Is the morpho-anatomical differentiation of dogwood (*Cornus* L.) endocarps reflected in the phylogeny of the genus?

Maria Morozowska[1], Agata Woźnicka[2], Renata Nowińska[1]

[1] Department of Botany, Faculty of Agronomy, Horticulture and Bioengineering, Poznań University of Life Sciences, Poznań, Poland

[2] ul. Radosna 94, Legnica, Poland

Corresponding Author:
Maria Morozowska [1]
Wojska Polskiego 71C, Poznań 60-625, Poland
Email address: maria.morozowska@up.poznan.pl

Abstract

Genus *Cornus* is known for its complicated taxonomy and an extreme polymorphism. However endocarps, the reproductive structures of potentially high taxonomic value, were examined in the limited range. Currently dogwoods are classified ~~-~~in four morphological groups and four reflecting them phylogenetic clades: blue- or white fruited dogwoods (BW), cornelian cherries (CC), big bracted (BB) and~~-~~ dwarf dogwoods (DW). The phylogenetic relationships within the genus are as follows (BW(CC(DW-BB))). The present study is a continuation in a series of the papers that started with examination of the diversity within endocarp morphology among BW species. We have implemented to our analyses the data obtained earlier and a compilation of these and presently obtained results was provided. We examined 17 morphological and four anatomical features of endocarps of 22 *Cornus* species with the aim to check the influence of endocarp traits on the understanding of relationships within the genus. Our present approach was extended to analyse endocarp sculpture. We hypothesized that the grouping of the studied dogwoods based on the morphology and anatomy of endocarp would be the same as their phylogenetic relationships. Data analyses included multivariate statistics (principal component analysis, discriminant function analysis, hierarchical cluster analysis, multivariate analysis of variance) as well as parametric and nonparametric tests of statistical significance (analysis of variance with post-hoc tests, chi-squared test, Kruskal-Wallis *H* test, Mann-Whitney U test). The hypothesis was positively verified on the basis of the obtained results. Hierarchical cluster analysis of the qualitative and quantitative traits of the endocarps revealed the highest morphological similarity of endocarps between DW and BB groups, which supported the phylogenetic relationships. The quantitative traits which most~~ly~~ influenced such grouping were the stone lengths, the endocarp

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length/width ratio and the number of vascular bundles on the endocarp surface. Among the qualitative characters the stone shape, the vascular bundles position on the stone surface, the apex and the base outline and the presence of a furrow on the side walls of the stone were the most important taxonomic traits. With regard to the anatomy of endocarps, the most important features were the germinating valve thickness, the ratio of the endocarp wall thickness divided by the endocarp diameter, the septum width and its structure, the presence or absence of crystals in the outer endocarp, the number of cell layers in the transition sclereids zone and the primary and secondary sculpture. Considering the described variation in morphology and anatomy of endocarps we believe that our results will be helpful in taxonomic studies of the ~~living and~~ extant *Cornus* species.

Introduction

The monophyly of ~~genus~~ *Cornus* L. is strongly supported by morphological and molecular data (Murrell 1993; Xiang et al. 1993, 1996, 1998; Fan & Xiang 2001), but due to the extreme polymorphism of the genus the relationships between different taxonomic units (subgenera, sections) into which the genus was divided in the past were unclear for almost a century (for details see Xiang et al. 2006). The whole genus comprises approx. 60 species classified in ten subgenera which belong to four morphological groups: blue- or white fruited dogwoods BW [*Yinquania* (Zhu) Murrell, *Kraniopsis* Raf., *Mesomora* Raf.]; cornelian cherries CC [*Afrocrania* (Harms) Wangerin, *Cornus* L., *Sinocornus* Q. Y. Xiang], big bracted dogwoods BB [*Discocrania* (Harms) Wangerin, *Cynoxylon* Raf., *Syncarpea* (Nakai) Xiang] and dwarf dogwoods DW (*Arctocrania* Endl. Ex Reichenb.). Similarly to the morphological classification, four major clades BW, CC, BB and DW were distinguished within the genus as the results of the phylogenetic studies based either on morphological, molecular or combined data (Xiang et al. 1996, 2006; Fan & Xiang 2001). However, the relationships of the particular groups, subgenera or species shown on the base of the morphological or molecular evidence were incongruent (Murrell 1993; Xiang et al. 2006).

Murrell (1993) on the ~~base~~ basis of his cladistic analysis of 28 morphological, anatomical, chemical and cytological characters performed the simultaneous analysis of the relationships within the genus and generated a hypothesis of the *Cornus* phylogeny as (BW(DW(CC-BB))). Such hypothesis in which the closest relationship was found between the CC and BB groups differed to some extend from those proposed earlier (Adams 1949; Bate-Smith et al. 1975; Jahne 1986; Eyde 1988), but the most unique in it was the placement of the dwarf dogwoods with the subg. *Arctocrania* as the sister group basal to the clade formed by the subg. *Afrocrania, Cornus,* *Discocrania, Cynoxylon* and *Syncarpea* (see Xiang et al. 2006, Figs. 1, 2). In turn, Xiang et al. (1998) and Fan & Xiang (2001) showed the phylogenetic relationships between four clades within genus *Cornus* as (BW(CC(DW-BB))). However, these relationships were supported by low bootstrap values, and thus they were not clearly resolved. According to Xiang et al. (2006) the differences between the molecular and morphological evidence arose because in none of the previous molecular analyses all of the ten subgenera of *Cornus* were included, and the samplings were limited. Furthermore, in Murrell's (1993) cladistic analysis of morphological characters the chosen outgroup taxa were not complete as only the genera *Mastixia* and *Diplopanax* were taken into consideration. Xiang et al. (2006) generated DNA sequences for *matK* and ITS with complete sampling of species with the aim to reconstruct a species level phylogeny of the genus. The authors reconsidered ~~also~~ Murrell's (1993) morphological data matrix and expanded it by introducing nine new characters and they added the genus *Alangium* (sister of *Cornus*) as an outgroup. As the result of ~~such complete~~this research the species relationships within the subgenera were mostly ~~clearly~~ resolved, however the relationships between subgenera and clades were still different depending on the type of the data taken into consideration. The results of phylogenetic analyses of the morphological data suggested the BB and CC groups to be sisters, but they were only moderately supported by the bootstrap values. According to the relationships suggested by the molecular evidence the *matK* trees showed that the DW-BB, and CC-BW were two pairs of sister clades, with the latter pair being weakly supported (65%). In turn, the ITS trees placed the CC group with the BB-DW clade, rather than with the BW group. The combined *matK*-ITS data set showed similar relationships to those of the *matK* and ITS trees, but with higher support for most of the nodes. Complex analyses performed with the use of the combined results of phylogenetic and morphological studies, together with the already published *rbcL* and 26S rDNA sequences resolved the relationships among clades as (BW(CC(DW-BB))) (Xiang et al. (2006).

