# *Carex ×kneuckeri*, a hybrid new for central Europe and neotypification of this name

***Carex ×kneuckeri*, nový hybrid pro střední Evropu a neotypifikace jeho jména**

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*Carex ×kneuckeri* (*C. hirta* × *C. rostrata*) is reported here for the first time to occur in south-eastern Poland and central Europe. Although both parents are widespread and common species in Europe, the hybrid is extremely rare or overlooked, worldwide only known from France (one locality), Sweden (two localities) and now also Poland (one locality). *Carex ×kneuckeri* has the general appearance of *C. rostrata*, but has hairy utricles and hairy leaf sheaths, like *C. hirta*. Phylogenetic analysis of plastid *mat*K and (cloned) nuclear ribosomal ITS sequences indicates a close relationship of *C. ×kneuckeri* with *C. rostrata*. The ITS alignment shows complex mutational patterns and a clear dominance of the *C. rostrata* sequence type, but also an indication of the presence of the *C. hirta* sequence type. As no original material of *C. ×kneuckeri* could be traced, a specimen from the Swedish Museum of Natural History (S) is chosen here as the neotype for this name.

K e y w o r d s: *Carex hirta*, *Carex rostrata*, distribution, habitat, ITS, *mat*K, morphology, neotype, new locality, sedges

## Introduction

Hybridization is a relatively common phenomenon and important mechanism in the evolution of vascular plants (e.g. Rieseberg 1995, Arnold 1997, Barton 2001, Wissemann 2005). This phenomenon may have several evolutionary consequences, e.g. increased intraspecific genetic diversity, origin of new ecotypes or species and reinforcement or breakdown of reproductive barriers (Rieseberg 1997, Soltis & Soltis 2009, Soltis 2013). The ability of hybrid species to occupy new niches also means that hybridization can contribute to adaptive radiations (Mallet 2007).

The percentage of species that hybridize varies among families, but around 25% of all plant species hybridize with at least one other species (Mallet 2007). Hybridization is especially frequent in the large genus *Carex* (e.g. Toivonen 1981, Koopman 2010, 2015, Więcław & Koopman 2013, Řepka et al. 2014, Pedersen et al. 2016). However, most of the *Carex* hybrids are restricted to a few sections: *Ceratocystis*, *Glareosae*, *Paludosae*, *Phacocystis* and *Vesicariae* (Cayouette & Catling 1992, Jermy et al. 2007). From a historical

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perspective, long-term research on the genus *Carex* has resulted in a large and increasing number of recognized hybrids (Řepka et al. 2014, Koopman 2015). Kükenthal (1909), for example, included 141 hybrids in the first worldwide *Carex* monograph, while Koopman (2015) mentions 296 *Carex* hybrids occurring in Europe. In addition, several *Carex* species are supposed to be of hybrid origin, e.g. *C. recta* and *C. salina* (Cayouette & Morisset 1985), *C. lucennoiberica* (Maguilla & Escudero 2016), *C. stenolepis* (Pedersen et al. 2016), *C. pseudobrizoides* and *C. curvata* (Koopman 2018).

*Carex* hybrids can usually be recognized by empty utricles and anthers that do not dehisce and are hidden under the male glumes (Rich 1998). Moreover, hybrids are generally morphologically and genetically intermediate in relation to their parental taxa or they show a mosaic of parental, intermediate and unique characters (Cayouette & Catling 1992, Řepka et al. 2013, Więcław & Wilhelm 2014).

*Carex* ×*kneuckeri* is a hybrid between *C. hirta*, in the section *Carex*, and *C. rostrata*, in the section *Vesicariae*. It was first found by Andreas Kneucker near Wissembourg, Alsace, France, about 40 km west of Karlsruhe (Germany); Kükenthal (1909) was the first to give a detailed description of this hybrid in the literature, which is cited below: "*C. hirta* × *rostrata* Kükenth. hybr. nov. – Gracilis. Culmis obtusangulus laevis. Folia 1½–2½ mm lata marginibus subinvoluta utrinque et ad margines cum vaginis pilis brevibus obsita aeque bracteae (perangustae) culmum longe superantia. Spiculae ♂ 2 lineares, harum squamae apice tantum ciliatae. Spiculae + 2 vel 3 tenuiter cylindricae parum remotae, squamae longe aristatae. Utriculi ovato-conici parce breviter hirti, in rostrum longum profunde bifurcatum subsensim attenuati, crura intus scabra. Stigmata 3. Elsass: Weißenburg (Kneucker!)."

Kneucker, who was very active with his exsiccatae during the early 1900s (Kneucker 1896-1911), did not include this hybrid in any of his emissions, or write an article about it. It was possibly present as a small population (most likely a polycormon of one individual) that could not be added to an exsiccatae emission, which requires a lot of material, or maybe Kneucker was not sure about its status and therefore sent it to Kükenthal, who subsequently mentioned it in his magnum opus (Kükenthal 1909).

The French botanist Fournier (1928) was the first to provide this hybrid with a name, *Carex* ×*kneuckeri*, in two lines in a footnote under *C. hirta*, on his newly described hybrid, in which he referred to Kneucker's finding: "*C. ampullacea* × *hirta*: feuilles étroites, peu velues; épi m. lineaire [= leaves narrow, sparsely hairy, masculine spike linear]. – RRR. – Als. (Wissembourg) … × *C. Kneuckeri* P. Fournier." (*Carex ampullacea* Gooden. is a synonym of *C. rostrata*). Furthermore, *Carex* ×*kneuckeri* has only been recorded twice in Sweden more recently (Gustafsson 1979, Edqvist & Karlsson 2007). In the present paper, the first locality in central Europe (and fourth locality in the world) of *C.* ×*kneuckeri* is reported (Fig. 1) and a neotype for this name designated.

*Carex* ×*kneuckeri* is a very rare and poorly known hybrid. Thus, the main aims of this study are to: (i) determine the morphological characters useful for recognizing this hybrid, (ii) identify morphological differences between the hybrid and its putative parental taxa, and (iii) indicate the parental species of *C.* ×*kneuckeri* based on both morphological and molecular evidence.

Fig. 1. – Map showing the distribution of *Carex* ×*kneuckeri* in Europe. ● previously known locality; ▲ newly found locality.

## Material and methods

*Field research, specimen collection and morphological analysis*

The presence of *C.* ×*kneuckeri* in Poland was revealed during a vegetation survey conducted in June 2015 near Chełm, Lubelskie province, south-eastern Poland. Subsequently, two phytosociological relevés were made in 2016 and 2017 using the Braun-Blanquet method (Braun-Blanquet 1964). The two relevés were made in approximately the same place, but in two subsequent years. The phytosociological units comply with the syntaxonomic nomenclature of the vegetation of Europe (Mucina et al. 2016). The nomenclature for vascular plants follows Mirek et al. (2002), Koopman (2015) for *Carex* hybrids, Ball et al. (2002) for *Carex* sections, and Ochyra et al. (2003) for mosses.

