bulletin de la société royale de botanique de Belgique, 1867).

In the book Annales générales des sciences physiques - Tome cinquième, Prodròme d'une monographie des rosiers de l'Amérique septentrionale (1820), Rafinesque describes species of roses in North America. Among the 33 species he describes, there are 15 new ones. Based on the shape of sepals (outer plumose sepals and sepals without lateral additions) and the fruit, he divides them into two groups (divisions) and eight sections.

That same year, Lindley (Shinwari et al., 2003) divides the genus Rosa into 11 sections (Simplicifolia, Feroces, Bracteatae, Cinnamomeae, Pimplinellifoliae, Centifoliae, Villosae, Rubiginosae, Caninae, Synshtuleae and Banksianae) and gives a detailed description of 76 species. Lindley also uses root sprouts to identify species: “The habit of roses, although not often of moment, may sometimes be employed with advantage, when its differences are caused by the manner in which the root-shoots grow. Their being bent like a bow distinguishes Caninae and Rubiginosae from Villosae; in which they are quite erect. The flagelliform shoots of arvensis prevent its being confounded with sylvestra; and their being climbing separates sempervirens from prostrata. Yet cinnamomea contains two plants, of which one has straight and the other curved root-shoots; and the same remark is applicable to tomentosae.”

In doing so, he differentiates roses based on the morphology of branches and lateral shoots, density of prickles on the branches, roughness of the branches, inflorescences, stipules, infrastipulary prickles, setae and hairiness of branches, stems or calyx...
depressions. Lindley points out of the setae (bristles): “Some of my most natural divisions depend on their presence on the branches.” He points out that the hairiness of branches, stems or depressions of calyx was the only invariable property in roses. He also monitors the hairiness of leaves as a secondary feature. The shape and concavity of leaflets can rarely be used for determination, but plication of leaflets is an important property. Under special circumstances, the serration of leaves can also be used. Form of sepals can rarely be considered, especially their degree of fragmentation. Petals rarely offer some significant differences; they are usually concave and wide. Stamens differ only in the number, and in this sense, can rarely be used, except as a secondary feature.

Considering the problems that occur during the determination, Lindley says: “It however unfortunately happens that few parts of the plant are more subject to variation, not only as to surface, but form and size. This remark is particularly applicable to tomentosa, canina, and rubiginosa, in which every diversity of form may be found. Yet there are some species in which it appears to be much less polymorphous; but whether from our having less knowledge of them, or from the absence of the predisposition to vary for which canina and its neighbours are so famous, I do not pretend to be able to judge. Cinnamomeae may be considered to offer examples of the greatest uniformity, and Caninae and Villosae of the greatest diversity of fruit.”

In describing certain species, he lists larger or smaller number of synonyms that are used by other authors. Determination is further complicated by descriptions of types of some species of roses (listing 6 types in the R. canina and 7 in the R. rubiginosa species) for which he uses the letters of the Greek alphabet.

The proposal of the group of authors in 1824 is the division of the genus Rosa according to nectar glands into four subgenera: Chamaerhodon, Cassiorhodon, Cynorhodon, Stylorhodon (Bulletin de la société royale de botanique de Belgique (1867), Historique de la classification des Roses).

In his Prodromus systematis naturalis regni vegetabilis – pars secunda, de Candolle (1825) divides Rosaceae into eight tribes, and the tribus Roseae into four sections (Synstylae, Chinenses, Cinnamomeae, Caninae).

In his study of German flora, Reichenbach (1830-32) describes 77 species of roses, which are divided according to density of shoot prickles, i.e. hairiness, into two main groups, setigerae and aculeosae.

Koch (1837) publishes Synopsis Florae Germanicae et Helvetica, in which roses are divided into four groups according to carpels which are sessile or on the stalk.

In the first part of the book Flore de France, Grenier and Godron (1848) present the first classification of roses based on stipulas.

