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A systematic conservation strategy for crop wild relatives in the Czech Republic

Nigel G. Taylor[1*], Shelagh P. Kell[2], Vojtěch Holubec[3], Mauricio Parra-Quijano[4], Karel Chobot[5] and Nigel Maxted[2]

[1] School of Biology, University of Leeds, LS2 9JT, UK. Email: ngltaylor@yahoo.com

[2] School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

[3] Gene Bank, Crop Research Institute, Drnovská 507/73, 161 06 Praha 6 – Ruzyně, Czech Republic.

4 The International Treaty on Plant Genetic Resources for Food and Agriculture, FAO, Via delle Terme di Caracalla, 00153 Rome, Italy

[5] Nature Conservation Agency of the Czech Republic, Kaplanova 1, CZ-140 00 Prague 4, Czech Republic

Corresponding author: Nigel G. Taylor (ngltaylor@yahoo.com)

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Ⓐ ABSTRACT

Aim To create a crop wild relative (CWR) conservation strategy for the Czech Republic: the first national CWR conservation strategy for Central and Eastern Europe.

Location Czech Republic

Methods We generated a CWR checklist for the Czech Republic and then prioritized taxa, using widely adopted criteria modified with input from local experts, to create a national CWR inventory. For 204 priority CWR species, we collated 206 760 presence records. We carried out spatial analyses to identify patterns in species richness, gaps in existing conservation actions, complementary conservation networks and collecting strategies to increase representativeness of gene bank accessions. We considered both specific and genetic conservation, using geographic and ecogeographic proxies for the latter.

Results Passive *in situ* conservation of CWR in the Czech Republic is comprehensive at present, with all but one priority CWR species being contained in protected areas. Active *in situ* CWR conservation could be focussed within eleven ca. 10km by 10km grid cells containing 94% of priority species, or their overlapping protected areas. To augment the genetic coverage of the *in situ* conservation network, active CWR conservation is encouraged within eleven supplementary areas. Meanwhile, there are huge gaps in *ex situ* collections, with no known conserved material for 134 of the 204 priority species. Furthermore, existing accessions are generally unrepresentative of genetic diversity.

Main conclusions In the Czech Republic, active *in situ* conservation of priority CWR should be instigated within the 22 recommended grid cell areas or their 14 overlapping protected areas. For *ex situ* conservation, strategic and targeted collection of germplasm would markedly increase the value of gene bank collections. Diversity of priority Czech CWR is concentrated in South Moravia, making this a particularly important CWR area for the country and for Europe.

Ⓐ INTRODUCTION

As the global population grows and the climate changes, concerns over food security are rising to the forefront of scientific and public agendas. Alongside reducing wastage and meat consumption, a key strategy for food security will be to increase crop yields (Godfray *et al.*, 2010). This must be implemented in the face of climate change reducing or negating the utility of current crop cultivars, in line with targets for reducing greenhouse gas emissions and increasing water efficiency, and in synchrony with changing market demands (Lusser *et al.*, 2012). However, since domestication is associated with genetic bottlenecks and reduced diversity (Tanksley & McCouch, 1997), the genetic base within cultivars and landraces of many crops is likely to be too narrow to facilitate future breeding and adaptation to change (Hajjar & Hodgkin, 2007).

Crop wild relatives (CWR) are wild plant taxa related to crops. They have potential use as gene donors in crop improvement programmes because many possess desirable traits, such as resistance to pests and diseases or tolerance to abiotic stresses like drought, heat and flooding (Hodgkin & Hajjar, 2008). Modern cultivars of most major crops already contain some genes from CWR (Heywood *et al.*, 2007; Lebeda *et al.*, 2009), and CWR will continue to provide a source of genetic material to improve crop yields, enhance nutritional qualities and modify husbandry requirements under future environmental change (Maxted *et al*., 2007; Ford-Lloyd *et al*., 2011; Maxted & Kell, 2009; Maxted *et al*., 2007).

However, like many other wild plants, CWR face threats such as intensive agriculture, urban development, pollution and biological invasions (Bilz *et al*., 2011; Kell *et al*., 2012b, 2015) and thus command urgent conservation attention (Maxted *et al*., 1997b; Heywood *et al*., 2007; Kell *et al*., 2008). This conservation need is recognized in international policy and legislation, including the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA; FAO, 2001), CBD Strategic Plan (SCBD, 2010) and Global Strategy for Plant Conservation 2011–2020 (SCBD, 2014), the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (FAO, 2011) and the EU Biodiversity Strategy to 2020 (EP, 2012).

Towards meeting European policy commitments, the European Union FP7-funded PGR Secure project (www.pgrsecure.org) sought to research novel characterization and conservation strategies for European CWR and landrace diversity (University of Birmingham, 2011–2015). This included the development of conservation strategies for individual nations within which practical conservation actions will be implemented, even when driven by policy at an international level (Maxted *et al*., 2015). Here, we develop one such conservation strategy for CWR in the Czech Republic as both a useful conservation tool in itself, and, as the first of its kind in Central and Eastern Europe, a catalyst for the development of other strategies in the region.

