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The hybrid *Centaurium erythraea* × *pulchellum* (Gentianaceae), newly recorded in the Netherlands and Belgium

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Key words

Belgium
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Abstract – In the summer of 2023, and again in 2024, a large population of an unidentified *Centaurium* was discovered at a recently excavated site along the River Meuse near Grevenbicht (Koeweide / Trierveld), the Netherlands. In 2024, morphologically identical plants were also found on the Belgian side of the river in Stokkem (Kerkeweerd), and in 2025 between Maasband and Meers, again on Dutch territory. The plants were morphologically highly uniform, fully fertile, and bore a striking resemblance to *C. tenuiflorum*, a species predominantly native to the Mediterranean region. However, several morphological traits also appeared intermediate between those of the native species *C. erythraea* and *C. pulchellum*. To determine the plants' identity, genetic analyses were conducted and their genome size was measured using flow cytometry. The results demonstrated that, despite their apparent resemblance and fertility, the plants do not belong to *C. tenuiflorum*, but to the hybrid between *C. erythraea* and *C. pulchellum*. This taxon has previously only been recorded in the British Isles, France, and Germany, and thus the recent finds represents a new record for both the Netherlands and Belgium.

Samenvatting – In de zomer van 2023, en opnieuw in 2024, werd een grote populatie van een onbekende *Centaurium* aangetroffen op een recent afgegraven terrein langs de Maas bij Grevenbicht (Koeweide / Trierveld), Nederland. In 2024 werden identieke planten waargenomen aan de Belgische zijde van de rivier, in Stokkem (Kerkeweerd), en in 2025 opnieuw in Nederland, tussen Maasband en Meers. De planten waren morfologisch zeer uniform, duidelijk fertiel, en vertoonden een opvallende gelijkenis met *C. tenuiflorum*, een soort die voornamelijk in het Middellandse Zeegebied voorkomt. Hun morfologische kenmerken bleken echter ook intermediair tussen die van de inheemse soorten *C. erythraea* en *C. pulchellum*. Om de identiteit van deze planten vast te stellen, werd een genetische analyse uitgevoerd en werd het genoomgewicht bepaald met behulp van flow cytometrie. Uit de resultaten bleek dat de planten, ondanks hun ogenschijnlijke gelijkenis en vruchtbaarheid, niet tot *C. tenuiflorum* behoren, maar tot de hybride van *C. erythraea* en *C. pulchellum*. Dit taxon was tot nu toe alleen bekend van de Britse Eilanden, Frankrijk en Duitsland, en is daarmee voor het eerst vastgesteld in zowel Nederland als België.

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INTRODUCTION

During fieldwork on a recently excavated site in the floodplains of the River Meuse near Grevenbicht (Koeweide / Trierveld), Province of Limburg, the Netherlands, an unfamiliar *Centaurium* was discovered on 15 July 2023. *Centaurium erythraea* Rafn (common centaury) was also present in the immediate vicinity, though in smaller numbers. The unknown plants exhibited smaller, darker pink flowers (Fig. 1) — a trait reminiscent of *C. pulchellum* (Sw.) Druce (lesser centaury) — yet differed markedly from the latter in habit: they reached heights of up to 30 cm and had more internodes (Fig. 2). Also striking was the abundance of seemingly fertile seeds, initially making the possibility of hybrid origin appear unlikely. But if not a hybrid, what species could it be?

Given the recent large-scale excavation and restoration works along the River Meuse — during which several non-native species were found (some potentially introduced via historical industrial activity along the River Vesdre upstream; see Verloove et al. 2025) — an alien origin was suspected. Using several European Floras (e.g. Díaz-Lifante 2012, Melderis 1972, Stace 2019, Tison & de Foucault 2014), the plants keyed out — albeit with

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some difficulty — as *C. tenuiflorum* (Hoffmanns. & Link) Fritsch. However, their morphological features did not fully match this species. For instance, in *C. tenuiflorum* the calyx is typically somewhat to significantly longer than the corolla tube (as illustrated by Pringle 2010, fig. 9), whereas the calyx lobes of the Meuse plants only reach the base of the corolla lobes (Fig. 3). Moreover, calyx-to-corolla ratio appeared to change over the course of the season, calling into question the taxonomic reliability of this character.

It is important to note that the aforementioned Floras do not include hybrid taxa, despite the fact that hybrids are relatively common in the genus *Centaurium* (see e.g., Banjanac et al. 2014, Valdés-Florido et al. 2024). Such hybrids are frequently fertile, and may give rise to hybrid swarms (Banjanac et al. 2019). Therefore, the possibility of a hybrid origin was also investigated. Given the morphology of the plants, the most likely candidate seemed to be the hybrid between *C. erythraea* and *C. pulchellum*. However, the absence of *C. pulchellum* at the site — although known from nearby locations (observation.org) — continued to raise questions. Additionally, the hybrid between *C. erythraea* and *C. pulchellum* (*C.* ×*ubsdellii* T.C.G.Rich, nom. inval.) had never before been recorded in the Low Countries, in contrast to other native hybrids such as *C.* ×*intermedium* (Wheldon) Druce [= *C. erythraea* × *littorale* (Turner) Gilmour; syn.: *C.* ×*klattii* P.Fourn. ex T.C.G.Rich) and *C.* ×*aschersonianum* (Seemen) Hegi (= *C. erythraea* × *C. littorale*) (den Bakker 1998, Duistermaat 2020, Verloove & Van Rossum 2023).