The combined analyses of Xiang et al. (2006) included 37 morphological characters and all DNA sequences using parsimony and Bayesian methods. From among all morphological traits most of them concerned inflorescence and flower structure, leaves and locules, as well as the chromosome number and selected chemical compounds. The stony endocarps, the reproductive structures of potentially high taxonomic value, were examined only in the limited range. Among all of the analyzed features only three of them were the endocarp characters: endocarp shape, cavities in endocarp and apex of the fruit stone. Recently the extensive studies on dogwoods morphology including the stony endocarps were performed by Schulz (2011, 2012), Zieliński et al. (2014) and Woźnicka et al. (2015). Schulz (2011) gave ~~in his work~~ an overview of the morphological differentiation within the genus including the fruit stones morphology, and discussed the various affinity of the particular groups and subgroups in the light of the ~~great~~ polymorphism of the *Cornus* genus. In his next work Schulz (2012) characterized the alternate-leafed dogwoods from the subgenera *Mesomora* and *Kraniopsis* (BW), including the cultivated taxa. The author stressed the importance of the endocarp morphology in taxonomy of dogwoods and proposed the new taxonomic approach for some of the very closely related species e.g. *C. alba* L., *C. sericea* L. (*C. stolonifera* Michx.) and *C. occidentalis* [(Torr. & A. Gray) Coville], *C. amomum* (Mill.) and *C. obliqua* (Raf.), C*. australis* ~~(~~C.A.Mey~~)~~ and *C. sanguinea* ~~(~~L.~~)~~, *C. foemina* ~~(~~Mill.~~)~~ and *C. racemose* ~~(~~Lam~~.)~~. Taking into consideration the earlier suggestions of Wangerin (1910), Schulz (2012) proposed the rank of the subspecies within *Cornus alba* as follows: *C. alba* L. subsp. *alba* and *C. alba* subsp. *stolonifera* ~~(~~Michx.~~)~~ ~~Little l~~Later Zieliński et al. (2014) summarized and described the history of *C. alba* and *C. sericea* taxonomy. The authors discussed the problems with the identification of these very similar and closely related species and agreed that the broad species concept of *C. alba* s.l. (including *C. sericea*) was most reliable. In justification of such position Zieliński et al. (2014) stressed also the possibility of the proper identification of the wild plants both in the generative and in the vegetative state as well as the easier and ~~not~~ less controversial classification of cultivars of uncertain origin.

Woźnicka et al. (2015), who described recently the qualitative and quantitative differences in the morphology of endocarps of 15 *Cornus* species from the BW group, proved the taxonomic and systematic importance of the endocarp morphology. However, the authors found some difficulties in sufficient enough explanation of the species status between very closely related taxa, as some of the observed morphological differences overlapped or were too subtle to distinguish particular species from the examined group. In the published dichotomous key based on the morphology of endocarps the authors adopted the new taxonomic treatments of very closely related species proposed by Schulz (2012). They also discussed ~~also~~ the taxonomic importance and systematic implications of the obtained results in a phylogenetic framework, and the partial congruence between the observed morphological differentiation of endocarps and a currently available species phylogeny within the BW clade of the genus *Cornus* was found.

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Apart from the modern dogwoods, the well preserved fruits and woody endocarps of the extant representatives of the Cornaceae family, and the whole Cornales clade, are by far the most taxonomically informative fossils which facilitate the better understanding of the initial phylogenetic diversification of Cornales (Eyde 1987, 1988; Manchester et al. 2010; Atkinson et al. 2016; Stockey et al. 2016; Atkinson et al. 2017). The present study is a continuation in a series of papers that started with Woźnicka et al. (2015) who examined the diversity within endocarp morphology among selected species from the blue- and white fruited dogwoods. In the present work we focused on the fruit stones morphology of 22 species representing the whole genus *Cornus* with the use of the same methods as Woźnicka et al. (2015). Additionally, our present approach was extended to analyze the anatomical structure of the endocarps as well as their sculpture. We provided a compilation of the earlier and presently obtained results to discuss our findings in a broader context regarding the phylogenetic relationships within the entire *Cornus* genus. We hypothesized that the grouping of the dogwoods studied based on the morphology and anatomy of the endocarps would be the same as the dogwoods phylogenetic relationships (BW(CC(DW-BB))) based on the combined molecular and morphological evidence described by Xiang et al. (2006).

Materials & Methods

Materials

Endocarps of 22 *Cornus* species representing four morphological groups (BW CC, BB and DW) were analyzed according to their morphology and anatomy (Table 1). Examination of the endocarp morphology was partly based on our earlier work, as we have ~~implemented~~ added to our analyses the results obtained by Woźnicka et al. (2015) who examined 2812 stones collected from 185 specimens representing 15 dogwood species of the blue- or white-fruited dogwoods from the BW group. Here we examined, with the same methods as Woźnicka et al. (2015), 1034 stones collected from 69 specimens representing seven red-fruited *Cornus* species of the CC, BB and DW groups. The combined data set of the endocarp morphology of 22 dogwood species representing all four groups (BW, CC, BB, DW) was used for the complex analysis based on the results of morphological measurements of 3846 stones collected from 254 specimens.

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In the next step the endocarp anatomy of 22 dogwood species was examined. The endocarps were extracted from the fully developed, ripened fruits. For the origin of endocarps of 15 blue- or white-fruited dogwoods see Woźnicka et al. (2015). The stones of red-fruited *Cornus* species were collected from the specimens growing in 10 Polish and 8 European, Asian or American botanical collections (cultivated materials) (Supplemental File 1, Supplemental File 2) as well as from 12 herbarium collections: BM, BG1, G, GH, H, K, KOR, KRAM, L, POZ, S, TRN (Thiers, *Index Herbariorum*) (herbal materials) (Supplemental File 3).

Plant material was collected between 2009 and 2012, from July to October, during the fruiting period of individual species. On collection the samples were prepared for morphological measurements with the same methods as described by Woźnicka et al. (2015). The original nomenclature of the examined dogwoods was considered appropriate and reliable. However, in order to increase the confidence of the study approx. 70% of the specimens were verified according to their morphology (Supplemental File 2, Supplemental File 3). The nomenclature of the examined species follows the The Plant List 1.1 (2013). The specimens and the fruit materials are deposited in the herbarium of the Department of Botany (POZNB), Poznań University of Life Sciences, Poland.

Plant measurements

- **endocarp morphology**

Seventeen morphological traits (8 qualitative and 9 quantitative) of the woody stones were analyzed (Table 2). Each species from CC, BB and DW group was represented by 6-12 specimens derived either from the herbal or cultivated collections. Within these collected materials in average 6-16 and 23 endocarps per specimen, respectively, were evaluated depending on their availability. The terminology used to describe 17 morphological traits of endocarps followed Woźnicka et al. 2015 (see Table 2; Fig. 3). Since few of the qualitative traits (ASH, BSH, SSH, VBP) were described in the different variants, the percentage share of the particular variant of the same trait was determined for each species under study. If the share of a given trait variant was greater than or equal 90%, the trait was regarded as typical for the species.

