Morphological characteristics as a key attribute for a successful determination of selected Cotoneaster species

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1 Introduction

According to the latest research, the Cotoneaster genus belongs to Rosaceae family, Spiraeoideae subfamily and tribe Pyreae (Potter et al., 2007). Overall, it includes approximately 300 species (Bartish et al., 2001). Many species, included in Cotoneaster genus, are cultivated as ornamental plants (Dickoré and Kasperek, 2010). Latest research also confirmed a presence of phenolic compounds and flavonoids in Cotoneaster integerrimus. Therefore, Cotoneaster integerrimus has a great potential in health promotion. Novel food ingredients and medication could be developed from its twigs and fruit (Uysal et al., 2016; Kicel et al., 2019).

A total of 81 Cotoneaster species were recorded in Europe – 17 native and 64 alien species (Kurtto et al., 2013). The western part of Carpathian Mountains includes 3 native Cotoneaster species – Cotoneaster integrerrimus Medik., Cotoneaster melanocarpus (Bunge) Fisch. et C. A. Mey and Cotoneaster tomentosus Lindl. (Marhold and Hindák 1998). Some authors distinguish Cotoneaster matrensis Domokos from Cotoneaster melanocarpus (Bunge) Fisch. et C. A. Mey. However, in this case, further research is needed (Baranec, 1992). The occurrence of a hybrid species Cotoneaster alaunicus × integerrimus in the area of the Low Tatras Mts., was also recorded (Baranec and Eliáš ml., 2004).

Cotoneaster tomentosus individuals usually grow in the community of Prunion spinosae and Calamagrostion variæ from sub-montane to montane level. Cotoneaster integerrimus individuals grow in the community of Seslerio-Festucion duriusculae and Prunion fruticosae from colline to alpine level. Cotoneaster melanocarpus individuals typically grow in the community of Seslerio-Festucion duriusculae and Prunion spinosae from colline to montane level (Baranec, 1992).

Keywords: pyrenes, pomes, infructescence, Cotoneaster, Western Carpathians

In this paper, morphological features, such as the number of pyrenes in pome and the number of pomes in infructescence, were used for determination of closely related tetraploid Cotoneaster species. Samples were collected from various localities in the Western Carpathians. The collection of samples, designed for counting of pyrenes in pome, included 2353 pomes of >130 individuals. Number of pyrenes in pome ranged from 1 to 5. Statistical analysis revealed a significant difference in pyrenes per pome mean values between C. integerrimus (3.01), C. melanocarpus agg. (2.46; including C. matrensis) and C. tomentosus (3.93). The collection of samples, designed for counting of pomes in infructescence, included 1019 infructescences of 141 individuals. Number of pomes in infructescence ranged from 1 to 5. Statistical analysis also revealed a significant difference in pomes per infructescence mean values between C. integerrimus (1.14) and C. melanocarpus agg. (1.54; including C. matrensis), and between C. integerrimus and C. tomentosus (1.50).

Keywords: pyrenes, pomes, infructescence, Cotoneaster, Western Carpathians

1 Introduction

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Cotoneaster tomentosus individuals usually grow in the community of Prunion spinosae and Calamagrostion variæ from sub-montane to montane level. Cotoneaster integrerrimus individuals grow in the community of Seslerio-Festucion duriusculae and Prunion fruticosae from colline to alpine level. Cotoneaster melanocarpus individuals typically grow in the community of Seslerio-Festucion duriusculae and Prunion spinosae from colline to montane level (Baranec, 1992).

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The classification of taxa included in *Cotoneaster* genus is relatively problematic. Morphological features, such as colour of fruit and bark or shape and size of leaves, are not always reliable attributes. A pome is a type of fruit specific to subfamily formerly known as *Maloideae*. In *Cotoneaster*, the ovary develops into the pyrene (Rohrer, 1991). The total amount of pyrenes in a single pome is variable. The number of pyrenes in pome and the number of pomes in infructescence are the most important morphological features for determination of previously mentioned *Cotoneaster* species. Other features include the presence of trichomes on leaves and pomes, and the colour of pomes (Baranec, 1992). Since we believe, that there is a lack of information and available data from contemporary articles, we decided to prove, whether these morphological characteristics are reliable for identification of selected *Cotoneaster* species. We also wanted to prove, whether it is possible to rely only on these selected characteristics. And last but not least, it should be mentioned, that such a large-scale study in the Western Carpathians has been lacking so far.