In 2024, the population was found to have expanded significantly and the largest subpopulation was estimated to consist of 1,000–2,000 individuals. In the same year, morphologically identical plants were also discovered five kilometers upstream in nature reserve Kerkeweerd, at Stokkem, on the Belgian side of the Meuse Valley and again, in 2025, on the Dutch side of the river between Maasband and Meers. Hence, a genetic analysis was carried out and, additionally, the genome size of the unknown taxon was determined. These analyses confirmed the identity of the plants as *C. erythraea* × *pulchellum*, a hybrid that is newly recorded for both the Netherlands and Belgium.

MATERIALS AND METHODS

Field observations were conducted in the floodplains of the River Meuse near Grevenbicht (Koeweide / Trierveld) and Maasband / Meers, the Netherlands, in 2023, 2024, and 2025 by author SG (with partial assistance from FV), and in Stokkem (Kerkeweerd), Belgium, in 2024 by WV. During these surveys, specimens of the unidentified *Centaurium* plants were collected and subsequently deposited in the herbaria of Naturalis Biodiversity Center (L), Leiden, the Netherlands, and the Meise Botanic Garden (BR), Meise, Belgium.

Identification of the material was performed using several authoritative Floras for Western and Southwestern Europe, including Melderis (1972), Díaz-Lifante (2012), Tison & de Foucault (2014), Stace (2019), Duistermaat (2020), and Verloove & Van Rossum (2023), among others. These Floras were used to key out morphological characters and compare diagnostic features with related taxa, including native and non-native species. The analysed *Centaurium* specimens are listed in Table 1 along with their respective localities.

In addition to a morphological analysis, molecular investigations were carried out to determine the taxonomic identity of the unknown plants. These included both DNA-based analyses and flow cytometric estimation of nuclear DNA content (2C-value), expressed in picograms (pg), to assess genome size.

The nuclear rDNA internal transcribed spacer (ITS) region, and the plastid *trnL* (UAA) intron and *trnL-trnF* intergenic spacer (*trnL-F*) were selected as markers for phylogenetic analysis and for guiding species identification. The selection of these markers was based on their availability from previous studies (Jiménez-Lobato et al. 2019, Mansion et al. 2005, Mansion & Struwe 2004). Total genomic DNA was extracted from silica-dried leaf material using a modified CTAB protocol (Doyle & Doyle 1987). The ITS region was amplified using primers ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) and ITS5 (5’-GAAGTAAAAGTCGTAACAAGG-3’) (White et al. 1990), the *trnL* intron was amplified using primers trnLc (5’-CGAAATCGGTAGACGCTACG-3’) and trnLd (5’-GGGGATAGAGGGACTTGAAC-3’), and *trnL-F* was amplified using primers trnLe (5’-GGTTCAAGTCCCTCTATCCC-3’) and trnLf (5’-ATTTGAACTGGTGACACGAG-3’) (Mansion et al. 2005). PCR conditions followed Mansion et al. (2005). PCR products were sequenced by Macrogen (Seoul, South Korea). Forward and reverse sequences were assembled using Geneious Prime v2023.2.1. (Biomatters, Auckland, New Zealand). Sequences have been deposited in EMBL/GenBank under project number PRJEB96298 and accession numbers OZ345114-OZ345117 (ITS), OZ345092-OZ345096 (*trnL* intron), and OZ345167-OZ345171 (*trnL-F*).

Three datasets were constructed: ITS (41 sequences, 635 positions), *trnL* intron (34 sequences, 430 positions), and *trnL-F* (42 sequences, 376 positions). These datasets included the sequences generated in this study, and publicly available sequences obtained from GenBank. Sequences were aligned using MUSCLE (Edgar 2004). Phylogenetic relationships were reconstructed using maximum likelihood and 1000 ultrafast bootstrap replicates in IQ-TREE 2.2.2.7 (Hoang et al. 2018, Nguyen et al. 2015) on the IQ-TREE server (Trifinopoulos et al. 2016).

Flow cytometry was performed on material collected from the Grevenbicht population (Koeweide / Trierveld) in 2024. For each analysis, a small segment of petiole was chopped finely together with a DNA standard in a modified chopping buffer

Table 1. The analysed *Centaurium* specimens with their respective localities. The herbarium abbreviation BR refers to the herbarium of Meise Botanic Garden in Meise (Belgium); the abbreviations of the names of the collectors WV and SG refer to, respectively, the authors Ward Vercruysse and Sipke Gonggrijp; BE refers to Belgium, NL refers to the Netherlands. Three of the five location names include a link to the corresponding record in either waarneming.be or waarneming.nl.