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- **endocarp anatomy**

Within 22 dogwood species under study 317 stones were examined anatomically. For each species 14-15 stones collected from the specimens examined within the particular species were tested. Four quantitative traits of the anatomical structure of endocarps were analyzed on the cross-sections made at half-length of the stone (Table 3, Fig. 1). The longitudinal sections of the stones were also made for SEM examination. The endocarps were sectioned with a Leica CM18050 Cryostat, in a cryochamber at -15°C. The ImageJ application (Rasband 1997-2018) was used for measurements. A Zeiss Axio Scope A1 stereoscopic microscope was used for photographic documentation.

Data analysis

- **endocarp morphology**

The comparative analyses of the obtained results were made at the three following levels: groups, subgenera and species.

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The statistical analyses were based on the average values of the measurements of 17 morphological traits obtained from 254 specimens taken into consideration in the present work. The quantitative traits of the stones were transformed logarithmically to obtain a normal or close to normal data distribution for statistical purposes (Sárnal et al. 1999; Howell 2007; Tabachnick & Fidell 2007).

The aim of the principal component analysis (PCA) was to estimate the diversity of specimens in relation to the quantitative morphological traits, to identify the main trends in the diversity of the set of specimens and to select the quantitative traits that were the most closely related with the observed gradient of diversity. First, the data were standardised due to the wide range of values. The calculations were based on the trait correlation matrix and varimax rotation. The Kaiser criterion was used to select the principal components (V) that significantly explained the variability of the set. Therefore, the principal components whose eigenvalue exceeded or was close to 1.0 were left. The PCA enabled the identification of the traits that were strongly correlated ($r \geq 0.60$) with previously selected principal components.

The multivariate analyses of variance (MANOVAs) based on the three PC scores was carried out to determine whether a separation of endocarps in terms of their belonging to clades, subgenera and species was statistically significant. To evaluate this differentiation, each PC axis was compared by analyses of variance (ANOVAs) with the Bonferroni correction.

At the next stage of statistical analysis the specimens from particular clades, subgenera and species were assessed for significant differences in the morphological traits selected by means of the PCA method. The homogeneity of variances of features was checked with Levene's test. As some of the traits did not meet the assumption of the homogeneity of variance, the significance of differences was verified by means of one-way ANOVA with the Welch correction and with Dunnett's T3 post-hoc test.

The statistical analyses of the eight qualitative traits were based on individual measurements of the traits of the stones (3846 specimens?). Chi-squared tests of independence were used to test relation between a specific qualitative trait and the belonging of the dogwood specimens to particular groups, subgenera and species. The Yates correction was used in the analyses with one degree of freedom ($df = 1$). This correction made the discrete distribution of the trait were better approximated by the continuous chi-squared distribution (ACZEL 2000). When the expected value was less than 5, the closest variants of the feature were combined into one class (in tests with $df>1$), or the Fisher's exact test was used (in tests with $df = 1$).

Hierarchical cluster analysis was applied to ~~check~~ determine whether all the qualitative and quantitative morphological features of the endocarps reflected the current relations within the

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Cornus genus resulting from the earlier studies based on the analyses of other morphological features and molecular evidence. The data used in the analysis were arithmetic means of the traits. The mean values of the quantitative morphological traits were calculated for the logarithmic values. The Manhattan distance was used to calculate the similarity matrix between the samples. The unweighted pair group method with arithmetic mean (UPGMA) was used to construct the dendrogram.

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Most of statistical analyses were performed using the program STATISTICA 11 (StatSoft, Poland). Analyses of variance with Welch's correction and Dunnett's T3 tests were calculated with IBM SPPS 21.0 software (SPSS Inc., Chicago, IL, USA). The Bonferroni correction was found at http://quantitativeskills.com/sisa/calculations/bonfer.htm. The chi-squared tests of independence with df>1were performed at http://www.quantpsy.org/chisq/chisq.htm.

- **endocarp anatomy**

Discriminant function analysis (DFA) was used to check which of the species under study were the most strongly discriminated on the basis of three anatomical traits of the endocarps, that occurred in all groups studied (GVT, SMW and WTP). Factor structure coefficients, i.e. simple correlations between two variables, were used to assess the links between the traits and discriminatory axes. Each trait was tested statistically to verify the initial assessment of diversity obtained through the DFA. Some of the data did not have a normal distribution after the logarithmic transformation~~, either~~. Therefore, nonparametric tests were used on non-transformed data. The +-Wallis *H* test was used for three combinations of categories (22 species, 4 groups, 6 subgenera). Numerous Mann-Whitney U tests were done to compare consecutive pairs within each category.

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Statistical analyses were performed using STATISTICA 11 (StatSoft, Poland).

SEM analysis

Endocarp cross- and longitudinal sections were made at half of the endocarps’ length or ~~thickness~~width, respectively. The whole endocarps and their cuttings were ~~covered with gold~~gold coated and examined with a Zeiss EVO 40 electron microscope. The following surface micromorphological traits were examined: cellular pattern, cell outline (tetragonal, polygonal, rounded, irregular), anticlinal walls (straight, sinuate), relief of the anticlinal cell boundary (raised, channeled), curvature of the outer periclinal cell wall (concave, convex, flat). According to the anatomy the following features were examined: the outer endocarp (OE) (absent, present), the type of sclereids in the OE (isodiametric, elongated), crystals in the OE (absent, present), the

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inner endocarp (IE) (absent, present), the transitional sclereids zone (TS) (absent, present/the number of cell layers), the structure of endocarp septum (S) (solid, openwork, partly openwork), crystals in S (absent, present).

Results

Endocarp morphology

- **quantitative traits**

The diversity of the quantitative morphological traits was initially tested by means of principal component analysis (PCA). The first three principal components together explained 77.95% of the traits (V1 = 35.08%; V2 = 31.98%; V3 = 14.74%). The scatter diagram of the first two components showed a clear cluster of specimens belonging to the same species as well as those belonging to the same subgenus and group (Fig. 2). The first principal component mostly separated the DW and CC groups (Table 4). Four traits were negatively correlated with the first principal component: the endocarp thickness (ST), the apical cavity width (ACW), the apical cavity length (ACL) and the endocarp width (SW) (Table 5). The second component separates the mentioned above groups from the species included in the BW group. The following traits were negatively correlated with the second component: the endocarp length (SL) and the ratio of the endocarp length to width (SL/SW). The following traits were positively correlated: the share of bifurcated vascular bundles (FV%) and the number of vascular bundles on the endocarp surface (VN). The third principal component noticeably separated groups BB and DW (Table 4). The following features were positively correlated: the apical cavity width (ACW) and the apical cavity length (ACL). The endocarp width-to-thickness ratio (SW/ST) was not significantly correlated with any of the principal components (Table 5). Therefore, this trait was not included in further analyses.