In 1993, the Ministry of Agriculture of the Czech Republic established the 'National Programme on plant genetic resources conservation and utilization' (Dotlačil & Stehno, 2008). The National Programme became law in 2003 (Act No. 148/2003 and Decree No. 458/2003) with an amendment bill following in 2013 (Act. No. 232/2013). Since 1993, more than 5000 accessions of CWR (mostly of grasses and fodder legumes) have been accumulated from the Czech Republic and neighbouring border regions (Holubec *et al*., 2010). However, there has not yet been any systematic planning for CWR conservation in the country.

Thus, we present a multifaceted conservation strategy for CWR in the Czech Republic which aims to efficiently but comprehensively conserve both taxonomic and genetic diversity of the most important Czech CWR. We follow a four-step, systematic (*sensu* Margules and Pressey, 2000) framework for the development of a CWR conservation strategy: (a) production of a CWR checklist (b) prioritization of this checklist (c) *in situ* conservation analysis for priority CWR species and (d) *ex situ* conservation analysis for priority CWR species. The results are formulated into a national CWR conservation strategy that provides a spatial and taxonomic blue print for practical CWR conservation.

Ⓐ METHODS

Ⓑ CWR checklist and inventory

A CWR checklist details all CWR present in a country as a starting point for conservation analysis (Maxted *et al.*, 2013, 2015). Following the methodology of Kell *et al.* (2008, 2015), the Czech CWR checklist contains 3283 species (or 3512 taxa, including subspecies and varieties). These are all taxa (excluding hybrids) from the Checklist of Vascular Plants of the Czech Republic (Danihelka *et al*., 2012) within any of 7430 genera on a global crop list, derived from the CWR Catalogue for Europe and the Mediterranean (Kell *et al*., 2005) and the Czech National Crop Database (EVIGEZ, 2012).

Priority CWR taxa (the most important targets for conservation) were then selected from the checklist to form a CWR inventory. During prioritization, all infraspecific taxa were considered separately (see Appendix S1 in Supporting Information), but were amalgamated to species for subsequent conservation analyses. Prioritization was based on the following five criteria, identified through discussions with local experts, data inspection and literature review. Taxa had to meet all criteria 1 to 3, and either 4 or 5, to be considered for prioritization.

1. Wild (not existing in solely cultivated populations).
2. Forms self-sustaining populations (not casual; Pyšek *et al.*, 2012).
3. Native, or naturalized archaeophyte (not neophyte; Pyšek *et al.*, 2012).
4. Related to a crop of high socioeconomic value to the Czech Republic. Local experts deemed that food and feed (i.e. forage and fodder) crops are most important economically and for food security. Major Czech food crop genera were identified using FAO crop value statistics for the Czech Republic (FAOSTAT, 2012). Feed crops were identified following use categories in EVIGEZ (2012) and GRIN (2012), with the least important for the Czech Republic rejected by local expertise. To reduce their dominance in the priority list, grasses with a large range across Europe were also rejected (occurring in 30 or more Euro+Med (2006–) geographic units, and thus likely to be conserved – if only passively – elsewhere).
5. Endemic (according to Gerža, 2009). By definition, the sole responsibility for *in situ* conservation of endemic taxa lies with the country in which they exist.

Final review by experts led to the removal of taxa for which it is difficult to justify investment of conservation resources: nationally widespread (recorded in more than 90% of ca. 10km by 10km grid cells covering the Czech Republic; AOPK ČR 2012) and common, weedy taxa. This yielded a final list of 222 priority taxa (in 204 species), characterized in the inventory (Appendix S1).

Ⓑ Distribution data

To facilitate spatial analyses, Czech presence records for the 204 priority species (or their synonyms in Kubát *et al.*, 2002) were collated from the species occurrence database of the Nature Conservation Agency of the Czech Republic (AOPK ČR, 2012), collecting databases at the Crop Research Institute, Prague (Holubec *et al.*, 2010) and GBIF (2012). Where applicable, records matched prioritized infraspecific taxa (Appendix S1). If these sources yielded fewer than 50 records for a species, additional location data were retrieved from Czech herbarium records (MZM, 2013) and georeferenced online (www.mapy.cz). Additionally, gene bank databases (EVIGEZ, 2014; GENESYS, 2014) were queried for accessions of wild Czech origin. Across all records, filters based on accession number or species, plus location and date of record, were used to remove spatiotemporal duplicates. For location data, records from before 1950, from gardens or to fewer than three decimal places were excluded.

The final distribution database contained 206 760 unique records (mean 1014, median 196, range 1 to 19 086 records per species), including 639 spatially distinct georeferenced accessions of 66 species. Most records (99.1%) came from AOPK ČR (2012) (full breakdown in Appendix S2).

Ⓑ *In situ* conservation analyses

Spatial analyses on priority CWR species were performed in DIVA GIS 7.5 (Hijmans *et al.*, 2011) and ArcMap 10.1 (ESRI, 2012). Statistical analyses were performed in R 3.1.0 (R Development Core Team, 2014) and SPSS 21.0 (IBM Corp. 2012).