Number	Barcode BR	Taxon name	Observer	Location	Date
M24-793	BR0000027059937V	*Centaurium erythraea* × *pulchellum*	WV	Stokkem, Kerkeweerd, Maas (BE)	26-08-2024
M24-794	BR0000027059906V	*Centaurium erythraea*	SG	Beverwijk (NL)	26-07-2024
M24-795	BR0000027059913V	*Centaurium pulchellum*	SG	Beuningen, Beuningse Plas (NL)	10-08-2024
M24-796	BR0000027059890V	*Centaurium erythraea* × *pulchellum*	SG	Grevenbicht, Koeweide (NL)	23-06-2024
M24-797	BR0000027059920V	*Centaurium* backcross	SG	Grevenbicht, Koeweide (NL)	14-07-2024

(based on Bharathan et al. 1994), using a razor blade. The released nuclei were stained with propidium iodide, a fluorescent dye that binds specifically to DNA. The mixture was then filtered through a 20 µm nylon mesh to remove debris. Fluorescence intensity, which correlates with DNA content per nucleus, was measured after approximately 30 minutes using a Becton Dickinson Accuri C6 flow cytometer. Between 1,000 and 5,000 nuclei were analysed per sample. Each measurement was repeated after one hour to confirm consistency. As an internal standard, *Agave americana* L. 'Aureomarginata' was used, with a known 2C-value of 15.9 pg, calibrated against human leukocytes (2C = 7.0 pg; Tiersch et al. 1989). The resulting DNA content values were expressed in picograms (pg) per diploid nucleus (2C). While small amounts of DNA are also present in mitochondria and chloroplasts, their contribution to total fluorescence is negligible due to the small size of their genomes (typically a few hundred kilobases). Although RNA also binds propidium iodide, its presence does not interfere with the measurements, as the chopping buffer contains RNase, an enzyme that degrades RNA. One picogram corresponds to 10^{-12} grams and represents approximately 978 million base pairs. For comparison, *Arabidopsis thaliana* (L.) Heynh. (thale cress) has a genome size of 0.32 pg per nucleus (2C), humans approximately 7 pg, and *Galanthus nivalis* L. (common snowdrop) up to 72 pg.

RESULTS

Morphology

The unknown *Centaurium* plants displayed a remarkably uniform morphology (Fig. 1, 2, 3, 4 & 5). The stems were relatively robust and erect, ranging in height from 4 to 30 cm, occasionally even taller. Branching from the base was rare, with most lateral branches arising either just below or above mid-height. These branches were typically angled at approximately 20° relative to the main axis. Vigorous specimens exhibited up to eight internodes, whereas smaller individuals had as few as five. A basal rosette was absent. The leaves resembled those of *Centaurium erythraea* in general shape (Fig. 2), but were distinctly narrower and more acute; in well-developed individuals, they measured 2–3 cm in length along the main stem and possessed three to five veins.

Flowers were numerous and arranged in broad, loosely paniculate inflorescences. Pedicels were short, ranging from 0.5 to 1(–1.7) mm in length, or sometimes the flowers appeared nearly sessile. The calyx was approximately equal in length to the corolla tube during anthesis but became only ¾ to ½ as long by the time the fruit matured. Corolla lobes measured around 3 to 4 mm (on average 3.5 mm) in length and were dark pink in colour. At flowering, the lobes were roughly as long as the tube, while in fruiting specimens, they were about half as long. The fruits were cylindrical, measuring approximately 8–10 mm in length. Seeds were 0.35–0.4 mm, irregularly angular, and finely pitted.

The overall suite of morphological traits was clearly intermediate between those of *Centaurium erythraea* and *C. pulchellum*, consistent with published accounts of their hybrid (Stace et al. 2015). Superficially, the plants strongly resembled *C. tenuiflorum*, which explains our initial confusion when we tried to identify the unknown *Centaurium* plants.

To facilitate the identification of *Centaurium* species and their hybrids occurring in Belgium and the Netherlands, we propose the following key. It is based on our field observations and standard Floras for Western Europe.

1a. Inflorescence and/or calyx clearly set with papillae; stem leaves linear to lanceolate with 1 or 3 veins **2**
b. Inflorescence or calyx without (or with barely visible) papillae; stem leaves broader, ovate to elliptic with 3–5(–7) veins **3**

2a. Stem leaves usually 1-veined, narrow, lanceolate to linear, often obtuse at apex; pedicel 0–1 mm; upper part of inflorescence and calyx with numerous papillae; petal 4.5–6.5 mm, dark pink; stigma lobes semicircular to ± rounded . . . . *Centaurium littorale* (Turner) Gilmour
b. Stem leaves usually 3-veined, variable in width and shape but usually narrow, often acute at apex; pedicel 0–1 mm; calyx with papillae; petal (4.5–)5.5(–6.2) mm, dark pink; stigma lobes ovate-triangular. Intermediate between *C. erythraea* and *C. littorale* . . . . *Centaurium ×intermedium* (Wheldon) Druce
c. Stem leaves 3-veined, variable, sometimes linear, sometimes ovate, rounded to obtuse at apex; pedicel often > 1 mm; upper part of inflorescence and calyx with (few) papillae; petal < 5 mm, dark pink; stigma lobes semicircular to ± rounded. Intermediate between *C. littorale* and *C. pulchellum* . . . . *Centaurium ×aschersonianum* (Seemen) Hegi