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Multivariate analyses of variance (MANOVAs) based on PC scores (V1-V3) with the taxonomic affiliations as a predictor variables confirmed that tested groups, subgenera and species differed in the quantitative characteristics of endocarps (groups: Wilks' lambda = 0.03, $F_{(3,9)}$ = 203.67, P<0.0001; subgenera: Wilks' lambda = 0.00, $F_{(3,15)}$ = 311.6, P<0.0001; species: Wilks' lambda = 0.00, $F_{(3,63)}$ = 113.05, P<0.0001). The statistically significant separation within taxonomic units occurred along all tree axes (ANOVAs for groups: $F_{(3,250)}$ = 69.37, 555.46 and 32.94, respectively, P<0.0001; ANOVAs for subgenera: $F_{(5,248)}$ = 262.58, 479,63 and 122.72, P<0.0001; ANOVAs for species: $F_{(21,232)}$ = 150.51, 164.95 and 51.62, P<0.0001).

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The differentiation of the particular quantitative traits of the endocarps between individual groups, subgenera and species ~~was~~ is shown in Figs. 3-5.

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The endocarp length (SL) and thickness (ST) were the best diagnostic traits as they significantly differentiated each group from the others (Figs. 3A, 3D; post-hoc tests, p <0.05). The longest and thickest endocarps were found in the specimens representing CC group, whereas the shortest and thinnest were found in the species from the DW group. The endocarp width (SW) and length-to-width ratio (SL/SW) also assumed the highest values in CC group and the smallest values in DW group. However, they did not differ significantly between the BW and BB groups (Figs. 3G, 4A). The highest number of vascular bundles (VN) were present on endocarps of species from BW group. This trait differed significantly BW species from CC, BB and DW species while BB and DW species did not differ each other according to VN (Fig. 4D).

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The endocarp length (SL) revealed also significant separation between subg. *Mesomora* and subg. *Kraniopsis* (BW) as well as between subg. *Cynoxylon* and subg. *Syncarpea* (BB) (Fig. 3B). Subgenera *Mesomora* and *Kraniopsis* differed also significantly according to endocarp thickness (ST) and width (SW) (Figs. 3E, 3H), but their endocarps were characterised by similar number of vascular bundles (VN) (Fig. 4E). The endocarp length/width ratio (SL/SW) separated significantly subg. *Cynoxylon* and subg. *Syncarpea* (BB) (Fig. 4B).

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Most of the species clustered in the same groups and subgenera exhibited great similarity in the quantitative traits under analysis, but with some exceptions. The endocarp length (SL) and the SL/SW ratio of *C. kousa* (*Syncarpea*) were significantly smaller comparing to *C. florida* and *C. nuttalli* (*Cynoxylon*) (BB) (Figs. 3C, 4C; species no. 18-20). The endocarps of *C. alba*, *C. bretschneideri*, *C. occidentalis* and *C. sericea* (BW) were significantly thinner (ST) in comparison with all other species from that group (Fig. 3F; species no. 1, 5, 11, 14), while *C. canadensis* endocarps were significantly narrower (SW) than endocarps of *C. suecica* (DW) (Fig. 3I, species no. 21- 22).

~~The~~ An apical cavity was observed on stones of 12 among 22 species under study, but for most of these species it occurred sporadically (*C. australis, C. bretschneideri, C. drummondii, C. foemina, C. macrophylla, C. obliqua, C. sanguinea, C. walteri,* and *C. officinalis*). Thus, the apical cavity length and width (ACL, ACW) were analysed in detail only for *C. alternifolia, C. controversa* (*Mesomora*; BW) and *C. mas* (CC). Both species of subg. *Mesomora* differed from each other in ACL and ACV ~~each other~~, furthermore their cavities were significantly longer and wider comparing to *C. mas* (Fig. 5A, B).

– **qualitative traits**

The results of the chi-square test of independence showed significant differences between examined dogwood groups, subgenera and species in all the qualitative morphological traits of the endocarps (Table 6).

To find possible association between the qualitative traits and group, subgenus or species assignment the Chi square test was performed based on completed data set of 22 *Cornus* species. Among all examined characters the endocarp shape (SSH), the apical shape (ASH), the position of vascular bundles on the endocarp surface (VBP) and the presence of a distinctive furrow (DF) (Figs. 6A, D,7D, J) enabled differentiation of each group from the others (chi-square tests; p<0.05). The basal shape of the endocarp (BSH), the presence of the apical cavity (ACP) and the presence of bifurcated vascular bundles (FV) differed examined groups one another, with an exception of BB and DW (Figs. 6G, 7A, G). The endocarp surface (SSF) enabled differentiation of all the groups except the CC and DW (Fig. 6J).

The qualitative traits of the endocarps were the least differentiated in DW group, where six typical traits of endocarps were identified: rounded base (BSH), smooth surface (SSF), ~~luck~~ lack of the apical cavity (ACP), flat vascular bundles (VBP), not forked vascular bundles (FV), absence of the distinctive furrow (DF). In CC group four typical traits of endocarp were observed: spherical=globose endocarps (SSH), rounded or flat apex (ASH), smooth endocarps (SSF), presence of the distinctive furrow (DF). In turn the individual species in that group were strongly differentiated according to the trait FV as 100% of *C. mas* fruit stones had not forked vascular bundles and in turn 58% of *C. officinalis* stones had at least one forked vascular bundle (Fig. 7I).

In BB group just the three following endocarp traits were found to be typical for all species: rounded or flat apex (ASH), luck of the apical cavity (ACP), no forked vascular bundles (FV). However, four other traits (SSH, BSH, SSF, VBP) separated significantly subgenera *Cynoxylon* and *Syncarpea* in BB group (Figs. 6B, H, K, 7E). Additionally, the irregular endocarp shape and rough surface (SSH, SSF) enabled fairly good differentiation of *C. kousa* from *C. florida* and *C. nuttallii* (BB) (Fig. 6C, L).

The endocarps of species from BW were the most heterogeneous, and no typical traits in that group were found. However, all of the examined characters, excluding SSH (Fig. 6J), differed significantly subg. *Mesomora* and subg. *Kraniopsis* each another. Analyses on the species level showed that most of the examined traits enabled differentiation of particular BW species from some other species in that group.