First, gap analysis identified priority CWR species un- or under- represented in existing protected areas (PAs) (Burley, 1988; Maxted *et al*., 2008a). We considered PAs that are designated nationally as Specially Protected Areas (IUCN Categories I – V) (EEA 2014a) or by the European Union as part of the Natura 2000 network (EEA 2014b). The location of every presence record was compared to shapefiles representing these PAs, and the number of PAs in which each species has been recorded was counted. Where a record was situated in overlapping PAs, only the largest PA was counted. Unrepresented species have no records in existing PAs. Underrepresented species have records in fewer than five spatially distinct PAs: below a threshold suggested to confer resilience to stochastic and anthropic species extinction, and sample the majority of common or widespread alleles in CWR (Marshall & Brown, 1975; Brown & Briggs, 1991; Dulloo *et al*., 2008). Species recorded in just a single PA, therefore most vulnerable to stochastic loss, were highlighted separately.

Second, patterns of priority CWR richness were explored. Observed species richness was described both on a grid of ca. 10km by 10km cells, and using a circular neighbourhood method (cell size 30 arc-seconds, diameter 10km) to reduce the influence of the arbitrary locations of grid cells (Scheldeman & van Zonneveld, 2010). Predicted species richness was examined using species distribution models (SDMs) created in MaxEnt version 3.3.3k (Phillips *et al*., 2004, 2006). Robust models were retained for further analyses: models based on more than 10 spatially distinct presence records and performing better than random (AUC > 0.7 and/or significantly > 0.5 in most replicate model runs; Appendix S2). Input data included thirteen relevant environmental variables and a bias file of the number of priority CWR records across the country to correct for uneven sampling effort (see also Appendix S2).

Third, complementarity analysis yielded a spatial network that most efficiently conserves priority CWR species. Complementarity analysis is an iterative selection procedure in which the location with the highest number of taxa is selected first, then these taxa are excluded from the analysis and the process is repeated until all target taxa have been included (Rebelo, 1994). Rebelo's reserve selection algorithm was applied to CWR presence records in DIVA GIS, terminating when all 204 priority species were included in a network of 10km by 10km cells. There are diminishing returns as cells are added to a complementary network, so we selected a subset as priorities.

Fourth, *in situ* conservation plans based on complementarity were augmented to increase the genetic diversity they contain. In the absence of comprehensive data on CWR genetic variation, two commonly used proxies were considered. A geographic proxy assumes that genetic variation in plants is structured across their geographical range, reflecting historical processes and current local regimes of selection, drift and gene flow (Loveless & Hamrick, 1984; Heywood, 1991; Eckstein *et al*., 2006; Eckert *et al*., 2008; Hargreaves *et al*., 2010). Thus, conserving species across the full extent of their range often provides a comprehensive sample of genetic diversity (Thomson *et al*., 2001; but see Ferguson *et al*., 1998). An alternative, ecogeographic proxy additionally incorporates explicit characterization of ecological variation that can influence genetic variation. Areas of similar geographic, ecological and climatic characteristics can be delimited as ecogeographic zones (EGZs), defining distinct evolutionary contexts amongst which adaptive genetic features are expected to vary (Maxted *et al*., 1995; Greene & Hart, 1999; Parra-Quijano *et al*., 2008, 2011).

Following Parra-Quijano *et al*. (2011), ecogeographic characterization identified EGZs appropriate to Czech priority CWR (see also Appendix S3). For each EGZ omitted from our complementary network, the area with the greatest predicted species richness was highlighted as a conservation target.

Subsequently, the entire *in situ* conservation network was reviewed and, appealing to a purely geographic proxy, a conspicuous spatial gap was filled by an additional species-rich area. Finally, PAs with the largest area of overlap with each grid cell, and therefore most likely to contain the species in each grid cell area, were highlighted as pragmatic candidate areas for *in situ* conservation (Maxted *et al*., 2008b).

Ⓑ ***Ex situ* conservation analyses**
First, gap analysis identified priority CWR species un- or under- represented in existing *ex situ* collections. First priorities for conservation are unrepresented priority species, lacking any gene bank accessions.

A subsequent goal is to ensure existing *ex situ* collections are representative of genetic resources within CWR species (Parra-Quijano *et al*., 2008). In order to conserve common alleles (frequency > 0.05) with a high probability (Pr > 0.90) and sample interpopulation variation, a minimum sample of ten individuals from five separate populations is recommended (Marshall & Brown, 1975; Brown & Briggs, 1991). Collections below this threshold were considered unrepresentative. Since most existing collections were unrepresentative, species were further prioritized using a combined index (sum of ranks) of geographic representativeness (GR) and ecogeographic representativeness (ER) as proxies for genetic representativeness. Priority for further collection is inversely related to genetic representativeness.