2 Material and methods

The majority of collected individuals grew in dry habitat with a permanent sunlight, mainly on grassy steppes or cliff edges. Some of the individuals grew in forest in the community of other shrubs and larger trees. Each population was relatively small and it included 1–5 individuals, rarely 6–10(–15). Pomes and infructescences were collected from various localities in four countries situated in the Western Carpathians – the Czech Republic, Hungary, Poland and Slovakia (Figure 1, 2). Infructescences were calculated in the field and from herbarium specimens stored in the herbarium NI (Herbarium Collection of Slovak University of Agriculture in Nitra). The list of localities selected for counting of pyrenes in pome and for counting of pomes in infructescence is listed in Appendix 1 and 2, respectively. Individuals were assigned to different species by the colour of pomes and the presence of trichomes according to determination key of Baranec (1992).

Samples were divided in two groups, one designed for counting of pyrenes in pome and the other for counting...
of pomes in infructescence. Overall, we collected 3–4 twigs per individual. Herbarium specimens from NI herbarium (Herbarium Collection of Slovak University of Agriculture in Nitra) were also used to increase the total amount of infructescences. The collection of samples, designed for counting of pyrenes in pome, included 2,353 pomes of >130 individuals. The collection of samples, designed for counting of pomes in infructescence, included 1019 infructescences of 141 individuals. The data were collected and further statistically analysed by Microsoft® Excel 2010 and STATISTICA, version 10 (StatSoft, Inc., 2011) data analysis software system (Tukey’s HSD test). Values were calculated as a mean per species.

Herbarium abbreviations are according to Thiers (2019). Nomenclature of flowering plants follows Marhold and Hindák (1998). The map was created by the software QGIS, version 3.2 (QGIS Development Team, 2018) with QuickMapServices plug-in and terrain background layer from Stamen Design with data by OpenStreetMap.

3 Results and discussion
Statistical analysis (Tukey’s HSD test; $\alpha = 0.01$) revealed a significant difference in pyrenes per pome mean values between C. integerrimus (3.01), C. melanocarpus agg. (2.46; including C. matrensis) and C. tomentosus (3.93). These results along with standard deviation are listed in Table 1.

| Species                | Mean per species | Standard deviation |
|------------------------|------------------|--------------------|
| C. integerrimus        | 3.01             | 0.67               |
| C. melanocarpus agg.   | 2.46             | 0.60               |
| C. tomentosus          | 3.93             | 0.73               |
Results, obtained by counting of pyrenes in pome of selected *Cotoneaster* species, compared with other published data, are listed in Table 2.

The majority of *C. integerrimus* pomes had 3 pyrenes (55.90%), 2 pyrenes were present in 21.57% of pomes and 4 pyrenes were present in 22.25% of pomes. Both, 1 and 5 pyrenes in pomes represented only 0.14 % of the collection. Number of pyrenes in *C. melanocarpus* agg. pomes ranged mainly from 2 to 3. Pomes with 2 pyrenes represented 56.01 % of the collection and pomes with 3 pyrenes represented 38.39 % of the collection. Pomes with 1 pyrene (1.32 %), 4 pyrenes (4.22 %) and 5 pyrenes (0.06 %) were also present in the collection. Number of pyrenes in *C. tomentosus* pomes ranged from 3 to 5.

| Species          | This paper | Baranec (1992) | Kovanda (1992) | Bartha (2009) |
|------------------|------------|----------------|----------------|---------------|
| *C. integerrimus*| (2–3–4)    | 3(–4)          | (2–3–4)        | 2–3(–5)       |
| *C. melanocarpus* agg. | 2–3      | 2(–3)          | 2(–3)          | 2(–4)         |
| *C. tomentosus*  | 3–5        | 3–5            | –              | 3–5           |