The following herbaria were asked for original material of *C.* ×*kneuckeri*: B, K, KR, NCY, P, STR and W, but all in vain. The only two other collections of this taxon, in the Swedish Museum of Natural History in Stockholm (S), were obtained on loan (Appendix 1).

Specimens collected in the field were deposited in the herbaria in Szczecin (SZUB), Leiden (L), Stockholm (S), as well as in the private herbaria of the first and the second author. Living material of the hybrid was collected and is now growing in the University of Warsaw Botanic Garden. Abbreviations of herbaria follow Thiers (2018).

Overall, 135 specimens were included in the morphological analysis (25 specimens of *C.* ×*kneuckeri*, 28 of *C. rostrata*, 29 of *C. hirta*, 28 of *C. lasiocarpa* and 25 of *C. atherodes* (Appendix 1). In addition, herbarium material of *C.* ×*grossii* [*C. hirta* × *C. vesicaria*] and *C.* ×*prahliana* [*C. lasiocarpa* × *C. rostrata*] from B and L was studied for comparison. The following morphological characters were recorded: stem height, leaf and sheath pubescence, leaf width, distribution of stomata on leaves of fresh material, ligule shape, number of male and female spikes, utricle length, width, shape and hairiness, beak length, teeth length, hairiness of male and female glumes, apex of female glumes. The measurements were made using a stereomicroscope with an accuracy of 0.01 mm (utricle size, beak and teeth length, length of the hairs on utricle), a Vernier calliper with an accuracy of 0.05 mm (leaf width) and a ruler with an accuracy of 0.1 cm (stem height).

*Molecular analysis*

DNA sequences were generated of two samples each of *Carex* ×*kneuckeri*, *C. hirta* (sect. *Carex*) and *C. rostrata* (sect. *Vesicariae*) and one of *C. lasiocarpa* (sect. *Paludosae*), all from Poland. These sequences were analysed together with sequences of 15 specimens of six *Carex* species, including C. *hirta*, *C. rostrata* and *C. lasiocarpa* as well as one other species each of sections *Carex* (*C. atherodes*), *Vesicariae* (*C. pseudocyperus*) and *Paludosae* (*C. riparia*), which were all part of the clade containing both parent species of *C.* ×*kneuckeri* in the analysis of Global *Carex* Group (2016). One species each of section *Ammoglochin* (*C. praecox*), *Holarrhenae* (*C. disticha*) and *Remotae* (*C. remota*) were used as outgroup representatives following the Global *Carex* Group (2016). All additional sequences originated from plants collected in the Netherlands, and were generated by the Naturalis DNA barcoding project of Dutch vascular plants (sequences stored in BOLD, Ratnasingham & Hebert 2007), except those of *Carex atherodes*, which were downloaded from GenBank. Specimen information for all samples included in the molecular analysis is summarized in Electronic Appendix 1.

Genomic DNA of the newly sequenced collections from Poland was extracted from air-dried or silica-dried leaf material, using either the NucleoMag® 96 Plant kit (Macherey-Nagel) on the KingFisher™ Flex Purification System (ThermoFisher Scientific) or a standard manual CTAB extraction. Two markers were PCR-amplified and sequenced, a part of the *mat*K gene of the plastid *trn*K/*mat*K region and the nuclear ribosomal ITS1-5.8S-ITS2 region. PCR amplifications were carried out in a final volume of 25 μl and contained 1.5 μl template DNA, 2.5 μl 10× buffer with $MgCl_2$ (or 1.0 μl 25 mM $MgCl_2$ added separately), 1 μl 100 mM BSA (for *mat*K only), 1.0-1.5 μl 2,5 mM dNTPs, 1.0 μl of both forward and reverse primers (10 pMol/μl; partial *mat*K: matK1RKim-f/matK3FKim-r, see Table S2 in de Vere et al. 2012; ITS: 18F/25R, Stech & Frahm 1999; all with M13F or M13R tails attached, respectively), and 1.0-1.25 U (5 U/μl) Taq DNA polymerase (Qiagen, Hilden, Germany). PCR programs were 1 min 96°C, 40 cycles (30 s 96°C, 40 s 53°C, 1 min 72°C), 5 min 72° for *mat*K and 5 min 96°C, 35 cycles (30 s 96°C, 30 s 50°C, 1 min 72°C), 7 min 72° for ITS. In addition, PCR products of ITS of both specimens of *Carex* ×*kneuckeri* were cloned, using the Supercoiled pUC19 control plasmid kit (ThermoFisher Scientific) and Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* cells (Thermo Fisher Scientific) according to the supplier's protocols. After overnight incubation, eight clones of the first and 10 clones of the second specimen were

PCR-amplified. All PCR products were purified and sequenced at BaseClear B.V. (www.baseclear.com) using the M13F and M13R primer tails. Sequences were assembled and edited using Geneious® v8.1.8 (Biomatters Ltd.), submitted to GenBank (Electronic Appendix 1), and manually aligned with the existing sequences in PhyDE v0.995 (Müller et al. 2006).

Phylogenetic reconstructions based on maximum parsimony (MP) and Bayesian inference (BI) were performed using PAUP 4.0b10 (Swofford 2002) and MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), respectively. Both markers were analysed separately. Gaps were treated as missing data. Heuristic searches under parsimony were carried out using random sequence addition with 1000 replicates and tree bisection-reconnection (TBR) branch swapping. Heuristic bootstrap searches were performed using 1000 replicates, and 10 addition sequence replicates per bootstrap replicate. The GTR model was inferred as best-fit model of nucleotide sequence evolution for both markers (GTR+Ă for ITS) according to the Akaike information criterion in jModeltest 2.1.4 (Darriba et al. 2012). Posterior probabilities under BI were calculated based on the Markov chain Monte Carlo method with the settings nst=6 (and rates=gamma for ITS), four simultaneous Markov chains, $2\times10^6$ (*mat*K) or $5\times10^6$ (ITS) generations, and trees sampled every 1000th generation. Fifty percent majority rule consensus trees and posterior probabilities of clades were calculated by combining the four runs and using the trees sampled after the chains converged, excluding the first 25% of trees as "burn-in".