GR provides a simple proxy for genetic representativeness under the assumption of spatial genetic variation (explained above). Accordingly, the greater the proportion of a species' range from which germplasm has been collected, the greater the genetic diversity likely to be sampled. For each species, GR was defined as the percentage overlap between its total coverage (SDM) and accession coverage (circular area of 20km diameter around accession locations) (Hijmans & Spooner, 2001; Ramírez-Villegas *et al*., 2010).

ER provides an alternative proxy for genetic representativeness assuming (as above) that evolution maintains a relationship between environmental characteristics of sites and genetic features of populations. ER was defined as the percentage of EGZs in which a species is predicted to occur (based on its SDM) from which germplasm has been collected (Ramírez-Villegas *et al*., 2010).

Finally, we designed a spatial strategy for efficient augmentation of existing collections. Efficient expeditions would be able to collect multiple taxa within a limited area so should focus on areas of high richness, whilst sequential expeditions should collect complementary material. Accordingly, locations to fill species gaps were identified through complementarity analysis of priority CWR lacking accessions. To increase GR of existing collections, sampling should concentrate on areas where the most geographic gaps overlap. Geographic gaps were calculated for each species by subtracting accession coverage from its SDM. ER of existing collections would best be filled by sampling from EGZs, and the sections within those EGZs, from which the greatest number of unrepresentative species need sampling.

Ⓐ **RESULTS**

Ⓑ **CWR checklist and inventory**

The complete CWR checklist of the Czech Republic contains 3283 species. It is dominated by four genera (*Taraxacum*, *Rubus*, *Hieracium* and *Carex*) which together contain 15.0% of the species, and by relatives of aromatic and medicinal (913 species) and cut flower crops (832; Table 1) – but note 292 crops have both of these uses so their relatives are double-counted.

The current Czech CWR inventory (summarized in Table 2, full inventory in Appendix S1) provides the identity of, and further information about, 204 high priority CWR species (6.2% of the checklist). The Poaceae and Fabaceae families are the richest in the inventory, containing numerous species of feed crop relatives. In contrast, 10 families and 32 genera are represented by only a single species. The inventory is comprised mostly of food and feed CWR (Table 3), reflecting their explicit prioritization. Additional use categories derive from the 25 prioritized endemic CWR, or a secondary use of food or feed CWR. The following analyses consider priority (as opposed to checklist) CWR species. Note that taxonomic revisions have generated slight differences to results quoted in Iriondo et al. (2016).

Ⓑ ***In situ* conservation**

Ⓒ *Gap analysis*

Owing to the extensive PA network of the Czech Republic, covering ca. 21% of the territory (UNEP-WCMC, 2015), all but one of the 204 priority species have been recorded in at least one PA. The exception is *Alchemilla obtusa* (subsp. *trapezialis*). Moreover, 160 species (78.4% of the inventory) occur in five or more spatially distinct PAs, providing some insurance against stochastic or anthropic extinction. However, sixteen priority CWR have been recorded in only one PA. Half of these are endemic to their respective PA and are thus especially vulnerable (Table 4a). For the other eight species (Table 4b), populations in unprotected land present opportunities for additional *in situ* conservation (Maxted *et al*., 2008b; Hunter & Heywood, 2011).

Ⓒ *Species richness*

Observed richness of priority CWR species is high across the entire region of South Moravia, especially in and around Pálava Protected Landscape Area (PLA), Podyjí National Park (NP) and Brno (Fig. 1a). Observed priority CWR richness is also high to the west of Prague, in the north-east Doupovské Mountains and in the south-west of České Středohoří PLA. These areas offer the opportunity to conserve multiple priority CWR in single sites – although complementarity of species should also be considered (see below).

171 robust, bias-corrected SDMs were retained for analysis. These were based on 11 to 8335 spatially distinct presence records. Average test AUCs ranged from 0.547 to 0.999. The SDMs predict high CWR richness in South Moravia but in slightly different locations to observed richness: *between* Podyjí NP and Brno, and around Slavkov u Brna. The observed richness around Pálava PLA and in the Doupovské Mountains somewhat reflects high sampling effort (Fig. S2) which is correlated with species richness (Spearman rank correlation between number of observations and species richness on 10km by 10km grid $r_s = 0.837$, $n = 868$, $p < 0.001$). SDMs also indicate considerable diversity of priority CWR remains to be explored across the Česká Tabule in the north of the country.

Ⓒ *Complementarity analysis*
A complementary network of 22 grid cells (10km by 10km) is the smallest that contains at least one population of all 204 priority CWR species. The extensive Czech PA network means all of these grid cells overlap with at least one PA (Appendix S4). However, given diminishing returns as cells are added to the network (inset, Appendix S4), a reasonable cost-benefit balance is perhaps achieved by 11 cells (Fig. 2) containing 191 species (93.6% of the inventory) and representatives of all but three genera. Notably, 110 species (53.9% of the inventory) are contained in the first complementary cell, overlapping Pálava PLA in the Pavlov Hills. For comparison, the 22 richest cells contain records of only 163 different priority species, whilst the richest 11 (Fig. 1a) contain 150.