None of the traits under study enabled differentiation of all subgenera from each other. However, among the examined endocarps of *Cornus*, *Mesomora*, *Cynoxylon* and *Syncarpea* specimens the stones with rounded or truncate apex (ASH) were predominant (Fig. 6E). The rounded base

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(BSH) was observed on 69-93% endocarps of *Kraniopsis, Mesomora, Cornus, Syncarpea* and *Arctocrania* specimens, while 74% fruit stones of *Cynoxylon* specimens had a wedge-shaped base (Fig. 6H). Among all subgenera under study the endocarp surface (SSF) was mostly smooth (78-100%), while the subg. *Syncarpea* was the only one in which 86% of the examined endocarps had rough surface (Fig. 6K). The vascular bundles (VBP) on all endocarps of *Mesomora* and *Syncarpea* specimens were sunken while they were flat in most of the fruit stones of *Kraniopsis* and *Arctocrania* specimens (83-100%) (Fig. 7E). Not forked vascular bundles (FV) were often or always observed on the endocarps of *Cornu*~~,s ~~s, *Cynoxylon*, *Syncarpea* and *Arctocrania* specimens (71-100%), while on endocarps of *Kraniopsis* and *Mesomora* individuals the forked vascular bundles were predominant (78-100%) (Fig. 7H). The absence or the low percentage of fruit stones with the distinctive furrow (DF) were observed for *Mesomora, Cornus* and *Arctocrania* specimens, and in turn the presence of such furrow was predominant among the endocarps of the *Cynoxylon* and *Syncarpea* representatives (86-91%) (Fig. 7K).

Commented [MZE33]: Why is this a range?

Commented [MZE34]: Why a range? Is this within an individual? Species?

Commented [MZE35]: specimens

Commented [MZE36]: So neither trait is uniform within clades, subgenera, or species??????

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- **qualitative and quantitative traits**

Hierarchical cluster analysis performed for the qualitative and quantitative traits of the endocarps (Fig. 8) revealed the highest morphological similarity of endocarps between the DW and BB groups which form a clear cluster. In the dendrogram, the CC and BW groups do not cluster, but form separate branches.

Endocarp anatomy

The dogwood species under study differed in the anatomical traits of the stones. Three of the examined traits had significant discriminatory power (Table 7). There was a strong positive correlation between the valve thickness (GVT) and the first discriminant function. Similarly positive, but less strong correlation was found for the next two traits (SMW, WTP). The first discriminant function clearly distinguished *C. mas* from the other species. The second discriminant function separated *C. officinalis* from the other species. The WTP coefficient was strongly negatively correlated with this function. The other two traits (GVT, SMW) were less strongly correlated. (Table 7, Fig. 9). The other species belonged to the same point cloud in the scatter diagram, but the specimens of the same species formed very clear clusters within the cloud. Moreover, the species from the DW and BB groups occupied a separate space in the cloud along the first discriminant function. The species from the BW group appeared in different areas of the cloud (Fig. 9).

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The Kruskal-Wallis tests (*H)* confirmed that GVT, SMW and WTP were significantly different within each analysed level: group, subgenus and species (Table 8).

The valve thickness (GVT) and the WTP coefficient significantly differentiated the stones of specimens from all four groups (Figs. 10A, G). The highest values of both traits were noted in the CC, whereas the lowest were in the DW. The third trait, i.e. the septum width (SMW), enabled only the distinction between the BW and BB from DW (Fig. 10D).

Subgenera from the BW group differed significantly according to the examined anatomical traits. The endocarps of *Mesomora* had a significantly thicker valve, a wider septum and a lower WTP ratio than *Kraniopsis* (Fig. 10B, E, H). In the BB group the *Cynoxylon* species had stones with a wider septum than *Syncarpea* representatives (Fig. 10E).

Among the particular species examined the anatomical traits differentiated clearly *C. mas* and *C. officinalis* according to the presence and absence of the septum (it was absent *C. officinalis*) and the WTP coefficient which was significantly higher in *C. officinalis* (Fig. 10F, I: species no. 16, 17). The last mentioned feature differed also *C. canadensi*s and *C. suecica* (Fig. 10I, species no. 21, 22).

The fourth examined character, the presence of secretory cavities in the endocarp wall was a species-specific trait. It occurred only in *C. mas* and *C. officinalis* fruit stones. The average number of cavities (DCN) was significantly higher in *C. mas* endocarps (U test: U = 9.5; Z = 4.15; p = 0.000; Fig. 11).

SEM analysis

The endocarp surface micro-ornamentation pattern was reticulate for most of the species under study. Surface cells were similar in size for the particular species, mostly quadrangular to polygonal, sometimes rounded or irregular in their outline (Figs. 12-14). The biggest cells were observed on *C. mas*, *C. officinalis* and *C.* ~~*Canadensis*~~ *canadensis* endocarps (Fig. 14B, D, H). For some species like *C. sericea*, *C. sanguinea* or *C. amomum* the cellular pattern was less distinct (Figs. 12D, N, 13J). The anticlinal cell walls were straight and raised for most of the species, ~~and they~~but were wavy on *C. racemosa* and *C. controversa* endocarps (Fig. 13B, P). In ~~few species such as~~ *C. alba, C. occidentalis, C. drummondii, C. bretschneideri*, *C. racemosa*, *C. foemina* the specific constrictions and spherical thickenings were present on the anticlinal cell walls (Figs. 12B, F, J, L, 13B, F). The outer periclinal cell walls were either flat or concave generally without the secondary sculpture (Table 9). The exceptions were the endocarps with the verrucose (*C. sericea C. occidentalis C. drummondii*), striate (*C. sanguinea*, *C. amomum*),

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Commented [MZE41]: In different subgenera, yet not discussed.

punctate (*C. australis*) or foveate (*C. kousa*) secondary micro-ornamentation pattern (Figs. 12D, H, J, N, P, 13J, 14N).

The endocarps of most of the examined dogwoods were composed of the isodiametric sclereids with evenly thickened and lignified cell walls with numerous pits. In the structure of the endocarps of *C. walteri* from the BW group and *C. florida*, *C. nuttallii*, *C. kousa* from the BB group the elongated sclereids perpendicular to the stone surface were also observed (Table 10, Figs. 16G, 17I, K, M).

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In the endocarps of the species from CC group (*C. mas*, *C. officinalis*) the outer (OE) and the inner endocarp (IE) were distinguished. The OE was ~~built~~ composed mostly of isodiametric sclereids and the elongated fibrous cells which were surrounding the secretory cavities. The IE, interpreted also as the internal epidermis system (sensu Kaniewski & Hausbrandt 1968), was composed of approx. six layers of the parallel fibers with evenly thickened cell walls and numerous pits, and it was surrounding the seed chambers (Fig. 17A, C).

It was difficult to distinguish the inner endocarp (IE) in most of the other species from the BB, BW and DW groups. However, in endocarps of two species, *C. sericea* and *C. walteri* the groups of cells with slightly thinner cell walls, comparing to the whole endocarp, and surrounding the seed chambers were observed (Figs. 15D, 16G). Additionally, in the OE of four BW species (*C. sericea, C. drummondii, C. australis, C. alternifolia*) the crystals were observed (Table 10, Figs. 15E, K, U, 16M).