## Results

### *New locality and habitat*

The new site for *C.* ×*kneuckeri* is located west of the village of Nowosiółki, county and municipality of Chełm, Lubelskie Province, south-eastern Poland. It is a fen on chalk substrate, bordering willow and birch thickets (*Salix cinerea*, *S. rosmarinifolia*, *S. aurita* and *Betula pubescens*) belonging to the class *Franguletea*. The hybrid occurs in a 50-m narrow strip of wet, meadow-like vegetation in a depression between crop fields on raised areas in swampy thickets. The water level at the locality is high, even during the summer months (ca. 20 cm above the ground early in July 2016), but it varies significantly over the years as well as seasonally. The fen is agriculturally extensively managed by irregularly mowing (last time 4-5 years ago). The vegetation at this locality is very dependent on hydrological conditions. Generally, it belongs to the classes *Molinio-Arrhenatheretea* and *Phragmito-Magnocaricetea*; a few species are from the *Scheuchzerio palustris-Caricetea fuscae* class (*Carex nigra*, *Epilobium palustre* and *Juncus articulatus*) (see Electronic Appendix 2). During periods of high water, it has the appearance of a loose sedge community with several *Carex* species (e.g. *Carex acuta*, *C. appropinquata*, *C. pseudocyperus*, and *C. rostrata*). In drier years, the share of species belonging to the *Molinio-Arrhenatheretea* class (e.g. *Carex cespitosa*, *C. hirta* and *Lysimachia vulgaris*) increases.

The population of *C.* ×*kneuckeri* at Nowosiółki covers an area of 35 $m^2$. The Polish data imply that the habitat conditions, which seem to be relevant for the occurrence and persistence of *C.* ×*kneuckeri,* are wet conditions, calcareous substrate and extensive agricultural management preventing succession.

Table 1. – Morphological characters of *Carex* ×*kneuckeri*, compared with those of *C. hirta* and *C. rostrata* (according to Chater 1980, Egorova 1999 and Jermy et al. 2007; supplemented with our data); in **bold** are the diagnostic characters.

| Morphological character | *C. hirta* | *C.* ×*kneuckeri* | *C. rostrata* |
|---|---|---|---|
| Stem height | 15–70 (–100) cm | 30–75 (–85) cm | 20–80 (–100) cm |
| Leaf and sheath pubescence | densely hairy | **sparsely hairy** | glabrous |
| Leaf width | 2.0–5.6 (–7.0) mm | (3.0–) 4.0–5.8 (–6.5) mm | 2.0–4.5 (–6.8) mm |
| Position of stomata on leaves | hypostomatic | **amphistomatic** | mainly epistomatic |
| Ligule shape | obtuse | obtuse or rounded | rounded |
| Number of male spikes | 2–3 | 2–4 | 2–4 |
| Number of female spikes | 2–3 | 2–4 | 2–5 |
| Hairiness of utricles | densely hairy, hairs 0.35–0.7 mm long | **sparsely, but clearly hairy, hairs 0.2-0.35 mm long** | glabrous |
| Utricle length | (4.8–) 5–7 (–7.6) mm | (4.6–) 5.0–6.2 mm | (3.4–) 4.0–6.5 mm |
| Utricle width | 1.8–2.5 mm | 1.7–1.9 (–2.1) mm | (1.8–) 2.0–2.5 mm |
| Utricle shape | ovoid, gradually tapering into beak | ovoid, rather abruptly tapering into beak | ovoid, abruptly tapering into beak |
| Utricle beak | scabrid, 1.7–2.6 mm long | scabrid or smooth, 1.4–1.8 mm long | smooth, 1.1-1.5(-1.7) mm long |
| Beak teeth length | 0.8–1.7 mm | 0.4–0.8 mm | 0.25–0.8 mm |
| Hairiness of male glumes | densely hairy | **sparsely hairy, usually on the upper edge or glabrous** | glabrous |
| Hairiness of female glumes | sparsely hairy | sparsely hairy or glabrous | glabrous |
| Apex of female glumes | acuminate-aristate, scabrous-awned | acuminate with short ciliate awn | acute to acuminate |
| Nuts | developed | **undeveloped** | (un)developed |

### *Morphology of Carex* ×*kneuckeri*

Comparison of the voucher specimens of the two collections of *C.* ×*kneuckeri* kept in S (Thiers 2018) shows without any doubt that the Polish plants are identical to the Swedish material, and proves that the former belong to *C.* ×*kneuckeri*. This hybrid is rhizomatous. Old leaf sheaths at the base are dark reddish. The leaves are bluish-green, as in *C. rostrata*, but sparsely hairy on the back side and they have sparsely hairy sheaths. In *C.* ×*kneuckeri* and *C. hirta* the leaves are more or less flat, while in *C. rostrata* the leaves can be flat, canaliculate or with involute margins. Generally, the hybrid resembles *C. rostrata* in habit, but the utricles, leaf sheaths and usually the male and female glumes are hairy (Table 1). The hairs on the utricles are much shorter than in *C. hirta*. Additionally, the female glumes are acuminate with a short ciliate awn and there are stomata on both surfaces of the leaves (visible on fresh material in the field).

### *Molecular analysis*

The *mat*K alignment comprised 25 sequences and 789 positions, of which 25 were parsimony-informative. The ITS alignment comprised 41 sequences and 673 positions, of which 75 were parsimony-informative. The un-cloned ITS sequence of the first *Carex* ×*kneuckeri* specimen showed several double peaks in the chromatograms, which resulted in ambiguous bases at the respective alignment positions (Table 2). Consequently, this sequence was excluded from the phylogenetic analyses. The double peaks corresponded to the bases found in the sequences of *C. hirta* and *C. rostrata*, respectively. All cloned

Table 2. – Positions in the ITS1-5.8S-ITS2 alignment of mutations between *Carex atherodes, C. hirta*, *C. rostrata* and *C.* ×*kneuckeri*. The *C. rostrata* sequence is used as a reference and only bases deviating from this sequence type are indicated. Nucleotide ambiguity codes: R = A or G, S = G or C, Y = C or T, W = A or T