Exploring the flora of the Jura mountain range, Godet (1869) divides roses into three groups (Heteracanthae, Diancanthae, Homoeacanthae). However, he points out that this division does not have a large scientific, but more a practical type of value, using prickles for his determination. He states that the use of the form of the fruit, especially in Canina, is of little value.

In his monograph Die Rosen der Schweiz, Swiss botanist Christ (1873) uses Crépin’s methodical table of European roses and divides them into six sections: Cinnamomeae, Pimpineillifolae (additional division into Alpinae and Pimpinellifolae), Sabineae, Caninae, Arvenses and Gallicanae. He divides the section Caninae into five subsections, with additional divisions: Vestitae (Villosae, Tomentosae), Rubigineae (Rubiginosae, Sepiaeae), Tomentellae, Trachyphillae and Caninae (Glanduliferae, Pilosae). He describes 34 types of roses in total.

Burnat and Greml (1886), exploring the roses in Italy, put them into two major groups, with division into sections and subsections. The Chlorystylae group is comprised of two sections, Gallicanae and Cymorhodon, of which the latter is divided into four subsections: Vestitae, Rubigineae, Tomentellae and Caninae. The Synstylae group is divided into two subsections, Repentes and Moschatae.

Shinwari et al. (2003) refer to Christ who in 1887 divides the genus Rosa into two main sections: Choristylae (species with free styles) and Synstylae (joined styles). Also, Crepin in 1889 (according Shinwari et al. 2003) divides the genus Rosa into 15 sections (Synstylae, Stylosae, Indicae, Banksiae, Gallicae, Caninae, Caroliniae, Cinnamomae, Pimpinellifolae, Luteae, Sericeae, Minutifolae, Bracteatae, Laevigatae and Microphyllae).

Classification systems in the 20th century

According to Gustafsson (1944), in the first decades of the 20th century two research systems of classification and phylogeny of the genus Rosa appear. Almquist proposes the existence of groups of species, as well as specific species, the division of which will be based on general characteristics of the flower, fruit or prickles, while Hurst’s division is based on the features of form and leaf serration. Explaining polymorphism, Gustafsson quotes the 1919 research by Almquist, who counts 64 species of the sub-group Villosae, 48 Tomentosae, 13 Eu-Rubiginosae, 8 Agrestes, 120 Afzelianae and 99 Eu-Caninae, a total of 352 representatives.

Matthews (1920) makes the primary division of the genus Rosa based on leaflet serration, the presence or absence of subfoliar glands, development or underdevelopment of hispid peduncles and leaf pubescence. Aware of the complexity of the genus, Matthews says: “There are probably no plants more variable than roses and it is perhaps not too much to say that no two bushes of the same ‘species’ are quite alike in all the technical characters relied upon by rhodologists for making a diagnosis... It is gradually being realized that external morphology alone is inadequate for the solution of such problems, yet it is remarkable that still very few “critical species” among British plants have been subjected to the test of experimental investigation. Only by culture, combined as far as possible with cytological study, will it become possible, I think, to determine finally the genetic relationships of the numerous micro-species into which old, well known species like R. canina Linn, have been split. “

Gustafsson uses taxonomic grouping of the so called canina complex (in the broad sense), which is used by a lot of researchers at the time, into subsections (Jundzillianae, Rubrifoliae, Vestiti, Rubiginosae, Stylosae and Caninae). He divides some of these subsections into series (Vestiti into Villosae and Tomentosae, Rubiginosae into Agrestes and Eu-Rubiginosae, Caninae into Afzelianae and Eu-Caninae series).

Boulerger publishes a monographic study of European (1924-32) and Asian (1933-36) species of roses and reduces the number of species to 121 (according to Erlanson, 1938). Crepin and Boulerger represent the synthetic trend in the study of the genera (according to Shinwari et al., 2003). At the same time, Wolley-Dod describes numerous varieties and forms in the research of British roses (according to Graham and Primavesi, 1990), deeming them very
variable, but not taking into account their great tendency towards hybridization. Later (in 1936) he expresses doubts about the importance of these varieties, stating that he would rarely find samples in particular areas which closely or even remotely correspond to the description of a named variety.