Straight under the surface of the endocarp the transitional sclereids zone (TS) was present in most of the species. It was composed of 5(3)-1 layers of thin-walled (in comparison with the rest of the endocarp) cells. These cells were slightly elongated and flattened in the plane parallel to the stone surface (Table 10, Figs. 15A, D, E, G, J, M, P, 16A, C, I, M, 17D, I).

Isodiametric and elongated sclereids with differently thickened cell walls appeared also in the septum which was present in the stones of all examined dogwoods except *C. officinalis.* The differences in the anatomical structure of the septum, depending on the degree of the cell walls thickness, were observed on the cross- and longitudinal sections of the stones. Three types of ~~the septum~~septa were distinguished according to the cell wall thickness: (1) solid septum built of the cells with strongly thickened cell walls (Figs. 15L, 16B, D, P, 17B, L), (2) partly openwork septum built in its outer part of the cells with strongly thickened cell walls, and in the middle of the cells with thinner cell walls (Figs. 15B, F, N, Q, T, 16F, H, N, 17F, H, N), (3) openwork septum entirely composed of the cells with slightly thickened walls and with large cell cavities (Figs. 15H, 16J, L, 17J). In most of the species from BW, BB and DW groups crystals were observed in the septum (Table 10; Figs. 15C, I, O, R, U, 16B, D, F, H, J, P, 17H, J, L, N). A little different septum structure was observed in *C. mas*, a representative of the CC group. The septum was built of narrow fibers elongated parallel to the shorter endocarp axis. Additionally, small secretory cavities were present in the septum (Fig. 17B).

Discussion

Current research has shown that the morphology and anatomy of *Cornus* endocarps are taxonomically and systematically important. The results obtained are related to recent studies on the taxonomy of the genus *Cornus* (Schulz 2011, 2012; Zieliński et al. 2014; Woźnicka et al. 2015). The examined morphological and anatomical features of the fruit stones either significantly ~~differed~~ differentiated the particular groups, subgenera and species or showed their close similarities. The set hypothesis that the relationships between dogwood groups BW, CC, DW and BB based on the morphological features of their endocarps exactly reflect their phylogenetic relations BW(CC(DW-BB)) shown by Fan & Xiang (2001) and Xiang et al. (2006) was verified positively. According to the results obtained the dwarf dogwoods (DW) and big bracted dogwoods (BB) were the most similar. The quantitative traits which mostly influenced such grouping were the stone lengths (SL), the endocarp length/width ratio (SL/SW) and the number of vascular bundles on the endocarp surface (VN). Considering these three characters, there were no differences between the SL and SL/SW of endocarps of *Syncarpea* (BB, *C. kousa*) and *Arctocrania* (DW) species, and VN did not differ all of the species one from the other in the DW-BB groups (Figs. 3B, 4B, E). Moreover, the features SL and SL/SW differed significantly endocarps of *Cornus* (CC) and the BB species (Figs. 3A, B, 4A, B). The results described support the results of Xiang et al. (2006) who showed the closest phylogenetic relations between DW-BB.

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Features SL and VN were considered taxonomically significant also because they allowed to distinguish almost all BW species from red-fruited species (CC, BB, DW). With regard to SL the only exceptions were the following pairs of species: *C. bretschneideri-C. canadensis*, *C. bretschneideri-C. suecica*, *C. foemina-C. suecica*, *C. macrophylla-C. suecica* (Fig. 3C). Moreover, the same feature differentiated significantly approx. 76% of the red-fruited dogwoods (CC, BB, DW). With reference to VN it was shown that the specimens representing *Kraniopsis-Mesomora* complex (BW) had significantly more vascular bundles than the specimens representing the subgenera of red-fruited dogwoods (*Cornus*, *Cynoxylon*, *Syncarpea, Arctocrania*) (Fig. 4E, F).

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Considering other studied quantitative features such as the endocarp width (SW) and its thickness (ST) the results were not so clear. The SW analysed at the subgenus level showed that *Mesomora*

(BW) and *Cornus* (CC) species did not differ in this trait which may highlight the close relationship between BW and CC found by Xiang et al. (2006). The stone thickness (ST) did not differ significantly groups CC (subgen. *Cornus*) and BB (subgenera *Cynoxylon* and *Syncarpea*).

Among the qualitative characters the stone shape (SSH), the vascular bundles position (VBP), the apex outline (ASH), the base outline (BSH) and the presence of a furrow on the side walls of the stone (DF) were the most important taxonomic traits.

The differentiation in the stone shape (SSH), which determines the visual assessment of the endocarp shape, did not allow the classification of the studied species to particular morphological groups, but allowed the selection of the species with flattened, intermediate, round, and irregular stones. The flattened fruit stones were most often typical for species from BW and DW groups, while not flattened endocarps were typical for CC group. The irregular stones were very rare and they allowed the distinction of *C. kousa* from the rest of the examined dogwoods.

The stone shape (SSH) together with the length-to-width ratio (SL/SW) and the degree of flattening of the stone, i.e. its thickness (ST) were often used in the past to analyse the diversity of the endocarps of very similar and phylogenetically closely related species e.g. *C. alba*, *C. sericea* and *C. occidentalis* from the BW group (Fosberg 1942; Szafer & Pawłowski 1959; Rehder 1967; Bean 1976; Seneta 1994; Rutkowski 2004; Xiang et al. 2006; Schulz 2012; Zieliński et al. 2014; Woźnicka et al. 2015). However, the results of recent studies concerning dogwoods morphology (Schulz 2012; Zieliński et al. 2014; Woźnicka et al. 2015) as well as the presented results have shown that the ranges of these traits mostly overlapped and thus they did not explain sufficiently the species status of these closely related taxa. In turn, the qualitative feature indicating a close similarity of *C. sericea, C. alba* and *C. occidentalis* (BW) ~~there~~ was the presence of the distinctive furrow running longitudinally on the lateral faces of an endocarp (DF). Considering these controversial dogwoods Schulz (2012) proposed the new taxonomic approach of *C. alba* s.l. with subsp. *alba* and subsp. *stolonifera,* and he treated *C. occidentalis* at the rank of variety below subspecies *stolonifera* of *C. alba*. This new taxonomic approach was then discussed and accepted by Zieliński et al. (2014), Woźnicka et al. (2015) and supported by the obtained result.

With reference to the other closely related species from the subgenus *Kraniopsis* (BW) like e.g. North American species *C. amomum* and *C. obliqua*, *C. foemina* and *C. racemosa* or European species C. *australis* and *C. sanguinea*, some of the obtained results like e.g. no significant differentiation in the endocarp apical shape (ASH) and its basal shape (BSH) support their close relationships. These results together with the other morphological similarities support Schulz

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(2012), who suggested that *C. australis* and *C. obliqua* should be classified as subspecies within *C. sanguinea* and *C. amomum*, respectively. The obtained results complement also the earlier findings describing morphological similarity of these species (Wilson 1964; Bean 1976; ~~BALL~~ Ball 2005; Xiang et al. 2006; Schulz 2012; Woźnicka et al. 2015).