| | ITS1 | | | | | | | | | | | | | | | | | | | 5.8S | | ITS2 | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alignment position | 54 | 58 | 78 | 88 | 106 | 124 | 148 | 158 | 162 | 174 | 198 | 206 | 211 | 224 | 243 | 249 | 252 | 265 | 267 | 310 | 313 | 424 | 452 | 457 | 458 | 762 | 463 | 521 | 560 | 570 | 578 | 587 | 591 | 643 |
| *C. rostrata* | T | A | C | T | T | G | C | T | T | T | A | A | T | A | T | G | A | A | A | A | A | T | T | T | A | A | A | C | A | T | A | C | C | T |
| *C. hirta* | C | G | | C | C | A | G | | | C | T | | | G | C | | G | G | | | | T/Y | C | C | G | | G | | G | C | | G | T | C |
| *C. atherodes* | | G | | C | C | A | | C | C | C | | | C | G | Y | | G | | | | | Y | | C | G | G | G | | G | C | | G | | C |
| *C.* ×*kneuckeri* 1 uncloned | Y | R | | | | R | S | | | Y | W | | | | Y | | R | | | | | | | | R | | R | Y | R | | | S | Y | Y |
| *C.* ×*kneuckeri* 1 clone 8 | C | G | | C | C | A | G | | | C | T | | | G | C | | G | G | G | | | | | | | | | | | | | | | |
| *C.* ×*kneuckeri* 1 clones 7/9/10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | T/Y | | | | | | |
| *C.* ×*kneuckeri* 2 clone 1 | | | G | | | | | | | | | G | | | | A | | | | | | | | | | | | | | | | | | |
| *C.* ×*kneuckeri* 2 clone 3 | | | | | | | | | | | | | | | | | | | | G | | | | | | | | | | | G | | | |
| *C.* ×*kneuckeri* 2 clones 5/6/10 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | G | | | |
| *C.* ×*kneuckeri* 2 clone 7 | | | | | | | | | | | | | | | | | | | | | G | | | | | | | | | | | | | |
| *C.* ×*kneuckeri* all other sequences | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

sequences of both *C.* ×*kneuckeri* specimens had the *C. rostrata* sequence type, except clone 8 of the first specimen, which had an ITS1 similar to *C. hirta* and an ITS2 similar to *C. rostrata* (Table 2). Additional mutations, not found in either *C. hirta* or *C. rostrata* were found in some clones, most of them representing autapomorphies.

The MP analysis of the *mat*K alignment resulted in a single most parsimonious phylogenetic reconstruction (Fig. 2A; length 30, consistency index [CI] 1.00, retention index [RI] 1.00). The respective ITS analysis yielded 10 most parsimonious phylogenetic reconstructions (length 143, CI 0.80, RI 0.90), of which the strict consensus tree is shown in Fig. 2B. The consensus trees of the respective Bayesian analyses are shown in Electronic Appendix 3 (*mat*K) and 4 (ITS), respectively, but Bayesian posterior probabilities (PP) are displayed together with maximum parsimony bootstrap support (BS) values in Fig. 2. In both trees, a clade comprising the representatives of sections *Carex*, *Paludosae* and *Vesicariae* was resolved with maximum support (BS 100%, PP 1.00). Within this clade, the species *Carex pseudocyperus* and *C. riparia* could be distinguished by both markers, with higher bootstrap support in the ITS tree (*mat*K: BS 86/62%, PP 1/0.97; ITS: BS 99/93%, PP1/0.97). *Carex atherodes* formed a clade nested within a *C. hirta* clade (both with a low BS of 64%/62% and PP 0.98/0.97) in the *mat*K tree. In the ITS tree, a clade of *C. atherodes*, *C. hirta* and one clone of C. ×*kneuckeri* was resolved with 0.96 PP. Within this clade, *C. atherodes* was sister to the *C. hirta* clade, the latter with 89% BS. *Carex lasiocarpa*, *C. rostrata* and C. ×*kneuckeri* had identical *mat*K sequences, but the former was clearly separated by ITS (BS 100%, PP 1). All cloned sequences of *C.* ×*kneuckeri* (except clone c8 of the first specimen) as well as the un-cloned sequence of the second specimen were grouped in one clade together with *C. rostrata* (BS 66%) in the ITS tree, forming a polytomy except for four cloned sequences that formed a separate subclade (BS 64%).

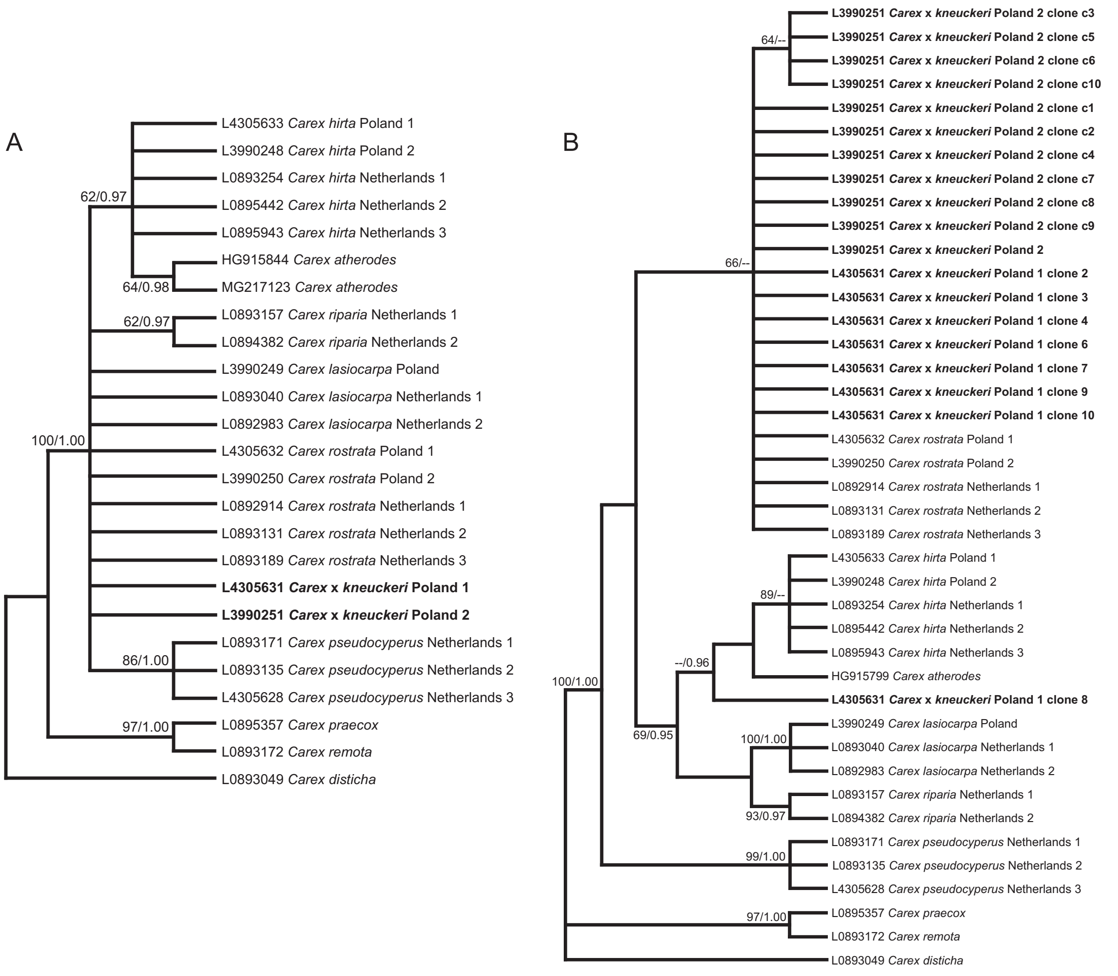


Fig. 2. – Maximum parsimony phylogenetic reconstructions of *Carex* species based on *mat*K (2A) and nuclear ribosomal ITS (2B) sequences, respectively. Bootstrap values >60% and Bayesian posterior probabilities >0.95 from respective Bayesian inference analyses are shown on the branches. Sample codes starting with "L" are herbarium numbers (herbarium L), other numbers are GenBank accession numbers. These numbers are linked to further information on the respective samples in Electronic Appendix 1.