Table 3 | A difference in pomes per infructescence mean values between selected species

| Species          | Mean per species | Standard deviation |
|------------------|------------------|--------------------|
| *C. integerrimus*| 1.14             | 0.38               |
| *C. melanocarpus* agg. | 1.54     | 0.71               |
| *C. tomentosus*  | 1.50             | 0.74               |

Table 4 | Number of pomes in infructescence presented in this paper, compared with other published data

| Species          | This paper | Baranec (1992) | Kovanda (1992) | Bartha (2009) |
|------------------|------------|----------------|----------------|---------------|
| *C. integerrimus*| 1(–2–3)    | 1(–2)          | –              | –             |
| *C. melanocarpus* agg. | 1–2(–3–5) | (1–)2–5       | –              | –             |
| *C. tomentosus*  | 1–2(–3–4)  | 3–7            | –              | –             |
5. Pomes with 3 pyrenes represented 29.63 % of the collection, 4 pyrenes were present in 48.15 % of pomes and 5 pyrenes were present in 22.22 % of pomes.

A significant difference (Tukey’s HSD test; \( \alpha = 0.01 \)) in pomes per infructescence mean values was also recorded between \( C. \text{integerrimus} \) (1.14) and \( C. \text{melanocarpus} \) agg. (1.54; including \( C. \text{matrensis} \)), and between \( C. \text{integerrimus} \) and \( C. \text{tomentosus} \) (1.50). There was no significant difference in pomes per infructescence mean values between \( C. \text{melanocarpus} \) agg. and \( C. \text{tomentosus} \). However, the determination of these two species is not problematic. \( C. \text{tomentosus} \) individuals have hairy and red pomes while pomes of \( C. \text{melanocarpus} \) agg. individuals are glabrous and dark red-violet or black (Baranec, 1992; Bartha, 2009). These results along with standard deviation are listed in Table 3.

We also compared results from this paper, obtained by counting of pomes in infructescence, with other published data. These results are listed in Table 4.

The vast majority of \( C. \text{integerrimus} \) pomes grew one by one and did not form infructescences. Single pome represented 86.96 % of the collection. Infructescences with 2 pomes represented 11.88 % and infructescences with 3 pomes represented only 1.16 % of the collection. Number of pomes in infructescences of \( C. \text{melanocarpus} \) agg. ranged mainly from 1 to 2. Single pome represented 57.42 % of the collection and infructescences with 2 pomes represented 33.09 % of the collection. Infructescences with 3 pomes (8.05 %), 4 pomes (1.25 %) and 5 pomes (0.18 %) were also present. Number of pomes in infructescences of \( C. \text{tomentosus} \) ranged mainly from 1 to 2. Single pome represented 61.74 % of the collection. Infructescences with 2 pomes represented 28.70 % of the collection. Infructescences with 3 pomes (6.96 %) and 4 pomes (2.61 %) were also present.

Nowadays, modern methods, such as flow cytometry method, are very important in taxonomy of plants (Hajrudinović et al., 2015; Macková et al., 2017; Žabka et al., 2018), which is a big step forward in systematic botany. These methods in combination with classic morphological and population biology research (Rohrer et al., 1991; Durišová, et al., 2016; Durišová and Baranec, 2016) give us the opportunity to solve many different problems in complicated groups, like Rosaceae.

The purpose of these morphological analyses was to determine a difference between two closely related tetraploid species \( C. \text{integerrimus} \) and \( C. \text{melanocarpus} \) agg.) (Rothleutner et al., 2016), since \( C. \text{tomentosus} \) is considered to be pentaploid (Macková et al., 2018). Flow cytometry method, used in the last study, revealed no significant difference in the genome size of tetraploid \( C. \text{integerrimus} \) and \( C. \text{melanocarpus} \) agg., including \( C. \text{matrensis} \) (Kšiňan et al., 2019). Therefore, we decided to prove, if these morphological characteristics are reliable for identification of selected tetraploid Cotoneaster species.
4 Conclusion

Results from this paper proved, that even in the era of modern cytological and molecular methods, classic morphological methods are still relevant in the field of botany. However, we do not recommend relying only on morphological characteristics, because in some cases a determination of problematic closely related species can be more complicated. We suggest a combination of available methods, from classic morphological research to modern methods, or at least a combination of several morphological characteristics.