In the description of the genus *Rosa*, Rehder (1940) claims that there are from 100 to 200 species in temperate and subtropical regions of the Northern Hemisphere. He also states: “The species of this genus are very variable and hybridize easily, and the conception of the species varies greatly according to the views of different botanists. While Bentham & Hooker recognize only about 30 species, Gandoger enumerates 4,266 species from Europe and Western Asia alone.” He divides the genus into four subgenera: *Eurosa* (69 species), *Hulthemia, Platyrhodon and Hesperhodos* with one species each. There are 10 sections within the genus *Eurosa* (*Pimpinellfoliaceae, Gallicanaceae, Caninae, Carolinaceae, Cinnamomaceae, Synstylaceae, Indicae, Banksianaceae, Laevigatae and Bracteatae*).

Rehder uses the following morphological features in his determination: branches, twigs roughness, lateral shoots, shoots, prickles, setae (“some of my most natural divisions depend on their presence on the branches”– he separates *Spinosissimae* from *Canina* based on setae), leaf glands, branch hairiness, pedicels and orifice - (“the only unchanging feature that I found on the roses – they are either permanent or deciduous”), stipules, leaves, leaf color, leaflets shape, flower and blossom, bracts, branching, form of orifice, sepals form, ovary; petals and stamens rarely offer significant differences.

Following Rehder’s classification, De La Roche (1978) divides roses into two genera, *Hulthemia* and *Rosa*, while further dividing the latter into three subgenera, *Rosa, Platyrhodon* and *Hesperhodos*. He further divides the subgenus *Rosa* into 10 sections. The section *Cinnamomaceae* is given the name *Rosa*, while the section *Indicae* is renamed *Chinenses*.

In his pollen key to the family *Rosaceae* (for Northwestern Europe), Eide (1981) explores the type of *R. canina* and finds that the pollen is quite variable in size, shape, structure and sculpturing, but does not determine solid morphological boundaries in relation to *R. rubiginosa* and *R. pimpinellifolia* species.

Shinwari et al. (2003) cite several authors from the second half of the 20th century who apply chemotaxonomic methods, which sometimes allows detection of differences in the chemical properties of morphologically similar species. Differences in the content of vitamin C, E and K, anthocyanins, flavonoids, carotenoids, tannins, pectins, phenolic acids, fatty oil, nicotinamide, steroids, terpenes and microelements can be noticed in flowers, fruits and leaves.

Classifications in the 21st century

In a research carried out by Wissmann and Ritz (2005) and Joly et al. (2006) the first source of taxonomic confusion is the use of morphology as the basis for the classification of roses. Morphological properties are frequently heavily influenced by environmental factors and selection pressure, for example when the growth conditions (rapidly) change. Selection pressure can, on the one hand, result in similarity of properties for evolutionary different species which are adapted to similar conditions, but on the other hand, in the striking morphological differences between related species adapted to different conditions. Another source of taxonomic confusion in the genus *Rosa* is complicated evolutionary history combined with a long history of cultivation and interbreeding of selected genotypes. Complexity is caused by several factors, often in combination: (1) extensive hybridization, ancient and recent; (2) the absence of clear differences between many species, partly due to their recent expansion; (3) incomplete sorting by origin (common feature of newly separated species); and (4) polyploidy - with multiple/hybrid origin for polyploids in some species at least (Koopman et al., 2008).

Exploring the Belgian wild rose taxa, De Cock et al. (2008) confirm high complexity of taxonomic structure of the polymorphic subgenus *Rosa* section *Caninae* due to the combination of some unusual properties: unique polyploid chromosomal constitution, heterogamous *canina* meiosis, the ability of hybridization between species and predominantly macrolinial inheritance. Both morphological and AFLP-based analyses support the subdivision of *Canina* into three well-defined, although partially overlapping groups (*Rubigineae, Vestitae* and *Caninae*), but there is no evidence to support the existence of separate subsection *Tomentellae*.