Ⓒ *Augmentation of conserved genetic diversity*
Twenty-two distinct EGZs, excluding urban environments and water bodies, were identified for priority CWR (Fig. S3). The top 11 priority cells identified through complementarity analysis contain 12 different EGZs, which comprise 98.1% of the area of all EGZs. The ten omitted EGZs demand conservation as they are likely to contain distinct genetic diversity, which is especially vulnerable owing to the limited extent of these EGZs.

Complementary cells 12 to 22 only contain three of the ten omitted EGZs. Alternatively, efficient conservation of CWR ecotypes in these EGZs could be achieved in ten different areas, each containing the greatest predicted richness of priority CWR within an EGZ (blue areas, Fig. 2). All but one of these areas overlaps one of six existing PAs, with Beskydy PLA notably containing rich expanses of four EGZs: 1, 2, 6 and 7 (Table 5). The richest area of EGZ 22 is not overlapped by a PA.

Considering a purely geographic proxy of genetic variation, the broad coverage of the *in situ* strategy should incidentally capture a broad range of genetic diversity. However, it neglects the south-west of the Czech Republic so an additional representative area from Plzeň or South Bohemia could incorporate potentially distinct genetic variation. A suitable area would be the Tábor Uplands, around the river valleys between Záhoří and Bechyně (Fig. 2), which has the greatest predicted priority CWR richness in these regions.

Ⓑ ***Ex situ* conservation**

Ⓒ *Gap analysis and genetic representativeness*
First priorities for *ex situ* conservation are the 134 priority species (65.7% of the inventory) without any known accessions of wild Czech origin (Appendix S1). These include all of the prioritized endemic species: our database contains no accessions of Czech endemic CWR.

The remaining 70 priority CWR species have existing *ex situ* collections, comprising 726 accessions. However, collecting effort is unevenly distributed amongst species (χ^2 test against equal number of accessions in each species $\chi^2 = 2229$, df = 69, $p < 0.001$). Being the explicit focus of collecting expeditions (Lebeda *et al*., 2009), *Lactuca serriola* dominates numerically (18.3% of accessions). *L. serriola* is also the only species with a clearly representative collection (by the standard of Brown & Briggs, 1991), comprising more than 50 accessions distributed across multiple populations. In contrast, collections of 39 species consist of fewer than five accessions. Thus, most collections are far from sampling an adequate range of genetic variation.

GR and ER were assessed for the 66 priority species with georeferenced accessions. GR scores were very low. Absolute values depend on the circular area diameter chosen but with a diameter of 20km, median GR is just 3.3%. *Festuca supina* and *L. serriola* have the highest GR scores of 55.6% and 24.2% respectively, reflecting the limited distribution of the former and numerous accessions of the latter (Fig. 3a). Median ER is 14.0%. 54 species have an ER < 30.0 and 27 species have been collected from just a single EGZ despite predicted wide distributions (Fig. 3b). Amongst species, priority for further collection is inversely related to genetic representativeness (Appendix S5). *Vicia sylvatica* is the species with the greatest scope for augmentation, with its wide distribution represented by just a single georeferenced accession.

Ⓒ *Sampling strategy*

Gap, species richness and complementary analyses were combined to suggest a strategy for efficient sampling to augment *ex situ* collections. Sites are proposed that facilitate collection of diverse but complementary CWR material (Fig. 4). Species gaps could best be filled by collecting in three complementary cells (containing 57, 39 and 22 species without existing accessions). Geographic gaps would be efficiently filled by expeditions to South Moravia and South Bohemia (to the east and west of Brno) and to south-east Central Bohemia. EGZs 12, 20 and 21 contain the most ecogeographic gaps (for 55, 56 and 55 species respectively), so collections from the most species-rich areas of these – all in the north of the country – would best fill ecogeographic gaps. Further targeted expeditions will be necessary to collect individual species omitted from this holistic sampling strategy.

Ⓐ DISCUSSION

The Czech CWR conservation strategy outlines synergistic *in situ* and *ex situ* conservation actions (MZP ČR, 2005; Maxted *et al*., 2007; SCBD, 2010; Maxted *et al*., 2012) for up to 204 priority CWR species. Both parts are ranked, such that conservation impact can be maximized for any level of resource input.

Consistent with global patterns (Castañeda-Álvarez *et al*., 2016), representation of Czech CWR in gene banks is poor. Creating *ex situ* collections is a matter of urgency for 134 priority CWR with no known accessions of Czech origin. Secondarily, further sampling is required to augment the genetic diversity in almost all existing collections. We suggest an efficient collecting strategy to meet these needs.

Although most priority CWR already occur in PAs, this protection is largely passive. We encourage active *in situ* conservation within CWR genetic reserves, which have an explicit remit for conservation of CWR genetic diversity (Maxted *et al.*, 1997a; Hunter & Heywood, 2011; quality standards in Iriondo *et al*., 2012). A comprehensive network of genetic reserves (Fig. 2) could be established across just eleven 10km by 10km grid cells (first priories, in their rank order), ten supplementary ecogeographic areas and one area to fill a spatial gap (second priorities). These contain 94% of priority species and 96% of genera and include all EGZs. Typically, a network of 5 to 30 genetic reserves conserves the majority of a nation's priority CWR (Iriondo *et al*., 2016). As a foundation for the Czech network, the complementary approach is preferred over a simple richness approach because of its greater taxonomic representation (191 vs. 150 priority species in the top 11 grid cells) and wider geographic coverage.