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Appendix 1 List of localities selected for counting of pyrenes in pome (sorted by different species, by country and alphabetically. Sites are listed in order: geomorphological unit name, village, local site name, geographic coordinates)

Cotoneaster integerrimus:

Czech Republic (1 locality)

Pavlovske vrch hills, Mikulov, Státy kopeček hill, 48° 48’ 23.2” N 16° 38’ 48.3” E
Cotoneaster melanocarpus agg.: Hungary (3 localities)
Börzsöny Mts., Kóspallag, Só-hegy, 47° 52' 28.0" N 18° 53' 29.4" E
Bük Mts., Bélapatfalva, Bél-kő hill, 48° 02' 33.5" N 20° 22' 33.2" E
Zempléni-hegysék hills, Hejce, Sólyom-kő hill, 48° 26' 12.7" N 21° 19' 09.5" E

Poland (2 localities)
Pieniński Pas Skalkowy hills, Gronków, Skalka Cisowa hill, 49° 26' 13.1" N 20° 06' 13.6" E
Pieniny Mts., Trzy Korony, Okraglica 49° 24'50.4"N 20° 24'49.8"E

Slovakia (12 localities)
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03' 55.9" N 18° 09' 04.5" E
Chočské vrchy Mts., Studničná, Sedem kostolov site, 49° 08' 04.8" N 19° 15' 52.5" E
Ľubovnianska vrchovina hills, Jarabina, rocks NW from the village, 49° 20' 51.6" N 20° 38' 30.4" E
Malá Fatra Mts., Terchová, Baraniarky hill, 49° 13' 23.0" N 19° 00' 42.0" E
Malá Fatra Mts., Terchová, Kraviarske hill, 49° 12' 34.2" N 19° 01' 00.5" E
Malé Karpaty Mts., Čachtice, Velký Plešivec hill, 48° 42' 11.1" N 17° 44' 17.8" E
Malé Karpaty Mts., Devin, the foothills of the Devínska Kobyla hills, 48° 10' 50.2" N 16° 59' 15.3" E
Nízke Tatry Mts., Liptovský Ján, near the cemetery, 49° 02' 35.6" N 19° 40' 45.0" E

Cotoneaster melanocarpus agg.: Czech Republic (1 locality)
Hostýnsko-vsetínská hornatina hills, Vsetín, Valova skála rock, 49° 21' 02.9" N 18° 01' 47.0" E

Hungary (3 localities)
Börzsöny Mts., Letkés, Nagy Galla hill, 47° 52' 31.5" N 18° 49' 37.4" E
Bük Mts., Bélapatfalva, Bél-kő hill, 48° 02' 33.5" N 20° 22' 33.2" E
Zempléni-hegysék hills, Hejce, Sólyom-kő hill, 48° 26' 12.7" N 21° 19' 09.5" E

Poland (2 localities)
Pieniny Mts., Trzy Korony, Okraglica 49° 24'50.4"N 20° 24'49.8"E

Slovakia (16 localities)
Biele Karpaty Mts., Lednica, ruins of the Lednický hrad castle, 49° 06' 38.0" N 18° 12' 40.8" E
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03' 55.9" N 18° 09' 04.5" E
Burda hills, Kamenica nad Hronom, Skaly site, 47° 49' 34.0" N 18° 44' 53.6" E

Cerová vrchovina hills, Hajnáčka, ruins of the Hajnáčka castle, 48° 13' 06.5" N 19° 57' 20.4" E
Muránska planina hills, Brdárka, Malý Radzim hill, 48° 46' 31.1" N 20° 19' 44.4" E