Ben Cheikh-Affene et al. (2015), in the analysis of morphometric variation and taxonomic identification of populations of wild roses in Tunisia, use the properties of high discriminatory value, such as the shape of the style, leaf and leaflet length, the number of flowers in the corymbs, the leaflet serration, the presence of glands on the leaflet, peduncle, receptacle and sepalas. They state that these properties are very successful in the morphological identification for sections *Synstylae* and *Caninae*. This study finds substantial phenotypic divergence of populations of wild roses in Tunisia.

Hybridization events and the evolution of the species within the genus

In the evolutionary history of the genus *Rosa* spontaneous hybridization was a common occurrence that generated new properties and speciation. Zhu et al. (2015), citing research from several authors, make some important conclusions: (1) hybridization for *Rosa* species is simple due to overlapping areas of distribution and timing of flowering, common general pollinators and fertile hybrids; (2) hybridization has occurred between closely related species, but also between species from different sections; (3) polyploidy is very common, especially in *Caninae* and *Rosa* sections; (4) hybridization/introgression is important for the evolutionary history of most plant taxa, which applies to taxa of the genus *Rosa*.

Number of chromosomes and genomes

The basic number of chromosomes for *Rosoideae* is mostly x=7. The number of chromosomes in the genus *Rosa* is based on a multiple of 7, ranging from 2n=2x=14 to 2n=8x=56, while aneuploids are rare.

According to Chromosome Atlas of Flowering Plants (Darlington and Wylie, 1955) which is widely accepted by numerous researchers (Lata, 1981; Yokoya et al., 2000; Grossi and Jay, 2002) subgenera *Hulthemia, Platyrhodon and Hesperhodos* have only one species in which the 2n=2x. The fourth subgenus *Eurosa* contains more than 120 species grouped in 10 sections (Rehder, 1960). Sections *Banksianaceae, Bracteatae, Indicae, Laevigatae* and *Synstylaceae* have 2n=2x, *Gallicanaceae* 2n=4x, *Carolinaceae* and *Pimpinellfoliaceae* 2n=2x and 4x. *Caninae* 2n=4x, 5x and 6x, and *Cinnamomaceae* 2n=2x, 4x, 6x and 8x. Wissmann and Ritz (2005), Werlemark et al. (1999), Nybom et al. (2004) and Fedorova et al. (2010) claim that all *Caninae* are alloploids with 2n=4x, 5x or 6x, n=7. However, only two genomes
of the 4, 5 or 6 in nucleus of the polyploid Canina match and form bivalents during meiosis. These genomes are transferred via haploid pollen and polyploid egg cells. During meiosis 2, 3 or 4 unpaired genomes form univalents, transfer exclusively via egg cells and get lost in pollen mother cells. Such unbalanced meiosis in section Caninae results in a very distorted, mainly matroclinal character of inheritance at the morphological and molecular level respectively (Fedorova et al., 2010). Matroclinal inheritance in Caninae is mostly due to the special reproductive system called “balanced heterogamy” as mentioned by Fagerlind, 1951 and Wissemann and Ritz, 2007 (according to Fedorova et al., 2010).

In research on ploidy, flow cytometry is a valuable method for studying variations. Application of this method can be useful for determining the ploidy of parents and hybrids in breeding programs, the selection of plants with double the number of chromosomes, the selection of haploids from another culture and ploidy research in wild populations.

Anatomical, micromorphological and palynological analyses

In the study of Rosa pisiformis in eastern Turkey, Kültür (2002-03) analyzes leaf and pollen structure and lists the following data as important in the anatomical differentiation of Rosa species: the properties of the cuticle, the presence of collenchyma, the shape of sclerenchyma layer, the properties of the cell wall of the upper and lower epidermal cells, the types of crystals and hairs, the form of stomata and the number of surrounding cells. In comparing the similarities and differences of anatomical properties with other Rosa taxa he lists the importance of: the general shape of the leaf section, the absence of collenchyma below the vascular bundle of the central vessel, the presence of several sclerenchyma cells, the undulating cells of lower epidermis, the density of stomata, as well as glandular and nonglandular hairs. By following the structure of the pollen, he describes the grain's shape and size, the structure and sculpture of exine, as well as the colpus and pores. Analyzing the phenomenon of polymorphism, he states that polymorphism of pollen in Rosa pisiformis does not exist.