3a. Stem leaves 3–5(–7)-veined, rounded to obtuse at apex; rosette usually present at flowering; main axis with ≥ 5 internodes; inflorescence corymbose; pedicel 0–1(–1.7) mm; petal 4.5–5.4 mm, usually light pink; stigma lobes ovate-triangular; corolla tube not narrowed in fruiting stage . . . . *Centaurium erythraea* Rafn
b. Stem leaves 3–5-veined, rounded to obtuse at apex; rosette absent at flowering; main axis with < 5 internodes, often branching below; inflorescence open with scattered flowers; pedicel 1–4(–12.7) mm; petal 2.5–4 mm, dark pink; stigma lobes semicircular to ± rounded; corolla tube narrowed in fruiting stage . . . . . . *Centaurium pulchellum* (Sw.) Druce
c. Stem leaves acute at apex, 3–5-veined; rosette absent (withering) at flowering; main axis with ≥ 5 internodes; inflorescence corymbose; pedicel 0.5–1(–1.7) mm; petal 3–4 mm, dark pink; stigma lobes ovate-triangular; corolla tube usually not narrowed in fruiting stage. Intermediate between *C. erythraea* and *C. pulchellum* . . . . ***Centaurium erythraea × pulchellum***

Molecular analyses

Molecular analyses were conducted to verify the taxonomic status of the unknown *Centaurium* plants. The phylogeny reconstructions are presented in Fig. 6.

Sequencing of the nuclear ITS marker revealed that the samples M24-0796 (collected as *C.* species cf. *tenuiflorum*) and M24-0794 (*C. erythraea*), both from Grevenbicht, belonged to the same clade. By contrast, true *C. tenuiflorum* and related taxa such as *C. bianoris* (Sennen) Sennen and *C. serpentinicola Carlström*, based on published sequences (e.g., Guggisberg et al. 2006), clearly formed a separate clade. One sequence of a plant from Stokkem (M24-0793, *C.* species cf. *tenuiflorum*) was of lower quality and placed separately in the phylogeny, but it still grouped more closely with the *C. erythraea* cluster. The *C. pulchellum* sample (M24-0795) formed a well-supported, distinct clade. These results strongly indicated that the unknown plants do not belong to *C. tenuiflorum*, despite superficial morphological similarities.

Fig. 1. One of the unknown *Centaurium* plants (left) and a plant of *C. erythraea* Rafn (right) in Grevenbicht, Province of Limburg (the Netherlands), 23 June 2023. The unknown *Centaurium* plants were later identified as the hybrid *Centaurium erythraea* × *pulchellum* (see text). The hybrid shows markedly smaller and darker corollas than *C. erythraea*, resembling those of *C. pulchellum* (Sw.) Druce. Photo: F. Verloove.

Sequencing of chloroplast markers (*trnL* intron and *trnL–F* spacer) produced a different pattern. The samples from Grevenbicht and Stokkem (M24-0793 and M24-0796) clustered with our *C. pulchellum* sequence (M24-0795) as well as with several sequences from the *C. tenuiflorum* group and one putative backcross individual (M24-0797), which morphological traits intermediate between the unknown plants and *C. erythraea*. Thus, a pronounced incongruence was observed between the nuclear and plastid markers, which indicates a hybrid origin.

Genome size measurements

Flow cytometric genome size estimation further supports the hybrid hypothesis (Table 2). The two unknown plants from Grevenbicht had 2C genome sizes of 2.48 and 2.12 pg, respectively, yielding an average of 2.30 pg. Previously reported values include 2.15 pg for *C. erythraea* (Zonneveld 2019, three measurements from different localities), 2.25 pg for *C. littorale* (two measurements), and 2.53 pg for *C. pulchellum* (five measurements). A theoretical hybrid between *C. erythraea* and *C. pulchellum* would be expected to have a genome size of (2.15 + 2.53) / 2 = 2.34 pg, which corresponds well with the observed value for the unknown plants. This genomic evidence corroborates the identification as *C. erythraea* × *pulchellum*.

Locations in the Netherlands and Belgium

Field populations of these hybrid plants were discovered on both sides of the River Meuse (Fig. 7). The first population was found in July 2023 at Koeweide / Trierveld near Grevenbicht, the Netherlands, on a recently excavated riverbank. This location had already yielded several notable plant discoveries following large-scale habitat restoration, including *Damasonium polyspermum* Coss. (Verloove & Gonggrijp 2025). The hybrid *Centaurium* plants were scattered across several microhabitats within the site, with the largest subpopulation estimated to contain between 1,000 and 2,000 individuals in 2024. Nearby, the parent species *C. erythraea* also occurred, albeit in smaller numbers and with unusually pale flowers.

In July 2024, plants identical to hybrid plants from Grevenbicht were located approximately five kilometres upstream on the Belgian side of the river, in the nature reserve Kerkeweerd at Stokkem. This population was much smaller, comprising only three individuals. A third site was discovered in June 2025, again on the Dutch side of the river, between Maasband and Meers, with approximately 80 individuals. Both the Stokkem and Maasband / Meers sites are comparable to Grevenbicht in habitat, consisting primarily of recently excavated riverbank terrain.