Twenty of the twenty-two abstract *in situ* priority areas are overlapped by PAs (Fig 2, Table 5). It would be pragmatic to incorporate CWR conservation into the scientific remit of existing PAs, although effects on taxa already managed in these PAs must be considered (Maxted *et al*., 2008b). However, *in situ* conservation outside of PAs is also necessary (Hunter & Heywood, 2011). Many CWR are associated with disturbed habitats, such as agricultural land, that fall outside the remit of PAs (Dotlačil *et al*., 2004; Lebeda *et al*., 2009; Hopkins & Maxted 2011; Jarvis *et al*., 2015), whilst some simply have few populations in existing PAs (e.g. Table 4). Surveys across the proposed *in situ* network are needed to confirm CWR occurrences and determine the state of habitats, and thus select exact locations for CWR conservation.

South Moravia stands out as the most important region for Czech CWR conservation, including germplasm collection and genetic reserve establishment. South Moravia contains the greatest richness of priority CWR, especially in the first complementary cell overlapping Pálava PLA. Further, the flora likely contains distinct genetic material (a) at a national scale, given that South Moravia is the only Czech region to overlap with the Pannonian biogeographical region and thus contains a nationally distinctive ecogeographical setting (Miko & Hošek, 2009) and (b) at the European scale, given that South Moravia contains the north-western extremity of the Pannonian region and plant genetic diversity tends to be distinctive at range margins (Eckstein *et al*., 2006; Eckert *et al*., 2008). Nonetheless, the Czech Republic's mountainous regions are also of value, harbouring disparate taxonomic and genetic diversity – hence the inclusion of cells two (Jeseníky PLA) and four (Krkonoše NP), for example, in our complementary network.

The Czech CWR checklist contains 88% of species occurring in the Czech Republic. It is not unusual for CWR checklists derived using a similar methodology to contain the majority of the national flora. Around 65% of UK native taxa (Maxted *et al*., 2007) and 70% of the Chinese flora (Kell *et al*., 2015) are CWR, owing to broad definitions of CWR (congeners of any global crop species) and crops (any plant species of use to humans when harvested anywhere in the world), and the inclusion of cultivated and non-native taxa that may subsequently be excluded from an inventory (Kell *et al*., 2008, 2015). We deliberately built a comprehensive checklist to provide a broad, informative baseline for national CWR conservation planning (Maxted *et al*., 2013). The inventory of prioritized CWR is only a small proportion (6.2%) of the checklist, but these are relatives of various major food and feed crop genera. Relatives of aromatic, medicinal and flower crops – which dominate the checklist – were not prioritized. Thus, conservation of priority CWR would make a substantial and disproportionate contribution to food security. Although the value of related crops to the Czech Republic was our primary concern in CWR prioritization, many of these crops are also great importance for global food security (e.g. relatives of wheat, barley and *Brassica*; FAO, 2001).

Being based on prioritization criteria chosen by national stakeholders, the Czech CWR inventory is inherently subjective. CWR prioritization methodologies will vary across nations, depending on the CWR present, the conservation resources available and the goal of the conservation strategy (Maxted *et al*., 1997b, 2007; Hunter & Heywood, 2011; Kell *et al.*, 2012a). The potential socioeconomic value of CWR is emerging as a standard criterion for prioritization (Iriondo *et al*., 2016), although whether value is viewed primarily from a national or global perspective is variable. The conservation of endemic CWR resources should be a priority for all nations. Within the context of the ITPGRFA (FAO, 2001), and by definition, every nation is solely responsible for the *in situ* conservation of its endemic CWR diversity. Threat status was not used as an explicit prioritization criterion for Czech CWR, on the basis that threatened taxa are more likely to be protected already, or may have a wider distribution across Europe (e.g. sub-Mediterranean elements in South Moravia; Miko & Hošek, 2009).

Meanwhile, prioritising by threat may exclude more common CWR, which still deserve proactive conservation if they contain valuable, broad or distinct genetic diversity (Frankham *et al*., 2009; Kell *et al*., 2012b; Maxted *et al*., 2015). Still, we encourage special conservation attention for the most highly threatened (Appendix S1) and apparently range-restricted (Table 4) priority CWR, especially those also threatened across Europe, to avoid complete loss of their genetic resource.

It is imperative that the conservation actions proposed here are seen through to practical implementation. The goal must be active conservation: positive action, beyond protection on paper alone, to promote the sustainability of target taxa (Maxted *et al*., 1997a). *In situ*, this involves monitoring of population and habitat changes, and ideally explicit monitoring of genetic changes, in order to identify and mitigate threats (SCBD, 1992; Maxted *et al*., 1997b; Iriondo *et al*., 2012). For *ex situ* conservation, collection of material is necessary but not sufficient. There must be a regular process of regeneration and evaluation – which can be challenging and time-consuming for CWR (Castañeda-Álvarez *et al*., 2016) – and accessions must be recorded in gene bank databases. In this way, the material will become generally available for use, with the capacity to aid both Czech and global food security in the face of contemporary environmental challenges (Ford-Lloyd *et al*., 2011; Dempewolf *et al*., 2014).