Considering the red-fruited dogwoods (CC, BB, DW) the rounded BSH observed in big bracted (BB) and dwarf dogwoods (DW) indicated that these two groups are very similar, which once again supports their close phylogenetic relationship shown by Xiang et al. (2006). The only exception among BB-DW species was *C. florida* with a wedge-shaped endocarp base (Fig. 6H, I). In turn, the noticeable variation in the form of ASH allowed the distinction of almost all of the pairs of the species within the BB, CC and DW groups. There were only two exceptions of the species with the same type of rounded or flat apex like *C. mas*-*C. officinalis* and *C. florida*-*C. kousa*, respectively. According to Bojnanský & Fargašová (2007), *C. mas* stones have a pointed apex, whereas *C. officinalis* stones have a blunt apex. It is most likely that the differences between the results of our study and the cited ~~up~~ data were caused by the inverse description of the stone apex and the stone base by Bojnanský & Fargašová (2007).

The vascular bundle position on the endocarp surface (VBP) was a feature that distinguished *Kraniopsis* and *Mesomora* species which confirmed the results of Woźnicka et al. (2015). The red-fruited dogwoods also differed each other to the vascular bundle position on the endocarp surface. In turn, the feature describing presence or absence of the forked vascular bundles on the endocarp surface (FV) showed distinct similarity between two groups of red-fruited dogwoods (BB-DW) as 100% of the species from these groups had not-forked vascular bundles. This result supports a close phylogenetic relationship of these dogwoods shown by Xiang et al. (2006).

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Another character considered to have a significant impact on relationships of the studied dogwoods on the basis of similarities in the morphological structure of endocarp was the presence of the distinctive furrow running longitudinally on the lateral faces of an endocarp (DF). The DF was present on endocarps of some BW species which was consistent with results of Fosberg 1942, Schulz 2012 and Woźnicka 2015. In reference to the red-fruited dogwoods the stones with furrows on sides were predominant among the *C. florida*, *C. kousa* and *C. nuttallii* endocarps (BB). Such results indicate that the presence of DF on the endocarps of most of big-bracted species may be helpful in distinguishing them from the other red-fruited dogwoods.

Among other examined qualitative traits of endocarps the presence or absence of the apical cavity (ACP) as well as its size (ACL, ACW) were taxonomically important features. They allowed for significant separation of two alternate leaved dogwoods *C. alternifolia* and *C. controversa* (subgen. *Mesomora*, BW) which was in line with earlier results by other authors (Eyde 1988; Xiang & Boufford 2005; Schulz 2011, 2012; Woźnicka et al. 2015). Our results confirmed also the presence of much smaller but noticeable apical cavity on the stones of several other *Kraniopsis* species which was described earlier by Woźnicka et al. (2015). Apart from the BW group, it was also shown that within red-fruited dogwoods (BB, CC, DW) the apical cavity was present on the stones of both species from the CC group. It was observed on almost all *C. mas* stones and on 16% of *C. officinalis* stones. We also observed a shallow depression on *C. volkensii* stones (unpublished data). Eyde (1988) did not clearly state whether the cavity could be found in these species, nor what form it had. As far as the species from the group of large-fruited edible dogwoods (CC) were concerned, the author described only the apical cavity which was wide and shallow in *C. volkensii*, whereas in *C. chinensis* it had the form of a V-shaped incision and it was absent on *C. sessilis* endocarps. According to Manchester et al. (2010) the apical cavity in *C. mas* and *C. officinalis* was inconspicuous, while Atkinson et al. (2016) confirmed the presence of the apical cavity on stones of *C. mas*, *C. officinalis* and *C. volkensii* together with few other living (*C. chinensis*, *C. eydeana*, *C. sessilis*) and extant species (*C. piggae*, *C. ettingshausenii*, *C. multilocularis*). According to the results obtained ACP on *C. mas* stones was significantly smaller than on endocarps of *C. alternifolia* and *C. controversa* (Fig. 5).

The extensive and detailed study of Eyde (1988) also includes an analysis of the variation in the surface of the stone. The author emphasized that the stones of the herbaceous species (DW) were easy to distinguish because they were smoother than the stones in the BW group. Similar results were obtained by Woźnicka et al. (2015) and they were confirmed in the present study. Conducted research also showed that the number of the vascular bundles (VN) is an taxonomically significant feature. It allowed to distinguish BW species from DW species. There were usually 7-8 vascular bundles on the stones of the blue- or white fruited species, whereas the stones of the dwarf dogwoods had two shorter bundles, one on the opposite walls of the stone. These differences may have indirectly resulted in the stones of the DW species being considered smoother. It was shown that the surface of the stone was smooth also in the other red-fruited dogwoods (CC, BB) with the exception of *C. kousa* (BB, *Syncarpea*) (Fig. 6L). These results are in agreement with Eyde (1988: Fig. 14 h, i; p. 276) who has shown that *C. kousa* stones were irregular and rough. Considering all three groups of the red-fruited dogwoods the average number

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of the vascular bundles on their endocarps (VN) differed significantly CC species with BB and DW species, with the exception of *C. nuttallii* (*Cynoxylon*) (Fig. 4D, E, F).

The hierarchical cluster analysis based on the combined data of the qualitative and quantitative features showed the greatest similarity between BB and DW groups (Fig. 8). Such results are consistent with the results of phylogenetic analyzes based on the combined morphological and molecular data using sequences of *rbc*L, *mat*K, cpDNA, 26S rDNA and ITS (Xiang et al. 1993; 1996, 1998; Fan & Xiang 2001; Xiang et al. 2006). It follows that the results based on the more comprehensive datasets are more reliable, especially when molecular data and a wide range of morphological data are included simultaneously.

The results obtained concerning the anatomical structure of endocarps of *C. mas*, *C. officinalis* and *C. volkensii* (unpublished data) and the available literature showed that the presence of the numerous secretory cavities in both the stone walls and the septum are typical for CC species (Kaniewski & Hausbrandt 1968; Eyde 1988; Manchester et al. 2010). According to Manchester et al. (2010), this feature was taxonomically important because it allowed for the correct identification and classification of fossil endocarps of these species. Recently Atkinson et al. (2016) examined the anatomy of permineralized fruits from the Campanian of Vancouver Island and showed that the presence of secretory cavities in the woody endocarps indicated that they were assignable to the *Cornus* subg. *Cornus*. Manchester et al. (2010) analyzed also the germinating valve thickness (GVT) and the WTP ratio (the endocarp wall thickness divided by the endocarp diameter). The author found that these parameters as very useful in comparative analyses of endocarps of living dogwoods and fossil materials representing the former taxa of the *Cornus* subgenus (CC). The same two features were also included in our research and the obtained results were comparable with regard to *C. mas* and *C. officinalis* stones. In *C. mas* endocarps the WTP was 21 and the GVT was 1.2 mm while in *C. officinalis* endocarps the WTP ratio was 28 and the GVT was 1.1 mm. According to Manchester et al. (2010) the analogous data were 20 and 0.8-1.2 mm for *C. mas* stones and 22-34 and 0.9 mm for *C. officinalis* endocarps.