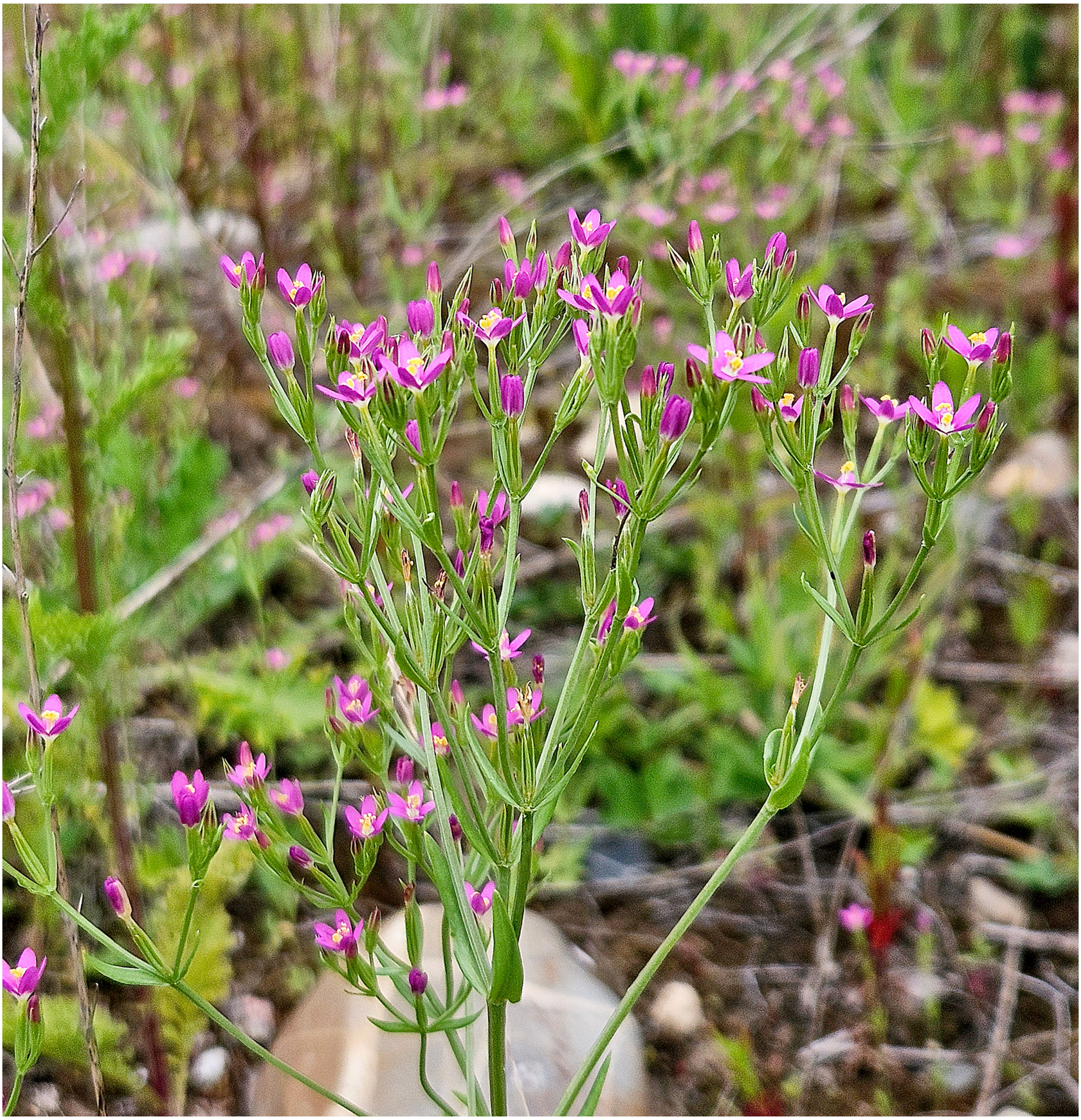

Fig. 2. A plant of the hybrid *Centaurium erythraea* × *pulchellum* in Meers, Province of Limburg (the Netherlands), 28 June 2025. The plants resemble those of *C. pulchellum* (Sw.) Druce but are taller, with more internodes. Photo: S. Gonggrijp.

Table 2. The 2C values of genome sizes measured by flow cytometry of the *Centaurium* species and the expected *Centaurium* hybrid under study.

Taxon / Plant	**Number of measurements**	**2C genome size (pg)**	**Source**
Unknown *Centaurium* plants (Grevenbicht, NL)	2	2.48 / 2.12 (average 2.30)	This study
Centaurium erythraea	3	2.15	Zonneveld (2019)
Centaurium littorale	2	2.25	Zonneveld (2019)
Centaurium pulchellum	5	2.53	Zonneveld (2019)
Expected hybrid (*Centaurium erythraea* × *pulchellum*)	–	2.34 (theoretical average)	calculated

Fig. 3. The hybrid *Centaurium erythraea* × *pulchellum* in Stokkem (Province of Limburg, Belgium), 22 July 2024. The calyx lobes are approximately as long as, or shorter than, the corolla tube. In *C. tenuiflorum* (Hoffmanns. & Link) Fritsch — with which these plants were initially confused — the calyx is typically somewhat to significantly longer than the corolla tube. Photo: W. Vercruysse.

