



Morphometric and flow cytometric evaluations of a putative natural hybrid of *Centaurium* (Gentianaceae) from Turkey

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Abstract

A putative natural hybrid of *Centaurium* was discovered in a population composed of *C. serpentinicola* and *C. tenuiflorum* subsp. *tenuiflorum* in the vicinity of Köyceğiz (Muğla province, SW Turkey). Morphometric analysis performed using 14 diagnostic characters supported that the putative natural hybrid as an interspecific hybrid between *C. serpentinicola* and *C. tenuiflorum* subsp. *tenuiflorum*. Flow cytometric measurements revealed that *C. serpentinicola* and *C. tenuiflorum* subsp. *tenuiflorum* have similar nuclear DNA amounts (2.61 and 2.53 pg DNA/2C), whereas the putative hybrid have a significantly higher nuclear DNA amount (2.77 pg DNA/2C), and they have the same ploidy level.

Key words: Köyceğiz, natural hybridization, nuclear DNA amount, numerical taxonomy

Introduction

The genus *Centaurium* Hill (1756: 62; subtribe Chironiinae, tribe Chironieae, Gentianaceae), includes about 20 species distributed mainly in the Mediterranean Basin (Mansion 2004). It is native to Europe and Western Asia and naturalized in America and Australia. These plants commonly known as “centaury”, are annual or biennial herbs with opposite and sessile leaves, cymose inflorescences, and salverform and pink to pinkish-reddish or purplish, rarely yellow or white corollas. Its anthers are spirally twisted after dehiscence.

In Turkey, *Centaurium* is represented by 8 species, two of which are recorded as doubtful or imperfectly known (Jakobsen 1978, Carlström 1986, Davis *et al.* 1988, Çiçek 2012). Two of them, *C. erythraea* Rafn (1800: 75) and *C. tenuiflorum* (Hoffmannsegg & Link 1813–1820: 354) Fritsch (1907: 97) are variable species widely distributed throughout Eurasia. *C. erythraea* includes 6 subspecies (4 of which occur in Turkey) and *C. tenuiflorum* includes 3 subspecies (2 of which occur in Turkey) (Melderis 1972).

Natural hybridization within *Centaurium* is a common occurrence both in nature and *in vitro*. According to literature (Ubsdell 1979; Mansion *et al.* 2005; Guggisberg *et al.* 2006; Banjanac *et al.* 2014), and records from IPNI (2014) and The Plant List (2014), several hybrids or putative hybrid species of *Centaurium* are recognized. The hybridizations are mostly among European *Centaurium* species, and are the following: *C. erythraea* × *C. tenuiflorum*, *C. bianoris* Sennen (1936: 175) (*C. maritimum* × *C. tenuiflorum*), *C. malzacianum* Maire (1939: 25) (*C. maritimum* × *C. pulchellum*), *C. centaurioides* (Roxburgh 1824: 283) Rao & Hemadri (1970: 357) (*C. tenuiflorum* × *C. pulchellum*), *C. ×aschersonianum* (Seemen 1897: 45) Fournier (1938: 856) (*C. littorale* subsp. *littorale* × *C. pulchellum*), *C. ×intermedium* (Pollini 1816: 16) Druce (1905: 48) (*C. erythraea* × *C. littorale*), *C. ×jolivetinum* (Fournier 1931: 26) Fournier (1938: 856) (*C. erythraea* × *C. pulchellum*), *C. ×litardierei* Ronniger ex Litardière (1948: 213) (*C. littorale* × *C. tenuiflorum*), and *C. ×klattii* Fournier (1938: 856) (*C. erythraea* × *C. littorale* subsp. *littorale*). These hybridizations generally involved *C. maritimum* (Linnaeus 1767: 55) Fritsch (1907: 97), *C. tenuiflorum* (Hoffmannsegg & Link 1813–1820: 354) Fritsch (1907: 97), *C. erythraea* Rafn (1800: 75), *C. littorale* (Turner 1805: 469) Gilmour (1937: 498) and *C. pulchellum* (Swartz 1783:85) Druce (1907: 242).

During fieldwork undertaken for the taxonomic revision of Turkish *Centaurium*, we collected *Centaurium* specimens from a locality at 10 km NW of the town Köyceğiz (Muğla province, SW Turkey). This locality is the same

TABLE 6. Nuclear DNA contents of *Centaureum* specimens as determined by flow cytometry and estimated genome size.

Analyzed specimen	Nuclear DNA content (pg/2C)	Estimated 1C genome size (Mbp)
<i>C. serpentinicola</i>	2.61 ^a	1276
<i>C. tenuiflorum</i> subsp. <i>tenuiflorum</i>	2.53 ^a	1237
Putative natural hybrid <i>Centaureum</i>	2.77 ^b	1355

Means with the same letter are not statistically different at $\alpha=0.05$ as per LSD.