Moreover, our research confirmed that the endocarp tissue of CC species consists of isodiametric and elongated sclereids. Takahashi et al. (2003) named them rise grain shaped sclereids. Additionally, in line with the previous results the outer and the inner regions in endocarp were distinguished (Manchester et al. 2010; Morozowska et al. 2013; Atkinson et al. 2016; Stockey et al. 2016; Atkinson et al. 2017). With reference to BW, DW, BB dogwoods the sclerenchyma cells comprising the wall and septa were also isodiametric to somewhat elongate.

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Apart from the described findings, several new results were obtained, potentially helpful in taxonomic examination of living and fossil woody fruit stones of different *Cornus* species. Considering the anatomical differentiation of endocarps it was found out that subgenera *Kraniopsis* and *Mesomora* (BW) as well as subgenera *Syncarpea* and *Cynoxylon* (BB) differed significantly according to GVT, SMW, WTP and SMW, respectively (Fig. 10B, E, H). Among red-fruited dogwoods the highest values of GVT and WTP were typical for endocarps of CC species (10A, G) while the narrowest septum (SMW) was typical for endocarps of DW species (Fig. 10D-F), which correlated with their lowest dimensions (SL, ST, SW) (Fig. 3A-I). Besides these differences, comparisons regarding the anatomy and micromorphology of endocarps of closely related species also provided taxonomically significant results. *~~C.~~Cornus alternifolia* and *C. controversa* (BW, subgen. *Kraniopsis* and *Mesomora*, respectively) endocarps differed according to such features as the presence or absence of crystals in the outer endocarp (OE), the number of cell layers in the transition sclereids zone (TS) and the septum structure (S). Moreover, the differences concerned the outline and anticlinal walls of the surface cells (Tables 12, 13). Considering other closely related species such as *C. alba*, *C. sericea* and *C. occidentalis*, *C. amomum* and *C. obliqua*, *C. foemina* and *C. racemosa* or *C. australis* and C. *sanguinea* the results obtained showed that some of them were very similar, while others clearly differed in terms of the examined anatomical and micromorphological features of endocarps (Tables 12, 13). For the first three species the primary sculpture was almost the same, however *C. sericea* and *C. occidentalis* endocarps stood out with the presence of the verrucose secondary sculpture (Fig. 12D, H). *~~C.~~Cornus sericea* was, moreover, the most different species as far as anatomy of endocarp was concerned. The differences included the presence of crystals in OE, absence of crystals in the septum, the presence of IE and the multi-layered zone of transition sclereids. The presence of IE was generally a rare feature in BW species. Besides *C. sericea* it was noticed only in *C. walterii* stones. According to Kaniewski & Hausbrandt (1968) the inner endocarp probably becomes destroyed during the ripening of dogwood fruit. With reference to *C. amomum* and *C. obliqua* no differences were found in anatomy of their endocarps which supports the close phylogenetic relationship of these species shown by Xiang et al. (2006). It should be noted, however, that the presence of the secondary sculpture on the stones of *C. amomum* differentiated this species from *C. obliqua* which may be a taxonomically significant feature (Fig. 13J, L). Another three closely phylogenetically related species *C. foemina*, *C. racemosa* and *C. drummondii* differed in the number of cell layers forming the transition sclereids zone, the type of the septum and the type of the periclinal and anticlinal cell walls (Table 10). The undulate anticlinal cell walls present on *C. racemosa* endocarps were exceptional among the examined dogwoods (Fig. 13B). ~~C.~~Cornus

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controversa was the second species of this kind. The undulate anticlinal cell walls differed that species significantly from *C. alternifolia* (Fig. 13N, P). In relation to *C. australis* and C. *sanguinea* the results obtained showed almost complete resemblance in anatomy and micromorphology of their endocarps. The only difference concerned the presence of crystals in OE in *C. australis* fruit stones (Table 10). Such findings support the close phylogenetic relationship of these species described by Xiang et al. (2006).

Within red-fruited dogwoods *C. mas* and *C. officinalis* were the species with the largest surface cells, and the correlation between their size and the endocarp size was additionally observed.

Similarly large surface cells were found on the endocarps of *C. canadensis*, however no similar correlation was found. In endocarps of the red-fruited dogwoods, as in BW species, no crystals were observed in OE, but their presence was found in the stones of *C. volkensii*, subgen. *Afrocrania* (unpublished data). Such observation suggests that confirming the taxonomic significance of this feature requires examination of endocarps of all CC species.

Conclusions

Our results showed that despite the large diversity in the morphological and anatomical structure of endocarps, selected features may be used to a greater or lesser extent in the taxonomy of the genus *Cornus* ~~genus both~~ for both living and fossil materials. Fruits are by far the most taxonomically informative fossils in Cornales (Eyde 1987, 1988; Atkinson 2016). They are used in analyses of phylogenetic relationships within ~~the~~ *Cornus* ~~genus~~ as well as in examinations concerning timing of the initial diversification and evolution of the family Cornaceae and basal asterid lineage, Cornales (Atkinson 2016). For contemporary species it was ~~proved~~ demonstrated that the morphology and anatomy of endocarp allow for the differentiation of particular species. In some cases only one trait was sufficient to distinguish one taxon from others, e.g. irregular stones found only in *C. kousa* (subgen. *Syncarpea*, BB), or 7-8 vascular bundles running along the entire perimeter of the stones of BW species, and can be considered synapomorphies for these lineages. Some specimens were differentiated on the basis of sets of traits, e.g. much longer than wider stones with a clearly visible valve, often with an apical cavity, which were characteristic of the *Cornus* subgenus. The micromorphology and anatomy of endocarps were also found to be potentially helpful in taxonomy of dogwoods~~,~~; however, apart from the traits of stones, it is also necessary to take other taxonomically important features of dogwoods into account. The microstructure of ~~dogwood~~ Cornus endocarp surface has not been studied ~~so far~~previously, so the presented results are new~~. E~~, whereas earlier ~~and recent~~ studies included the micromorphological structure of leaves of the selected dogwood species to assess the degree of diversity of the traits under analysis and verify their taxonomic and phylogenetic usefulness (Hardin & Murrell 1997; Schulz 2012; Zieliński et al. 2014; Gawrońska et al. 2019). Due to the fact that our findings are new, in order to confirm their taxonomic and systemic importance, further comparative studies based on more broad sampling of the material including the largest possible number of species from particular groups and subgenera are needed.

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Acknowledgements

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