DISCUSSION AND CONCLUSION

Our molecular data offer evidence for a hybrid origin of the unknown *Centaurium* plants along the River Meuse. As in many other species within the genus *Centaurium*, the observed phylogenetic incongruence between nuclear and chloroplast markers (Fig. 6) is best explained by complex evolutionary processes involving both reticulation and polyploidy (Guggisberg et al. 2006, Mansion et al. 2005, Valdés-Florido et al. 2024). In our study, the nuclear ITS sequences placed the unknown individuals firmly within the *C. erythraea* clade, whereas *C. tenuiflorum* and its close allies consistently formed a separate group. However, chloroplast markers (*trnL* intron and *trnL–F* spacer) revealed a different phylogenetic signal, placing the unknown plants instead in a clade with *C. pulchellum* and related species.

This incongruence supports a hybrid origin, and we infer that the unknown plants do not represent a form of the morphologically variable *C. tenuiflorum*, but correspond to the hybrid between *C. erythraea* and *C. pulchellum*. The clustering of the unknown individuals with *C. erythraea* in the nuclear dataset and with *C. pulchellum* in the chloroplast dataset further implies that *C. pulchellum* was the maternal parent in these hybridisation events, having donated its chloroplast genome.

Flow cytometric measurements of genome size further support this interpretation. The two Grevenbicht specimens yielded 2C values of 2.48 and 2.12 pg, respectively. While their simple mean (2.30 pg) falls between the published averages for *Centaurium erythraea* (2.21 pg across two Dutch populations) and *C. pulchellum* (2.55 pg across five Dutch populations) reported by Zonneveld (2019), the considerable discrepancy between the two individuals is particularly noteworthy. With such a small sample size, the average is of limited significance, but the observed variation itself may be more informative. This divergence could indicate genetic heterogeneity within the hybrid population, consistent with ongoing segregation and backcrossing processes expected in the early stages of a hybrid swarm. The genome size of *C. tenuiflorum* subsp. *tenuiflorum* was measured in Turkish material at 2.53 pg (Çiçek et al. 2015). One of our Grevenbicht plants (2.48 pg) falls close to this value, but the genome size of the second (2.12 pg) is much lower. Thus, although the average (2.30 pg) lies well below *Centaurium tenuiflorum*, it is the pronounced discrepancy between the two plants that is most informative. This heterogeneity is inconsistent with an identification as *C. tenuiflorum*, but rather aligns with expectations for hybrid material.

The question of fertility in *Centaurium* hybrids is also relevant here. Wheldon & Salmon (1925) already noted that this particular hybrid was 'quite fertile', suggesting that it is capable of sexual reproduction and potential backcrossing with parent taxa. Consistent with this, we observed occasional individuals with intermediate morphological traits (M24-0797) and detected variation in DNA content among the hybrid plants, both of which point to ongoing hybridisation and the possible formation of a hybrid swarm.

To date, confirmed occurrences of hybrids between *C. erythraea* and *C. pulchellum* have been limited to the British Isles (Stace et al. 2015, Rich et al. 2019, Wheldon & Salmon 1925), France (Fournier 1938, Tison & de Foucault 2014), and Germany (Werner & Hugo 2011). A possible occurrence has also been noted for Switzerland (POWO 2025), although the evidence for this is unclear. Our records from the Netherlands and Belgium appear to represent the first documented and genetically confirmed presence of this hybrid in the Benelux region.

Despite its relative rarity, the hybrid has a complicated nomenclatural history. Over the past century, it has been described under three different names. Druce (1928) proposed *Centaurium ×wheldonianum* Druce, and Fournier (1938) later suggested *C. ×jolivetinum* P.Fourn. However, neither name was accompanied by a valid description: both must be regarded as nomina nuda and are hence invalid (Art. 38.1; Turland et al. 2025). Rich et al. (2019) attempted to formalise the hybrid's name as *C. ×ubsdellii*, based on the earlier work by Wheldon & Salmon (1925). Yet, since they failed to designate a type specimen, this name also remains invalid under the rules of botanical nomenclature (Art. 40.1; Turland et al. 2025).

From an ecological and floristic perspective, the discovery of the hybrid *C. erythraea* × *pulchellum* along the River Meuse is notable. The species appears to favour recently disturbed, gravel-rich alluvial habitats, which are also frequented by both parental taxa. The presence of *C. erythraea* in low numbers nearby, including forms with very pale flowers, may hint at ongoing hybridisation or backcrossing. Furthermore, the detected differences in DNA content among the hybrids could also reflect the emergence of a hybrid swarm. The successful establishment of relatively large hybrid populations, such as at Grevenbicht, suggests that the species possesses sufficient ecological plasticity and reproductive capacity to persist and possibly spread further. Given current patterns of riverine habitat restoration and species introductions via water dispersal, further populations may yet emerge in suitable habitat patches along the river corridor.