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References

- Alan, A.R., Zeng, H., Assani, A., Shi, W.L., McRae, H.E., Murch, S.J. & Saxena, P.K. (2007) Assessment of genetic stability of the germplasm lines of medicinal plant *Scutellaria baicalensis* Georgi (Huang-qin) in long-term, *in vitro* maintained cultures. *Plant Cell Reports* 26: 1345–1355.
<http://dx.doi.org/10.1007/s00299-007-0332-9>
- Arumuganathan, K. & Earle, E.D. (1991) Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* 9: 208–218.
<http://dx.doi.org/10.1007/BF02672069>
- Baack, E.J., Whitney, K.D. & Rieseberg, L.H. (2005) Hybridization and genome size evolution: timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. *New Phytologist* 167: 623–630.
<http://dx.doi.org/10.1111/j.1469-8137.2005.01433.x>
- Banjanac, T., Šiler, B., Skorić, M., Ghalawenji, N., Milutinović, M., Božić, D. & Mišić, D. (2014) Interspecific *in vitro* hybridization in genus *Centaureum* and identification of hybrids via flow cytometry, RAPD, and secondary metabolite profiles. *Turkish Journal of Botany* 38: 68–79.
<http://dx.doi.org/10.3906/bot-1211-58>
- Bureš, P., Wang, Y.F., Horova, L. & Suda, J. (2004) Genome size variation in Central European species of *Cirsium* (Compositae) and their natural hybrids. *Annals of Botany* 94: 353–363.
<http://dx.doi.org/10.1093/aob/mch151>
- Carlström, A. (1986) New taxa and notes from the SE Aegean area and SW Turkey. *Willdenowia* 16: 73–78.
- Çiçek, M. (2012) *Centaureum*. In: Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (Eds.) *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, pp. 512–513.
- Davis, P.H., Mill, R.R. & Tan, K. (Eds.) (1988) *Flora of Turkey and the East Aegean islands. Vol. 10. (Supplement)*. Edinburgh Univ. Press, Edinburgh, 590 pp.
- Dolezel, J., Bartos, J., Voglmayr, H. & Greilhuber, J. (2003) Nuclear DNA content and genome size of trout and human. *Cytometry* 51: 127–128.
- Dolezel, J. & Bartos, J. (2005) Plant DNA flow cytometry and estimation of genome size. *Annals of Botany* 95: 99–110.
<http://dx.doi.org/10.1093/aob/mci005>
- Druce, G.C. (1905) Notes on the new edition of the Babington's "Manual of British Botany" *The Annals of Scottish Natural History* 14: 47–51.
- Druce, G.C. (1907) The nomenclature of British plants. *The Annals of Scottish Natural History* 16: 241–244.
- Fournier, P.V. (1931) × *Erythrae jolivetina* P.F. (*E. centaureum* × *ramosissima*) P.F. *Le Monde des plantes. Intermédiaire des Botanistes* 32me. Année (3e Série), pp. 1–26.
- Fournier, P.V. (1938) *Centaureum*. In: Fournier, P.V. (Ed.) *Les Quatre Flores de la France, Corse comprise (générale, alpine, méditerranéenne, littorale). Edition 1*. Poinson-lès-Grancey (Haute-Marne), pp. 833–896.
- Fritsch, K. (1907) *Centaureum maritimum* (L.) Fritsch and *C. tenuiflorum* (Hoffmanns. & Link) Fritsch In: Janchen, E.E.A. (Ed.) Einige durch die internationalen Nomenklaturregeln bedingte Änderungen in der Benennung mitteleuropäischer Pflanzen. *Mitteilungen des Naturwissenschaftlichen Vereines an der Universität Wien* 5: 83–100.
- Gilmour, J.S.L. (1937) Notes on the genus *Centaureum* 1. The nomenclature of the British species. *Bulletin of Miscellaneous Information*