Podtatranská kotlina basin, Primovce, Primovské skalky Nature Reserve, 49° 00' 56.2" N 20° 22' 57.5" E
Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03' 47.6" N 19° 18' 30.6" E
Podunajská nížina lowland, č ifaře, Podskalie site, 48° 14' 22.5" N 18° 25' 40.9" E

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Vtáčnik Mts., Podhradie, ruins of the Sivý Kameň castle, 48° 41′ 08.8″ N 18° 38′ 19.8″ E

**Cotoneaster tomentosus:**

**Slovakia (5 localities):**

- Malé Karpaty Mts., Devin, Devínska Kobyla hills, 48° 10′ 54.9″ N 16° 59′ 04.5″ E
- Nízke Tatry Mts., Hranovnica, Hranovnická dubina Nature Reserve, 49° 00′ 26.8″ N 20° 17′ 09.0″ E
- Oravská vrchovina hills, Oravský Podzámok, Oravský hrad castle, 49° 15′ 46.9″ N 19° 21′ 32.9″ E
- Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03′ 47.6″ N 19° 18′ 30.6″ E
- Nízke Tatry Mts., Svit, near the cemetery, 49° 03′ 15.5″ N 20° 12′ 22.2″ E

**Appendix 2 List of localities selected for counting of pomes in infructescence (sorted by different species, by country and alphabetically. Sites are listed in order: geomorphological unit name, village, local site name, geographic coordinates, collector of herbarium specimen, year of collection):**

**Cotoneaster integerrimus:**

- **Czech Republic (1 locality):** Pavlovske vrchy hills, Mikulov, Svatý kopeček hill, 48° 48′ 23.2″ N 16° 38′ 48.3″ E

**Cotoneaster melanocarpus agg.:**

- **Czech Republic (1 locality):** Hostýnsko-vsetínská hornatina hills, Vsetín, Valova skála rock, 49° 21′ 02.9″ N 18° 20′ 01.7″ E
- **Hungary (12 localities):**
  - Bükk Mts., Alsóhámor, Molnár-szikla hill, 48° 06′ 42.8″ N 20° 38′ 52.2″ E
  - Bükk Mts., Bél-kö hill, 48° 02′ 28.7″ N 20° 22′ 07.5″ E
  - Bükk Mts., Uppony, SW slopes of Kalica-tető, 48° 12′ 57.0″ N 20° 26′ 12.2″ E