## Discussion

### *Relations between hybrid and putative parental species*

At first glance, this newly recorded hybrid for central Europe from south-eastern Poland has the general appearance of *C. rostrata*. However, it differs from the latter by its hairy utricles, hairy leaf sheaths, usually hairy male glumes, as well as stomata on both surfaces of the leaves. According to Wallnöfer (2006) and Jermy et al. (2007), the distribution of stomata on the surface of leaves is sometimes useful for identifying carices and their hybrids. Almost all *Carex* species have stomata on the lower surface of their leaves (hypostomatic or hypostomous), but *C. nigra* and *C. rostrata* are among the very few sedges that have stomata on the upper surface of their leaves (epistomatic or epistomous) (Wallnöfer 2006). As a consequence, hybrids involving one of these two species have stomata on both sides of the leaves (amphistomatic or amphistomous) (Wallnöfer 2006). However, this character is useful only as a character on fresh specimens in the field, since the stomata are not clearly visible in dried material. Moreover, *C. rostrata* is mainly epistomatic, but occasionally has a few lines of stomata on the lower leaf surface (Jermy et al. 2007). The utricles of the hybrid from Poland do not have well-developed nuts (they are "empty"), but this is also relatively often the case in *C. rostrata*. Because of this similarity with *C. rostrata*, in combination with the hairy utricles, the Polish plants are probably a hybrid of *C. rostrata* with another, closely related species of *Carex* with hairy utricles.

The only tall, marshy sedges in Poland with hairy utricles are *C. atherodes*, *C. hirta* and *C. lasiocarpa*. Figert (1898) found *C. lasiocarpa* × *C. rostrata* in present-day Poland, near Rokitki (then: Reisicht), NW of Legnica (then: Lignitz) in Lower Silesia (Dolnośląskie), and gives a clear description of this hybrid. Afterwards, Junge (1906) provided this hybrid with the name *C.* ×*prahliana*. Since then it has been reported occurring in several countries: Finland, France, Norway, Poland, Russia, Slovakia and Sweden (Koopman 2015). However, as our hybrid has sparsely hairy leaves and leaf sheaths, it is unlikely that the other parent is *C. lasiocarpa*, which does not have hairy leaves or hairy leaf sheaths (Chater 1980). Of the three tall, marshy sedges with hairy utricles mentioned above, only *C. hirta* and *C. atherodes* have hairy leaves and leaf sheaths (Chater 1980). The latter has only a few single hairs, if any, on the utricles (Chater 1980), while the utricles of the Polish hybrid are clearly hairy. Furthermore, *C. atherodes* is extremely rare in eastern Poland and it does not occur in the area where this hybrid is recorded (Rutkowski 2014). *Carex hirta*, in contrast, occurs at the site where the hybrid plants were found. In addition, the hybrid has usually hairy male glumes, a character diagnostic of *C. hirta* (Chater 1980, Egorova 1999).

The molecular data clearly support a position of *C.* ×*kneuckeri* in the same clade as *C. atherodes*, *C. hirta*, *C. lasiocarpa* and *C. rostrata* (Fig. 2). The *mat*K results are generally consistent with uniparental inheritance of chloroplast DNA, identifying *C. rostrata* or *C. lasiocarpa* as one parent of *C.* ×*kneuckeri*. The ITS sequences seem to support biparental inheritance of the nuclear DNA. They support *C.* ×*kneuckeri* as closest to *C. rostrata*, and favour *C. rostrata* over *C. lasiocarpa* as the maternal parent, which accords with the morphological similarity. The *C. rostrata* ITS type is clearly dominant in the hybrid, since it is present in all but one of the sequenced clones and even in the uncloned sequence of the second specimen, indicating that *C.* ×*kneuckeri* is a mother-close primary entity. Based on the available data, the hybrid nature of the second specimen is thus only visible morphologically. The position of the single deviating sequence (clone 8 of the first *C.* ×*kneuckeri* specimen) in the phylogenetic tree suggests either *C. atherodes* or *C. hirta* as the second parent. The mutations occurring in ITS1 are more similar to *C. hirta* than to *C. atherodes* (Table 2), which, together with the non-molecular evidence (morphological similarity to *C. hirta*, absence of *C. atherodes* at the locality in Poland, presence of *C. hirta* at the site with *C.* ×*kneuckeri*) supports *C. hirta* as the second parent and pollen donor of *C.* ×*kneuckeri*. However, the sequence of clone 8 seems to be of hybrid origin itself, since ITS2 is again of the *C. rostrata* type, which probably causes the intermediate and weakly supported position of clone 8 in the phylogenetic reconstructions. The existence of double peaks in the un-cloned sequence suggests that the ITS2 sequence of *C. hirta* is present in *C. ×kneuckeri* as well, but has not been detected among the relatively small number of clones sequenced. A more extensive study of the mutational patterns in ITS and possibly other (nuclear) markers, including more specimens, especially of *C. atherodes*, is desirable. *Carex hirta* hybridizes also with *C. vesicaria* within the section *Vesicariae* to which also *C. rostrata* belongs (Nelmes 1947, Wallace 1975). The hybrid, *C. ×grossii*, resembles *C. hirta* in habit but it is overall much less hairy (Jermy et al. 2007). Thus, some *Carex* hybrids are much closer to one parental species (see above) or sometimes intermediate between the parents, e.g. *C. ×boenninghausiana* (*C. paniculata* × *C. remota*; Kobierski et al. 2018) and *C. ×rotae* (*C. appropinquata* × *C. paniculata*; Jermy et al. 2007).