Wrońska-Pilarek (2010) carries out research on pollen morphology on 16 species of the genus Rosa at 16 natural sites in Poland. She studies 13 quantitative traits of pollen grains and design of exine, as well as some qualitative characteristics (contour, shape and structure of the operculum). The collected data does not fully confirm the current taxonomic division of the genus Rosa into sections nor does it confirm the closeness of the relationship between the studied species of the section Caninae. The listed morphological features of pollen grains make only the isolation of Rosa gallica species possible. The diagnostic significance of the shape of exine and operculum on the levels of section and species is not supported either. Section Caninae is confirmed as the most polymorphic group of the genus Rosa. Modern Caninae are inherently many hybrids of R. canina and present a link that combines all taxa in this section. Wrońska-Pilarek quotes and confirm Zielinski’s thesis that there are no established morphological boundaries between section Caninae and groups that have contributed to its development, particularly the section Rosa. Based on these studies, it can be said that the pollen grain morphology can be used only as an aid for diagnosing sections and species of the genus Rosa.

Fatemi et al. (2012) analyze the anatomical, micromorphological and statistical data on the R. canina species. Because of widespread distribution and high potential for hybridization of Rosa canina with other species, it is difficult to find constant morphological properties. Due to highly variable features of four inducement properties of leaflet, peduncle, leaf glands and petiole in particular, serial forms of this species are studied. The analysis shows that the listed properties were valuable for separation of serial forms within a species.

Identification of species and varieties by the help of biotechnological methods

Many studies have sought to expand and clarify the botanical classification of the genus Rosa. Gudin (2000) reviews attempts to describe the genus by different methods such as flow cytometry to determine ploidy levels, RAPD analysis of DNA samples, statistical analysis of floral phenolic compounds, characterization of volatile products, computed canonical discriminant analysis and cluster analysis of phenotypic data. In conclusion, the identification of certain species of roses and classification based on morphology remain unsatisfactory, so that molecular approaches should be of great help in solving the problem of identification of species and varieties (Gudin, 2000).

Application of molecular genetic methods in taxonomy

In the 1990s molecular markers are developed for identifying varieties of roses, and many of them are tested to identify the relationship of the genus Rosa species. Many authors have used RAPDs and RFLPs for testing relationships between varieties and wild species; distinguishing a group of varieties and a group of wild species; grouping of wild accessions in accordance with sectional affinities, etc. Also, mitochondrial and chloroplast RFLPs have been used to study the relationship between wild Rosa species and ITS sequences as the basis for the phylogeny of roses.

Due to certain disadvantages of RAPD and RFLP markers, alternative markers are developed, among which the most important ones are microsatellites or SSR (Morgante and Olivieri, 1993) and AFLP (Vos et al., 1995). Koopman et al. (2008) suggest that both types of markers combine high reproducibility with high variability, potentially increasing both reliability and the resolution of phylogeny. On top of that, both AFLP and microsatellites allow sampling at the level of the whole genome.

Page and Holmes (1998) claim that the phylogenetic interpretation of microsatellite data is complicated due to size limitations of alleles, unsafe and poorly understood mechanisms of resolving alleles and the uncertain impact of mutational events on the length of alleles. However, regarding the microsatellites, set of markers originally developed for the identification of varieties (Esselink et al., 2003) prove to be useful for determining the relationship between varieties and species, giving dendrograms with considerable support and resolution.

For the analysis of local differentiation and hybridization events in populations of wild roses in Western Ukraine, Fedorova et al. (2010) use 20 morphological properties, chloroplast markers (chloroplast intergenic spacer Trnv-ndhC) and ISSR markers for the study of DNA polymorphism. They conclude that morphological variability does not correlate with ISSR markers diversity.