Where grid cells or PAs extend beyond the Czech border (Fig. 2), coordinated transboundary action could improve the success of active conservation. Krkonoše NP is included in the Czech CWR complementary network because it contains important, distinct Czech CWR diversity. It is already twinned with the Polish equivalent Karkonosze in the inaugural UNESCO Transboundary Biosphere Reserve (Štursa, 2011). Both Czech and Polish CWR populations should be considered as conservation targets. Similarly, CWR richness around Pálava PLA presents opportunities for successful Austro-Czech collaborative conservation.

To work towards implementation, Czech stakeholders must consider practical issues such as land ownership and management and existing conservation actions. CWR occurrences on which the strategy is based must be confirmed in the field. Further, periodic review is recommended in light of changing taxonomy, objectives, policy and environmental factors (such as climate change and biological invasions) and the availability of novel data (Kell *et al*., 2012a; Maxted *et al*., 2015). In particular, we encourage genetic analyses to test our predictions regarding genetic variation made using ecogeographic proxies.

More generally, there is a need to develop CWR conservation strategies for other nations. Methods similar to ours can (and have) been applied in many countries around the world (e.g. Kell *et al*., 2015; Iriondo *et al*., 2016). As more national strategies are developed, consideration of other national, regional and global strategies becomes imperative: conservation of a nation's resources for its own use must be balanced with systematic, coordinated and complementary conservation of CWR genetic diversity across Europe and the world (Maxted & Kell, 2009; Maxted *et al*., 2010, 2012, 2015). Finally, because the need to conserve CWR stems from their explicit utilitarian value, once CWR are effectively conserved their diversity must be made available for use. In turn, sustainable use should stimulate long-term term conservation of CWR diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1: Complete CWR inventory (spreadsheet)
Appendix S2: Species distribution modelling methods
Appendix S3: Ecogeographic land characterization methods
Appendix S4: Full results of *in situ* conservation analyses
Appendix S5: Genetic representativeness of *ex situ* collections

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Biosketch

This project united researchers with an interest in the conservation and use of plant genetic resources. The Birmingham Plant Genetic Resource Group (led by N.M.) researches the development and implementation of agrobiodiversity conservation strategies, helping to secure plant genetic resources to mitigate the impact of climate change, maintain consumer choice and underpin food security. The Czech Crop Research Institute (CRI) conducts strategic and applied research in crop production, with a focus on sustainable development and environmental protection. The CRI coordinates the Czech National Programme on Plant Genetic Resources, and houses the Czech national gene bank (led by V.H.). The Nature Conservation Agency of the Czech Republic is a governmental institution responsible for a wide spectrum of official nature conservation activities across the entire state territory, from administration and management to surveillance and reporting of habitats and species – including CWR.

Author Contributions: N.M. and S.P.K. conceived the idea; S.P.K, N.G.T., V.H., and N.M. developed the idea; V.H., N.G.T., S.P.K and K.C. collected or provided data; N.G.T., S.P.K., V.H. and M.P-Q. analyzed the data; N.G.T. wrote the manuscript. All authors edited the manuscript.

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TABLES

Table 1 Number of species in the Czech CWR checklist related to crops with different uses in the Czech Republic, according to EVIGEZ (2012). Note that some CWR are related to crops in more than one use category. For 875 checklist species there are no congeners in the Czech National Crop Database: these species are included because their relatives are used elsewhere in the world according to Kell *et al*. (2005).

Code	Crop use	Checklist species
A	Aromatic and medicinal plants	913
D	Flowers	832
G	Grasses	310
F	Fruit	250
H/B	Vegetables	205
T	Fodder	132
Z	*Zea* and alternative cereals	83
L	Food legumes	56
R/W	Ornamental woody plants	46
O	Oil plants	43
X	Industrial plants	40
C	Cereals	29
S	Potatoes	19
V	Grapes	3

Table 2 Overview of priority CWR in the Czech Republic. Use categories for related genera according to EVIGEZ (2012): A – aromatic and medicinal; C – cereals; D – flowers; F – fruit; G – grasses; H – vegetables; O – oil plants T – fodder; W – ornamental woody plants; X – industrial plants. Note that grasses were restricted to relatives of feed grasses according to GRIN (2012). All taxa – all infraspecific taxa explicitly prioritized (and listed in inventory).