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Cserhát Mts., Hollókő; Szár hegy hill, 47° 59’ 12.5” N 19° 35’ 50.3” E
Karancs-Medves hills, Salgóbánya, Kis Salgó (Boszorkánykő) 48° 8’ 24.40” N 19° 50’ 57.86” E
Mátra Mts., Mátraalmás, ruins of the Gyla-vár castle, 47° 55’ 31.40” N 19° 54’ 24.65” E
Mátra Mts., Mátraháza, Sas-kő hill, 47° 52’ 24.63” N 20° 1’ 50.12” E
Mátra Mts., Mátrakerestes, Bárány-kő hill, 47° 54’ 27.00” N 19° 49’ 6.17” E
Mátra Mts., Sástó, 47° 50’ 44.06” N 19° 56’ 50.48” E
Mátra Mts., Tar, Fehérkő-bánya stone pit, 47° 57’ 9.89” N 19° 45’ 52.50” E
Zemplényi-hegysék hills, Hejce, Sólyom-kő hill, 48° 26’ 12.7” N 21° 19’ 09.5” E
Poland (2 localities)
Pieniny Mts., Falsztyn, Zielone Skałki hills, 49° 25’ 55.5” N 20° 17’ 40.5” E
Pieniny Mts., Trzy Korony, Okraglica 49° 25’ 50.4” N 20° 24’ 49.8” E
Slovakia (16 localities)
Biele Karpaty Mts., Vršatské Podhradie, ruins of the Vršatec castle, 49° 03’ 55.9” N 18° 09’ 04.5” E
Muránska planina hills, Brdárka, Malý Radzim hill, 48° 46’ 31.1” N 20° 19’ 44.4” E
Malé Karpaty Mts., Driny hill, 48° 30’ 03.3” N 17° 24’ 14.3” E, Eliáš jun. 2014 NI
Malé Karpaty Mts., Buková, near ruins of the Ostrý Kameň castle, 48° 31’ 20.0” N 17° 22’ 24.2” E, Eliáš jun. 2010 NI
Podunajská nížina lowland, čifáre, Podskalie site, 48° 14’ 22.5” N 18° 25’ 40.9” E
Podtatranská kotlina basin, Ružomberok, memorial of the Slovak National Uprising, 49° 03’ 47.6” N 19° 18’ 30.6” E
Strážovské vrchy Mts., Rokoš – Košútova skala, 48° 46’ 39.3” N 18° 26’ 06.5” E, Eliáš jun. 2003 NI
Strážovské vrchy Mts., Uhrovské Podhradie, Zrubiská site, 48° 44’ 52.6” N 18° 23’ 35.1” E, Eliáš jun. 2003 NI
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56’ 42.3” N 20° 27’ 34.3” E, Eliáš jun. 2017 NI
Tribeč Mts., Partizánske, Podbralie site, 48° 37’ 07.0” N 18° 21’ 43.5” E, Eliáš jun. 2003 NI
Veľká Fatra Mts., Belianska dolina, near the cottage of Havranovo, 48° 58’ 08.8” N 19° 04’ 39.0” E, Širáň 2003 NI
Veľká Fatra Mts., Morávková hill, 48° 59’ 48.9” N 19° 07’ 59.8” E, Talapka 1995 NI
Veľká Fatra Mts., Folkušová, Pekárová hill, 48° 57’ 28.4” N 18° 58’ 07.9” E, Talapka and Jelšovský 1996 NI
Veľká Fatra Mts., Vlkolínec, 49° 02’ 30.6” N 19° 16’ 36.3” E, Baranec and Eliáš jun. 2003 NI

Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56’ 42.3” N 20° 27’ 34.3” E, Eliáš jun. 2017 NI
Vtáčnik Mts., Podhradie, ruins of the Sivý Kameň castle, 48° 41’ 08.8” N 18° 38’ 19.8” E
Zvolenská kotlina basin, Vígľaš, elevation point 419 m, 48° 33’ 26.5” N 19° 17’ 45.4” E

Cotoneaster tomentosus:

Slovakia (15 localities)
Kremnické vrchy Mts., Zvolen, Poštárka hill, 48° 33’ 37.7” N 19° 05’ 40.6” E, Galvánek 2018 NI
Malé Karpaty Mts., Devin, Devínska Kobyla hills, 48° 10’ 54.9” N 16° 59’ 04.5” E
Muránska planina hills, Muráň, Cigánka hill, 48° 45’ 27.5” N 20° 03’ 27.2” E, Baranec and Eliáš jun. 1999 NI
Nízke Tatry Mts., Liptovský Ján, near the cemetery, 49° 02’ 35.6” N 19° 40’ 45.0” E
Nízke Tatry Mts., Demiánová, Siná hill, 48° 59’ 58.9” N 19° 35’ 15.7” E, Talapka 1996 NI
Nízke Tatry Mts., Svit, near the cemetery, 49° 03’ 15.5” N 20° 12’ 22.2” E
Oravská vrchovina hills, Oravský Podzámok, Oravský hrad castle, 49° 15’ 46.9” N 19° 21’ 32.9” E
Strážovské vrchy Mts., Rokoš – Košútova skala, 48° 46’ 39.3” N 18° 26’ 06.5” E, Eliáš jun. 2003 NI
Strážovské vrchy Mts., Uhrovské Podhradie, Zrubiská site, 48° 44’ 52.6” N 18° 23’ 35.1” E, Eliáš jun. 2003 NI
Slovenský raj region, Spišské Tomášovce, Tomášovský výhľad site, 48° 56’ 42.3” N 20° 27’ 34.3” E, Eliáš jun. 2017 NI

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