In the study of genetic polymorphism of the genus Rosa in Hungary, Deák et al. (2005) use AFLP as a molecular marker technique. As the main advantage of molecular markers in comparison
to taxonomic interpretation of properties they emphasize their stability in relation to morphological variation and the effects of environmental factors. Joly and Bruneau (2007) successfully use AFLP to characterize the genetic constitution of individuals at the genomic level and to set the boundaries of species within the Cinnamomeae section.

Koopman et al. (2008) conclude that AFLP can be a valuable source of phylogenetic information, even in a group of species with a complex evolutionary history, such as Rosa species.

In the research of phylogeny and biogeography of wild roses for the study of phylogenetic relationships Fougère-Danezan et al. (2015) use psbA-trnH spacer, trnl. intron, trnL–F spacer, trnS-G spacer and trnG intron, using sequences from the plastids. They use nuclear GAPDH (glyceraldehyde 3-phosphate dehydrogenase) to identify potential allopolyploids and draw conclusions about their possible origin.

In attempt to identify the relation between diploids and researching the origin of polyploidy within North American rose population (sections Cinnamomeae and Carolinae) Joly et al. (2006) give priority to methods of genotyping (microsatellites, AFLP, isozymes) with genealogical approach. In doing so, they use a single-copy nuclear GAPDH gene and determine that the separation of these two sections is artificial. As the solution, they propose to treat the section Carolinae as a synonym for Cinnamomeae and recommend removing the section Carolinae from further taxonomic procedures. Due to the unreliability of morphological properties, they support research of biochemical and molecular properties. Highly polymorphic polyploid taxa (R. arkansana, R. carolina, R. virginiana) from the complex Rosa carolinae, which have different evolutionary past, should be verified by a large number of markers.

The problem of contemporary division in the taxonomy of the genus Rosa

According to various authors, Rehder’s classification system is still widely accepted and used as the basis for the modern debates (Ben Cheikh-Affène et al., 2015). Koopman et al. (2008) argue that there is currently a broad consensus for the use of Rehder’s system from 1940 that is renewed by Wissemann (2003). According to Fougère-Danezan et al. (2015), this renewed system contains four subgenera: Hulthemia (1 species), Rosa (about 180 species), Hesperhodos (2 species) and Platyhyodon (1 species). Subgenus Rosa is divided into 10 sections (Pimpinellifoliae, Rosa, Caninae, Carolinae, Cinnamomeae, Synstylae, Indicae, Banksianae, Laevigatae and Bracteatae), and the largest section, Caninae, is divided into six subsections (Trachyphyllae, Rubrifoliae, Vestitae, Rubiginae, Tomentellae and Caninae). Wissemann has kept Rehder’s division into subgenera and sections but has defined six new subsections.

According to De Cock et al. (2008), the apparent morphological similarities allow taxa to merge into smaller, but more diverse group of species in accordance with the preferences of taxonomists. According to their morphological and AFLP analyses, they accept division of Graham and Primavesi from 1990.

Fougère-Danezan et al. (2015) list a larger number of authors who were trying to build a phylogeny and give a new perspective on this genus. They found only several clades which are partly consistent with currently recognized (Wissemann’s) sections and stress allopolyploidy as important factor in stabilizing intersectional hybrids.