*Distribution and habitat*

*Carex hirta* and *C. rostrata* are both common species, widespread in Europe (Koopman 2015), but occupying different habitats (Ellenberg 1991). *Carex rostrata* grows at oligotrophic-mesotrophic sites with a high water level for most of the year and usually occurs in peat bogs, wet meadows, banks of lakes and watercourses, whereas *C. hirta* grows in more mesotrophic and especially eutrophic habitats, dry or wet. It tolerates short-term flooding but not standing water for most of the year like *C. rostrata* (Jermy et al. 2007). Occasionally, in wet habitats both species grow together, consequently, such places should be screened for the occurrence of their hybrid. Unfortunately, there are no details of the habitat, vegetation or population size of *C. ×kneuckeri* at the French site in Wissembourg, Alsace, where Kneucker found this hybrid; even the date of the collection is unknown, but obviously it was before 1909 (Kükenthal 1909). It is rather unlikely that this taxon currently exists at its locus classicus because there has been a significant transformation of the habitat around Wissembourg over the course of the last one hundred years.

The more recent Swedish localities are much better described and documented (Gustafsson 1979, Edqvist & Karlsson 2007). The first report of these localities dates back to 1968 when L.-Å. Gustafsson (1979) found *C. ×kneuckeri* at Högestad parish in Skåne (Scania), the southernmost province of Sweden. There, the plants formed a very vigorous stand in a small fen 400 m SW of Allevadstorp. However, when Gustafsson revisited the site in 1978, its hydrology and species composition had changed because the area was no longer used for grazing and there were spruce plantations on the surrounding meadows, resulting in a strong reduction in the size of the patches of *C. ×kneuckeri*, which was threatening its very existence (Gustafsson 1979). There is no material in S collected by Gustafsson. The site at Skåne was visited in 1977 by Th. Karlsson who collected material and recalls that the locality was a small fen on the slope of a hill on calcareous clay (Karlsson, pers. comm.). This place has probably never been visited again since 1978.

The other Swedish population was found in 1985 by J. Josefsson in Tranås municipality, Säby parish, at a horse-grazed fen, 75 m from the northernmost farm in Haga village. This stand was small, covering only ca. 2 $m^2$ (Edqvist & Karlsson 2007). According to its finder, *C. ×kneuckeri* was still present there in the late 1990s (Karlsson, pers. comm.).

*Carex ×kneuckeri* is very rare, probably because its parental species usually do not grow together. However, when parental species occupy overlapping habitats (sympatric


Fig. 3. – Neotype of *Carex* ×*kneuckeri* (photographed B. Kurnicki).

species), spontaneous hybrids appear relatively frequently, e.g. *C.* ×*boenninghausiana*, *C.* ×*elytroides* (*C. acuta* × *C. nigra*), *C.* ×*evoluta* (*C. lasiocarpa* × *C. riparia*) and *C.* ×*involuta* (*C. rostrata* × *C. vesicaria*) (Koopman 2015).

We have observed the *Carex* ×*kneuckeri* population in Poland for three years during which time it has been in good condition, which indicates it is able to thrive at this locality. This hybrid is completely sterile and does not tend to spread. A lot of hybrids within the genus *Carex* are also sterile (Toivonen 1981, Cayouette & Catling 1992), but others are partially fertile, especially in the sections *Phacocystis*, *Vesicariae* and *Ceratocystis* (Schmid 1982, Cayouette & Morisset 1985, Jermy et al. 2007, Pedersen et al. 2016). In the latter case, there is continuous hybridogenesis with the result that there are large populations of mother-father-like individuals (hybrid swarms), sometimes morphologically intermediate and capable of spreading in the landscape as separate living entities (Wallace 1975). Fertility is one of the factors stabilizing hybrid-derived individuals and in effect this gives rise to new species, i.e. an independent, morphologically and genetically recognisable and self-reproducing entity. Thus, spontaneous recurrence and survival of hybrids under natural conditions are a driving force of plant speciation (e.g. Barton & Hewitt 1989, Mallet 2007, Soltis 2013).

## Neotypification

All our efforts to detect Fournier's/Kneucker's *C.* ×*kneuckeri* in European herbaria (B, K, KR, NCY, P, STR and W), where original material might have been kept, were in vain. It is known that a lot of herbarium material was destroyed in the Second World War, especially in Berlin (B) and Karlsruhe (KR). This is unfortunate as the greatest chance of finding any of Fournier's/Kneucker's material of *C.* ×*kneuckeri* was in these two herbaria, as Kneucker lived in Karlsruhe and Berlin had, and still has, the largest herbarium in Germany. Accordingly, it is necessary to choose a neotype because there is no material found nor known that can be identified as having been seen by Fournier when describing this hybrid, which could be selected as a lectotype, following Art. 9.8 of the ICN (Turland et al. 2018). Therefore, the oldest known material of *C.* ×*kneuckeri*, a specimen from Stockholm (S), Sweden, was chosen here as the neotype for the name of this hybrid:

*Carex* ×*kneuckeri* P. Fourn., Fl. Compl. Plaine Franç.: 560, 1928 (*Carex hirta* L. × *Carex rostrata* Stokes) – **Neotype** (**designated here**): "Sweden, province of Skåne; Högestad, Allevadstorp; 29 Jul 1977; *T. Karlsson* [S: No. N08/93-860]" (Fig. 3).

See www.preslia.cz for Electronic Appendices 1–3

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## Souhrn

V jihozápadním Polsku byla nalezena *Carex ×kneuckeri*, kříženec ostřic *C. hirta* a *C. rostrata*. Jedná se o první údaj ze střední Evropy. Přestože jsou oba rodičovské druhy široce rozšířené a běžné, tento jejich kříženec je buď velmi vzácný, nebo přehlížený, protože byl předtím zaznamenán pouze na jedné lokalitě ve Francii a na dvou ve Švédsku. *Carex ×kneuckeri* se nejvíce podobá druhu *C. rostrata*, od které se liší chlupatými mošničkami a listovými pochvami, což jsou znaky zděděné po druhém rodiči. Fylogenetická analýza sekvencí úseku *matK* chloroplastové DNA a klonů úseku ITS jaderné DNA prokázala podobnost křížence s *C. rostrata*. Alignment sekvenačních dat z ITS odhalil hybridní strukturu s dominancí signálu *C. rostrata*. Protože nebyl nalezen žádný originální materiál vztahující se ke jménu *Carex ×kneuckeri*, byl jiný sběr tohoto křížence ze Švédska vybrán jako neotyp.

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Appendix 1. – List of specimens included in the morphological analysis.

*Carex ×kneuckeri*
**Poland**: Lubelskie province, county and municipality of Chełm, W of the village of Nowosiółki, NW of Chełm, N 51.18436, E 23.32405, very wet swamp, bordering a *Salix*-thicket, 25 Jun 2015, P. Kalinowski (herb. P. Kalinowski, herb. Jac. Koopman); idem, 1 Jul 2016, P. Kalinowski, H. Więcław & Jac. Koopman (herb. P. Kalinowski, herb. Jac. Koopman); idem, 17 Jun 2017, P. Kalinowski, H. Więcław & Jac. Koopman (L, S, SZUB, herb. P. Kalinowski, herb. Jac. Koopman; 25 specimens for measurements). – **Sweden**: Province of SkÍne, Högestad, Allevadstorp, 29 Jul 1977, T. Karlsson (S: No. N08/93-860/861; 860: neotype); Province of SmÍland, Säby par., Haga, 1985, J. Josefsson, det. T. Karlsson, 2001 (S: No. N391-1).