- (*Royal Gardens, Kew*) 1937: 497–502.
<http://dx.doi.org/10.2307/4107159>
- Guggisberg, A., Bretagnolle, F., Mansion, G. (2006) Allopolyploid origin of the Mediterranean endemic, *Centaurium bianoris* (Gentianaceae), inferred by molecular markers. *Systematic Botany* 31: 368–379.
<http://dx.doi.org/10.1600/036364406777585937>
- Hill, J. (1756) *The British Herbal: an history of plants and trees, natives of Britain, cultivated for use, or raised for beauty*. T. Osborne & J. Shipton, London, 533 pp.
<http://dx.doi.org/10.5962/bhl.title.51133>
- Hoffmannsegg, J.C. von & Link, H.F. (1813–1820) *Centaurium tenuiflorum*. In: *Flore Portugaise ou description de toutes les plantes qui croissent naturellement en Portugal. Vol. 1 (11)*. Imprimerie de C.F. Amelang, Berlin, pp. 354–355.
- IPNI (2015) The International Plant Names Index. Available from: <http://www.ipni.org/> (accessed 12 January 2015)
- Jakobsen, K. (1978) *Centaurium*. In: Davis, P.H. (Ed.) *Flora of Turkey and the East Aegean islands. Vol. 6*. Edinburgh Univ. Press, Edinburgh, pp. 178–183.
- Linnaeus, C. (1767) *Mantissa Plantarum. Generum editionis VI. et specierum editionis II*. Impensis Direct. Laurentii Salvii, Holmiae, 588 pp.
- Litardière, R.V. (1948) *Centaurium ×litardierei* Ronniger ex Litard. *Candollea* 11: 213.
- Maire, R. (1939) *Sertulum orientale. Notulae Systematicae* 8: 24–26.
- Marques, I., Feliner, G.N., Martins-Loução, M.A. & Aguilar, J.F. (2012) Genome size and base composition variation in natural and experimental *Narcissus* (Amaryllidaceae) hybrids. *Annals of Botany* 109: 257–264.
<http://dx.doi.org/10.1093/aob/mcr282>
- Mansion, G. (2004) A new classification of the polyphyletic genus *Centaurium* Hill (Chironiinae, Gentianaceae): description of the New World endemic *Zeltnera*, and reinstatement of *Gyrandra* Griseb. and *Schenkia* Griseb. *Taxon* 53: 719–740.
<http://dx.doi.org/10.2307/4135447>
- Mansion, G., Zeltner, L. & Bretagnolle, F. (2005) Phylogenetic patterns and polyploid evolution within the Mediterranean genus *Centaurium* (Gentianaceae-Chironieae). *Taxon* 54: 931–950.
<http://dx.doi.org/10.2307/25065479>
- Melderis, A. (1972) *Centaurium* Hill In: Tutin, T.G. (Eds.) *Flora Europaea. Vol. 3*. Cambridge University Press, Cambridge, pp. 56–59.
- Morgan, E.R., Burge, G.K., Seelye, J.F., Hopping, M.E. & Grant, J.E. (1998) Production of inter-specific hybrids between *Limonium perezii* (Stapf) Hubb. and *Limonium sinuatum* (L.) Mill. *Euphytica* 102 (1): 109–115.
<http://dx.doi.org/10.1023/A:1018384329409>
- Pollini, C. (1816) *Viaggio al Lago di Garda e al Monte Baldo in cui si ragiona delle cose naturali di quei luoghi aggiuntovi un cenno sulle curiosità del bolca e degli altri monti veronesi*. Dalla Tipografia Mainardi, Verona, 152 pp.
<http://dx.doi.org/10.5962/bhl.title.53793>
- Rafn, C.G. (1800) *Danmarks og Holsteens Flora: systematisk physisk og oekonomisk bearbejdet et priiskrivt. Vol. 1. Förfte Deel*, Copenhagen, 722 pp.
- Rao, R.S. & Hemadri, K. (1970) *Centaurium centaurioides* (Roxb.) S.R. Rao & Hemadri. *Journal of the Bombay Natural History Society* 67: 357.
- Rayburn, A.L., Biradar, D.P., Bullock, D.G. & McMurphy, L.M. (1993) Nuclear DNA content in F₁ hybrids of maize. *Heredity* 70: 294–300.
<http://dx.doi.org/10.1038/hdy.1993.42>
- Roxburgh, W. (1824) *Flora Indica, or Descriptions of Indian Plants. Vol. 2*. Mission Press, Serampore, 588 pp.
- Seemen, K.O. von (1897) Mitteilungen über die Flora der ostfriesischen Insel Borkum. *Allgemeine botanische Zeitschrift für Systematik, Floristik, Pflanzengeographie etc.* 3: 43–45.
- Sennen, F. (1936) *Diagnoses des nouveautés parues dans les exsiccata Plantes d'Espagne et du Maroc de 1928 à 1935*. Impr. Anglada, Melilla, 308 pp.
- Swartz, O. (1783) *Gentiana pulchella*, en ny Svensk Vaxt. *Kongliga vetenskaps academiens nya handlingar* 4: 85–87.
- The Plant List (2015) The Plant List A working list of all plant species, version 1.1. Published on the Internet. Available from: <http://www.theplantlist.org/> (accessed 12 January 2015)
- Turner, D. & Dillwyn, L.W. (1805) *The Botanist's Guide Through England and Wales. Vol. 1–2*. Phillips & Fardon, London, 804 pp.
- Ubsdell, R.A.E. (1979) Studies on variation and evolution in *Centaurium erythraea* Rafn and *C. littorale* (D. Turner) Gilmour in the British Isles 3. Breeding systems, floral biology and general discussion. *Watsonia* 12: 225–232.
- Zhou, X., Ma, J., Wang, W., Gong, N. & Liu, J. (2010) Genome size of the diploid hybrid species *Hippophae gonioarpa* and its parental species, *H. rhamnoides* ssp. *sinensis* and *H. neurocarpa* ssp. *neurocarpa* (Elaeagnaceae). *Acta Biologica Cracoviensia Series Botanica* 52: 12–16.
<http://dx.doi.org/10.2478/v10182-010-0018-4>