Species determination of the genus Rosa

The modern classification systems are based on the use of morphological and molecular analyses. Controversy and disadvantages of the classification of angiosperms are resolved by phylogenetic approaches based on APG analyses. APG system is based on the analysis of the chloroplast and ribosomes coding genes in conjunction with morphological properties (Folta and Gardiner, 2009). APG IV (2016) classification system of flowering plants is the fourth version of the modern, mostly molecular-based, system of plant taxonomy of angiosperms developed by an Angiosperm Phylogeny Group. According to this system, the genus Rosa belongs to class Equisetopsida, subclass Magnoliidae (APG classification systems do not use formal botanical names above the order), clades Eudicotidae, Superrosidae, Rosidae, Eurosidae, Fabidae, order Rosales, fam. Rosaceae, subfam. Rosoideae. APG classification systems do not problematize relationships at lower taxonomic units, such as genera and species. According to the List of Rosa Species, the genus Rosa is divided into four subgenera (Hulthemia, Hesperhodos, Platyhyodon, Rosa), subgenus Rosa is divided into 11 sections (Banksianae, Bracteatae, Caninae, Carolinae, Chinesis, Gallicianae, Gymnocarpaceae, Laevigatae, Pimpinellifoliae, Rosa, Synstylae).

Working list of all plant species (The Plant List) includes 4,389 scientific plant names of species which belong to the genus Rosa. Of that number, 366 names of species are accepted, 852 species are synonyms, 19 species are not placed, and 3,152 species have not been evaluated (Anonymous 2, 2017).

Riaz et al. (2007) believe that plant descriptors for Rosa species were not very well defined. In the study of wild rose populations, different keys were used to determine taxonomic properties. Most authors use several different keys of national and regional flora characteristics in the determination process.

Research of the genus Rosa in Croatia

There has not been any systematic work on the taxonomy of the genus Rosa in Croatia so far. Roberto de Visianii conducted the first significant research in the 19th century. In the book entitled Stirpium Dalmaticarum specimen, de Visianii (1826) only mentions Rosa canina species and its Illyrian names (rusa diviša, rusa pasia). In his book Flora Dalmatica, de Visianii (1852) lists nine species of the genus Rosa, with a detailed description of plant organs and habitat, synonyms and their Illyrian names.

In the book entitled Syllabus florae Croaticae, Schlosser and Vukotinović (1857) list 19 types of roses with a very brief description of the locations, but without specifying morphological properties. Horvatić’s “Iliustrirani bilinar” (1954) mentions the genus Rosa “... with many species”, and describes its basic characteristics, giving an example of the wild rose or dog rose without species description.

In his book entitled “Mala flora Hrvatske”, Domac (1979) describes 20 species of roses through the analysis of the main morphological characters, with a dichotomous key for determination. He also adds a note: “The genus Rosa is unusually polymorphic, many species are highly variable, and many are connected by transitional forms; hybrids are quite frequent, which are sometimes very difficult to distinguish from the transitional forms. Therefore, the key only took the main species of this genus into consideration.” In the 2002 edition (“Flora Hrvatske”), there are no changes introduced for the genus Rosa.
Trinajstić (2002) cites Vukotinović’s research (1884) on the R. zalana species discovered near Varaždinski Toplice. He briefly writes about this species, which has not been found to date.

While studying Phytogeographical features of Slatina munici-pality Prilić (2013) mentions five species of roses in the list of vascular plants (R. arvensis, R. canina, R. corymbifera, R. jundzillii, R. nitidula). He describes them as cosmopolitan species (Central European, European or Asian floral element, depending on the species), nanophanerophytes that grow in thickets. He connects the species of R. gallica to forests of deciduous oaks beyond the reach of floods.

Conclusion

The taxonomy of the genus Rosa has been intensively studied over the last 200 years. Since the first scientific classifications in the 19th century, the complexity of this genus has been established. Due to marked polymorphism and predisposition to interspecies hybridization, attempts at determination by analyzing the morphological properties did not yield in widely accepted systematics. Since traditional studies are not sufficient, anatomical, micromorphological and pollen analyses, as well as various molecular markers are used for the analysis of samples in recent times. In the study of the genus Rosa phylogeny, the use of morphology and molecular tools has created a better insight into the intersectional and interspecies relations, although the final solution has not been reached yet. The diversity of the genus Rosa has not been extensively studied in Croatia until now. According to own preliminary analyses of Rosa species populations in Croatia, assessed both by morphological and molecular properties, there might be significant pomo-logical and genetic variation among them.

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