*Carex atherodes*
**Armenia**: Lake W of village of Getavan, near old airport, lake border, 1481 m, N 41°3'12.96", E 44°19'30.828", 8 Jul 2018, Jac. Koopman (herb. Jac. Koopman; 2 specimens). – **Estonia**: Tartumaa, Nõo municipality, along the gravel road, along the River Elva, at the Elva-Vitipalu MKA border, N 58°11'33.396", E 25°26'27.132", 25 Jun 2011, T. Kull (TAA 0046883; 2 specimens); Võrumaa, Haanja Municipality, Ala-Suhka, Upper River Piusa and lake, N 57°41'6.36", E 27°8'49.2", 15 Sep 2012, T. Kull (TAA 0046884); 2 specimens). – **Poland**: Lower Silesia, Ścinawa municipality, Odra valley, SE of the village of Lisowiec, 12 Jun 2007, E. Kozioł (WRSL); West Pomerania, Mielęcinek, Jezioro Jasne (lake), N 53°2'34.174", E 14°53'58.085", 21 Jun 2011, H. Więcław & Jac. Koopman (SZUB; 8 specimens); West Pomerania, Ińsko, wet meadow, N 53°27'9.972", E 15°33'25.934", 29 May 2012, H. Więcław & Jac. Koopman (SZUB; 8 specimens); West Pomerania, E of Storkowo, N of Insko, along *Phragmites* in depression of a former lake, N 53°27'7.2", E 15°37'58.8", Jac. Koopman 43.2.98.5 (herb. Jac. Koopman; 2 specimens).

*Carex hirta*
**Armenia**: Geghark'unik' mars, NE side of Lake Sevan, near Pambak, wetland near village, 1965 m, N 40°22'55", E 45°31'34", 04 Jul 2015, G. Fayvush, M. Oganesian, Jac. Koopman, H. Więcław & E. Vitek 43.2.45.21 (herb. Jac. Koopman; 3 specimens). – **Austria**: NE of Jedlersdorf Vienna, along Marchfeldkanal along motorway Donaufer Autobahn, N 48°10'12", E 16°12'36", 30 May 2015, H. Więcław & Jac. Koopman 43.2.45.22 (herb. Jac. Koopman; 3 specimens). – **Bulgaria**: NE of Kolarovo, SW Bulgaria, on river valley slope; wet seepage meadow, 299 m, N 41°13'12", E 23°3'36", 07 May 2013, Jac. Koopman 43.2.45.14 (herb. Jac. Koopman; 3 specimens). – **Germany**: Between Dessau and Aken, N side of road L63; Hirschheckerdam, rough roadside along forest path, 27 May 2012, Jac. Koopman 43.2.45.13 (herb. Jac. Koopman). – **Netherlands**: Wyldemerk, Gaasterland, Fryslân, N 52°52'8.368", E 5°31'31.908", 13 Jun 1989, Jac. Koopman 43.2.45.1 (herb. Jac. Koopman; 2 specimens); Wons, along A7, by petrol station, Fryslân, roadside, N 53°5'32.55", E 5°25'33.661", 23 May 2011, Jac. Koopman 43.2.45.6 (herb. Jac. Koopman; 2 specimens). – **Poland**: West Pomerania, between Zamecin and Lubiana, W of Choszczno, wet seepage meadow, north of road, 04 Jun 2008, Jac. Koopman 43.2.45.5 (herb. Jac. Koopman); West Pomerania, near Żensko, 8 km S of Choszczno, roadside alongside a *Brassica*-field, 14 May 2010, Jac. Koopman 43.2.45.2 (herb. Jac. Koopman); West Pomerania, along Jezioro Miedwie (lake), S of Wierzbno, N of Pyrzyce, wet meadow along the lake, 02 Jul 2011, Jac. Koopman 43.2.45.10 (herb. Jac. Koopman); Lubuskie,near old cemetery of Moczele, Drawienski Park Narodowy, mixed forest border, along sandy path, 10 May 2012, Jac. Koopman 43.2.45.12 (herb. Jac. Koopman); West Pomerania, SW of Żensko, 8 km S of Choszczno, shore of small lake, in meadow, 28 May 2012, Jac. Koopman 43.2.45.11 (herb. Jac. Koopman); West Pomerania, NW of Zatom, Drawienski Park Narodowy, wet meadow, open place along river Drawa, N 53°4'48", E 15°30'0", 14 Aug 2012, Jac. Koopman 43.2.45.19 (herb. Jac. Koopman); West Pomerania, E of Nowa Korytnica, along main road, side of road running through forest, N 53°7'48", E 16°0'36", 09 Jun 2013, Jac. Koopman 43.2.45.18 (herb. Jac. Koopman); Pomerania, Sławoszynko, forest edge, N 54°29'24", E 18°8'24", 21 Jul 2013, H. Więcław, B. Kurnicki & Jac. Koopman (SZUB); Mazowieckie, E of Zaleś, N of road, Korczew, wet meadow, 05 Jun 2014, N 52°13'48", E 22°19'48", Jac. Koopman 43.2.45.20 (herb. Jac. Koopman); West Pomerania, Choszczno, ruderal roadside on sand in abandoned field, N 53°5'24", E 15°15'0", 10 Jun 2015, Jac. Koopman 43.2.45.23 (herb. Jac. Koopman); West Pomerania, E of Giżyn, municipality Pyrzyce, wet meadow along Lake Miedwie, N 53°13'22.404", E 14°51'41.292", 05 Jun 2016, Jac. Koopman 43.2.45.24 (herb. Jac. Koopman); West Pomerania, near Wierzbno, SE of Lake Miedwie, shaded grassland near road, N 53°13'18.66", E 14°55'44.58", 28 May 2017, Jac. Koopman 43.2.45.26 (herb. Jac. Koopman). – **Switzerland**: Graubünden, S of Tarasp Fontana, road to Alp Plavna, roadside in *Picea*-forest, a few hundred metres S of Parking, 1498 m, N 46°46'21.432", E 10°15'14.292", 30 Jun 2017, Jac. Koopman 43.2.45.25 (herb. Jac. Koopman; 3 specimens).