Finally, the case of the studied hybrid also invites reflection on the challenges of taxonomy based on morphology alone. Although calyx-to-corolla ratios and floral pigmentation are useful diagnostic characters, they may vary widely in hybrids and backcrosses. In this light, the integration of molecular data, flow cytometry, and classical morphology appears essential to resolve the identity and relationships of taxa within such reticulate complexes. Further research into these complex past

Fig. 4. A plant of the hybrid *Centaurium erythraea* × *pulchellum* in Grevenbicht, Province of Limburg (the Netherlands), 29 June 2024. The inflorescences are broad, lax, and many-flowered and have a paniculate or corymbose structure. Photo: S. Gonggrijp.

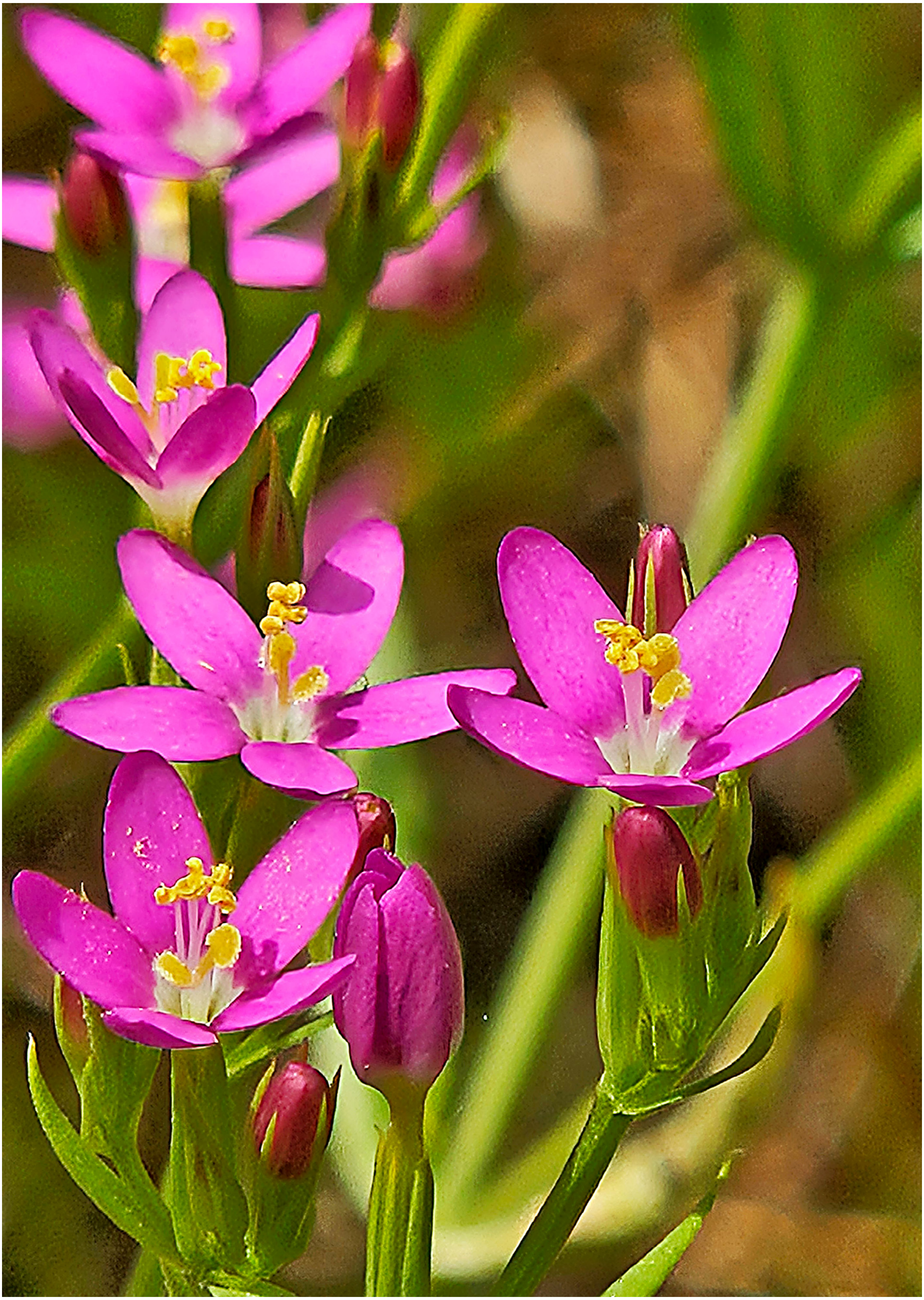

Fig. 5. A plant of the hybrid *Centaurium erythraea* × *pulchellum* in Grevenbicht, Province of Limburg (the Netherlands), 29 June 2024. A close-up of the corollas. Photo: S. Gonggrijp.