*Carex lasiocarpa*
**Netherlands**: Zuidveen, N of Bramenweg, Overijssel, depression in grassland, N 52°45'28.141", E 6°9'6.109", 27 Jun 1987, Jac. Koopman 43.2.30.1 (herb. Jac. Koopman; 2 specimens); Hoannekrite, Eernewoude, Fryslân, wet, peaty grassland, N 53°6'50.918", E 5°56'37.378", 28 May 1988, Jac. Koopman 43.2.30.2 (herb. Jac. Koopman; 2 specimens); Weerribben, Ossenzijl, Overijssel, *Phragmitetum sphagnetosum*, 29 Jun 2002, N 52°47'11.638", E 5°58'17.494", Jac. Koopman 43.2.30.3 (herb. Jac. Koopman; 4 specimens); Twijzeler Mieden, Twijzel, Fryslân, wet marsh, N 53°13'51.665", E 6°6'49.331", 26 May 2006, Jac. Koopman 43.2.30.4 (herb. Jac. Koopman; 4 specimens). – **Norway**: Killingkjolen, Mali-fjellet, NW of Budor, Hedmark, open forest bog on slope, 700 m, N N 60°34'48", E 11°8'24", 17 Jul 2011, Jac. Koopman 43.2.30.6 (herb. Jac. Koopman; 2 specimens). – **Poland**: West Pomerania, road to Łowiska, little lake of Łowiska (Jezioro Jeleń), kettlehole bog along lake shore, *Scheuchzerietum*, 12 Jun 2006, Jac. Koopman 43.2.30.5 (herb. Jac. Koopman; 4 specimens); Lubuskie, near Chłopiny, N of Gorzów, wet place along Road 3, 31 May 2008, Jac. Koopman 43.2.30.7 (herb. Jac. Koopman; 4 specimens); West Pomerania, reserve Kamienna Buczyna, Ińsko, 29 May 2012, Jac. Koopman 43.2.30.8 (herb. Jac. Koopman; 2 specimens); Pomerania, Białogóra, edge of dune valley, N 54°29'24", E 17°3'28.8", 20 Jul 2013, H. Więcław, B. Kurnicki & Jac. Koopman (SZUB; 2 specimens); West Pomerania, Forestry Jacinki, N of Polanów, W of road 205, border of small lake, N 54°9'58.284", E 16°40'15.6", 08 Jun 2017, Jac. Koopman 43.2.30.9 (herb. Jac. Koopman; 2 specimens).

*Carex rostrata*
**Armenia**: Lorri mars, c. 8.5 km WWNW of Stepanavan, road Urasar – Katnaghbyur, c. 1 km NW of Urasar, c. 300 m N of road, depression in wetland, 1560 m, N 41°01'24", E 44°16'59", 05 Jul 2015, G. Fayvush, M. Oganesian, Jac. Koopman, H. Więcław & E. Vitek 43.2.48.13 (herb. Jac. Koopman; 3 specimens); Road Vorotan pass to Sisian, N of Spandarian reservoir, dry slopes and gravel hills with humid depressions, along former fish ponds, 2072 m, N 39°41'06", E 45°46'40", 15 Jun 2016, H. Więcław & Jac. Koopman 43.2.48.15 (herb. Jac. Koopman; 2 specimens). – **Estonia**: 4 km W of Kohila, border of bog, 03 Aug 1992, Jac. Koopman 43.2.48.5 (herb. Jac. Koopman; 2 specimens); N of Riisipere, dry forest bog on calcareous soil, 26 Jul 1999, Jac. Koopman 43.2.48.2 (herb. Jac. Koopman; 2 specimens). – **Germany**: Rottforde, Westerstede, wet forest, 20 May 1989, Jac. Koopman 43.2.48.1 (herb. Jac. Koopman). – **Italy**: San Valentino Alla Muta, along lake Haide, Val Venosta, lake shore, 1452 m, N 46°45'32.04", E 10°32'10.716", 26 Jun 2017, Jac. Koopman 43.2.48.17 (herb. Jac. Koopman; 2 specimens). – **Norway**: W of Dravlaus, W of Dalsfjorden, mun. Volda, peaty shore around mountain lake, 360 m, 05 Aug 2000, Jac. Koopman 43.2.48.3 (herb. Jac. Koopman; 2 specimens); Killingkjolen, Mali-fjellet, NW of Budor, Hedmark, open forest bog on slope, 700 m, N 60°34'48", E 11°8'24", 17 Jul 2011, Jac. Koopman 43.2.48.8 (herb. Jac. Koopman). – **Poland**: West Pomerania, road to Łowiska, small lake of Łowiska (Jezioro Jeleń), Kettlehole bog along shore of lake, *Scheuchzerietum*, 12 Jun 2006, Jac. Koopman 43.2.48.4 (herb. Jac. Koopman; 2 specimens); Lubuskie, Chłopiny, NW of Gorzów Wlk., along *Alnetum*, in ditch, 31 May 2008, Jac. Koopman 43.2.48.16 (herb. Jac. Koopman; 2 specimens); West Pomerania, W of Bonin, N of Choszczno, bog (torfowisko), 04 Jun 2009, Jac. Koopman 43.2.48.12 (herb. Jac. Koopman; 2 specimens); West Pomerania, near Przytocko, E of Polanów, marsh around a small lake/pond, in small ditch, 13 Jun 2009, Jac. Koopman 43.2.48.9 (herb. Jac. Koopman; 2 specimens); Pomerania, near Władysławowo, edge of bog, N 54°28'48", E 18°8'24", 20 Jul 2013, H. Więcław, B. Kurnicki & Jac. Koopman (SZUB; 2 specimens); West Pomerania, Inoujście, mun. Goleniów, *Magnocaricion*, N 53°32'0.096", E 14°38'44.844", 25 May 2016, H. Więcław & Jac. Koopman 43.2.48.14 (herb. Jac. Koopman). – **Switzerland**: E of Vna, Graubünden, wet seepage place in meadow, 1900 m, N 46°50'37.864", E 10°22'35.789", 14 Jul 2008, Jac. Koopman 43.2.48.11 (herb. Jac. Koopman; 2 specimens).

*Carex ×grossii* (*C. hirta* × *C. vesicaria*)
**Ireland**: Cult. Kew Herbarium Garden, orig. coastal marsh, Magherabeg, Co. Wicklow, 1944, J. P. Brunker, det. E. Nelmes, 1 Jul 1951 (L). – **Poland**: West-Preußen [Pomorskie], Tiegendorf [Nowy Dwór Gdański], along ditch, 13 Jun 1897, R. Gross (B).

*Carex ×prahliana* (*C. lasiocarpa* × *C. rostrata*)
**Poland**: Dolnośląskie, Liegnitz [Legnica]: Reisicht [Rokitki] im Torfstick (?), unter d. Stammeltern l. cl., 2 Jul 1898, Figert (B).