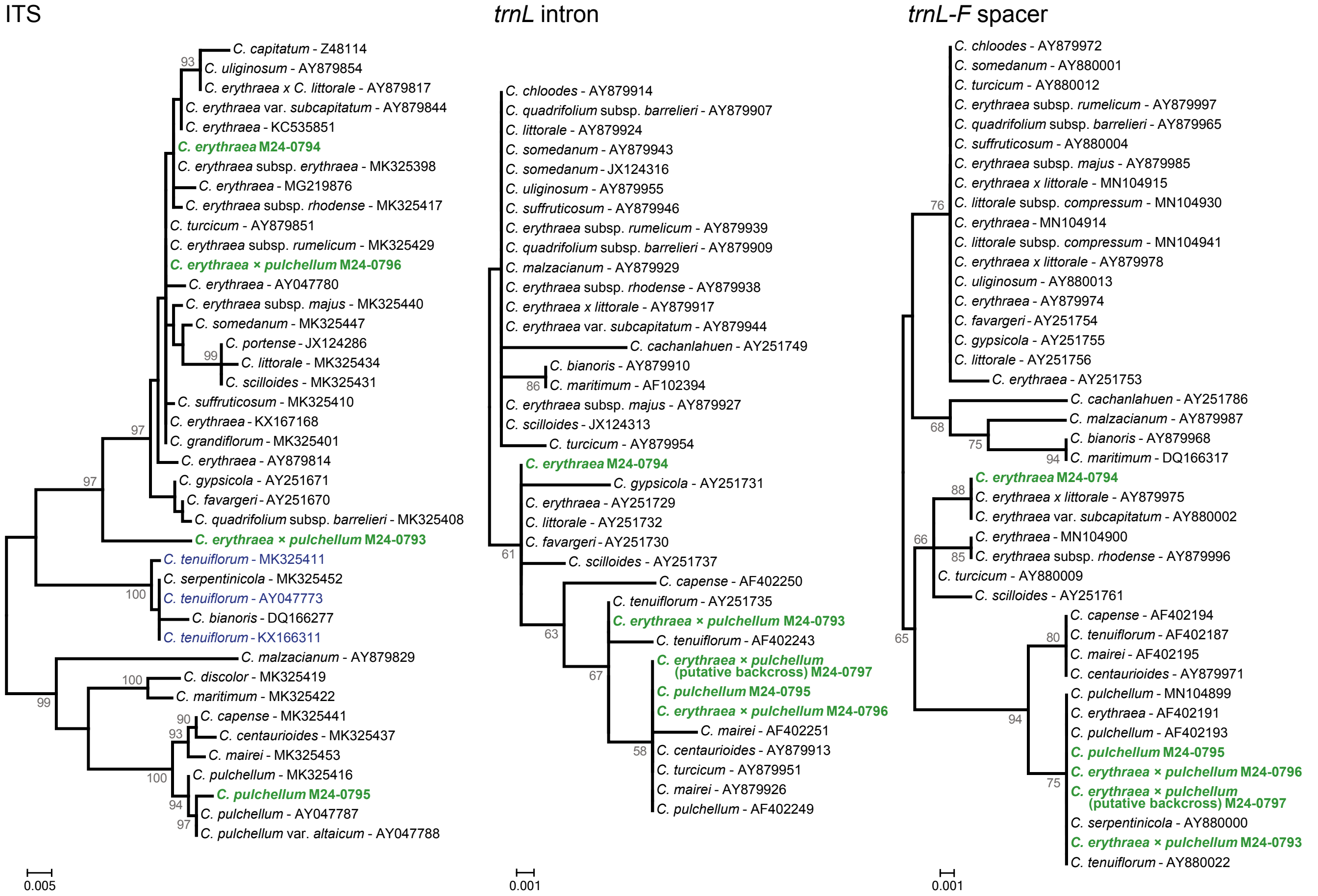

Fig. 6. Maximum-likelihood trees (IQ-TREE) based on the nuclear rDNA internal transcribed spacer (ITS) region and the plastid *trnL* intron and *trnL-F* spacer, illustrating the phylogenetic position of the sequenced *Centaurium* specimens (*C. erythraea* Rafn, *C. pulchellum* (Sw.) Druce, and *C. erythraea* × *pulchellum*). The incongruent phylogenetic placement between nuclear and plastid markers indicates a hybrid origin. Branch values represent UFBoot2 support (1000 replicates). The scale bar denotes the estimated number of substitutions per site.

and possibly ongoing evolutionary processes would benefit from combined population genetic and morphometric analyses across the distribution range of the species and their hybrids.

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Fig. 7. Known distribution of the hybrid *Centaurium erythraea* × *pulchellum* in the Meuse Valley in Belgium and the Netherlands. From north to south: 1. Grevenbicht / Koeweide / Trierveld (Province of Limburg, the Netherlands), 2. Stokkem / reserve Negenoord-Kerkeweerd (Province of Limburg, Belgium), 3. Maasband / Meers (Province of Limburg, the Netherlands). Source background map: www.openstreetmap.org; source of the inset images: PDOK Viewer of the Dutch governmental organisation 'Publieke Dienstverlening Op de Kaart' (PDOK).

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