Family	Use 1	Use 2	Use 3	Use 4	Endemic taxa	Genera	Species	All taxa
Amaryllidaceae	D	H			–	1	13	14
Apiaceae	H				–	1	1	1
Asteraceae	A	D	H		2	3	7	7
Brassicaceae	H	O			–	6	13	14
Campanulaceae	D				4	1	4	4
Cannabaceae	X				–	1	1	1
Caryophyllaceae	D	T			3	2	6	6
Fabaceae	A	D	L	T	–	17	67	76
Grossulariaceae	F				–	1	4	5
Iridaceae	A	D			1	1	1	1
Lentibulariaceae	A				1	1	1	1
Malvaceae	A	D	T		–	2	5	5
Papaveraceae	D	O			–	1	4	4
Plantaginaceae	A				1	1	1	1
Poaceae	C	G			–	17	48	52
Polygonaceae	D	T			–	1	3	3
Primulaceae	A	D			1	1	1	1
Ranunculaceae	A	D			1	1	1	1
Rosaceae	A	F	W		8	7	20	22
Rubiaceae	A				1	1	1	1
Saliaceae	W	X			1	1	1	1
Saxifragaceae	D				1	1	1	1
				TOTALS	**25**	**69**	**204**	**222**

Table 3 The number of species of priority CWR per crop use category (EVIGEZ, 2012). Note that some CWR are related to crops in more than one use category.

Crop group	Crop use	Priority species
Feed	Fodder	62
	Forage grasses	43
Food	Vegetables	32
	Fruit	23
	Oil	17
	Food legumes	16
	Cereals	5
Other	Flowers	37
	Aromatic and medicinal	17
	Industrial	2
	Ornamental	2

Table 4 Priority CWR that have only been recorded in one spatially distinct protected area (PA) within the Czech Republic. PAs in parentheses are contained within larger PAs outside parentheses (e.g. *Agrostis alpina* occurs in Praděd NNR, which is entirely contained within Jeseníky PLA).

PA status: NNM – National Nature Monument; NNR – National Nature Reserve; NP – National Park; NR – Nature Reserve; PLA – Protected Landscape Area; SCI – Natura 2000 Site of Community Importance; SPA – Natura 2000 Special Protection Area.

(a) Priority CWR that are endemic to a single PA

Taxon	Protected Area
Agrostis alpina	Jeseníky PLA (Praděd NNR)
Campanula gelida	Jeseníky PLA (Praděd NNR)
Carlina biebersteinii (subsp. *sudetica*)	Jeseníky PLA (Praděd NNR)
Festuca versicolor	Krkonoše SCI (NP/SPA)
Plantago atrata (subsp. *sudetica*)	Jeseníky PLA (Praděd NNR)
Poa riphaea	Jeseníky PLA (Praděd NNR)
Salix lapponum (var. *daphneola*)	Krkonoše SCI (NP/SPA)
Sorbus hardeggensis	Podyjí NP

(b) Priority CWR that have been recorded in a single PA and unprotected land

Taxon	Protected Area
Danthonia alpina	Bílé Karpaty PLA (Čertoryje NNR, Kútky NR, Machová NR)
Dianthus arenarius (subsp. *bohemicus*)	Kleneč NNM
Festuca drymeja	Hostýnské Vrchy SCI
Papaver lecoqii	České Středohoří PLA
Sorbus alnifrons	Údolí Jihlavy SCI
Sorbus eximia (s.l.)	Český Kras PLA (Karlštejn NNR, Koda NNR, Mramor SCI)
Sorbus rhodanthera	Chlum NR
Vicia dalmatica	Blanský Les PLA (Vyšenské Kopce NNR)

Table 5 Ecogeographic augmentation of the *in situ* conservation network for Czech priority CWR. Ten EGZs are omitted from the complementary network (top 11 cells). Conservation within priority areas of these EGZs would generate an ecogeographically complete *in situ* conservation network. EGZ – ecogeographic zone; PA – protected area; pSR –predicted species richness (based on 171 species distribution models). PA status abbreviations as in Table 4.

EGZ	**% Czech Area**	**Priority Area of Ecogeographic Zone (EGZ)**				
		Size (cells)	**% EGZ area**	**Max. pSR**	**Median pSR**	**Primary overlapping PA**
1	0.984	104	5.8	45	38	Beskydy PLA
2	0.314	142	24.9	37	31	Beskydy PLA
3	0.054	32	32.7	51	39	Bílé Karpaty PLA
6	0.189	48	14.0	40	28	Beskydy PLA
7	0.090	57	34.8	37	26	Beskydy PLA
8	0.015	19	70.4	24	17	Králický Sněžník NNR/SCI/SPA
17	0.097	12	6.8	54	47	Vapenice-Basa SCI
18	0.008	4	28.6	58	56	Ralsko NR/SCI
21	0.124	11	4.9	57	46	České Středohoří PLA/ Lužické Hory PLA (boundary)
22	0.010	11	61.1	57	48	–

FIGURES

Figure 1 (a) Observed and (b) Predicted richness of priority CWR species in the Czech Republic. (a) is based on circular neighbourhood analysis. Light blue boxes outline the eleven cells with the greatest species richness (based on analysis using a grid of ca. 10km by 10km cells). (b) was generated by summing robust binary SDMs for 171 priority CWR species. Mts. – mountains; SM – South Moravia (region). Projection: Transverse Mercator 33N.

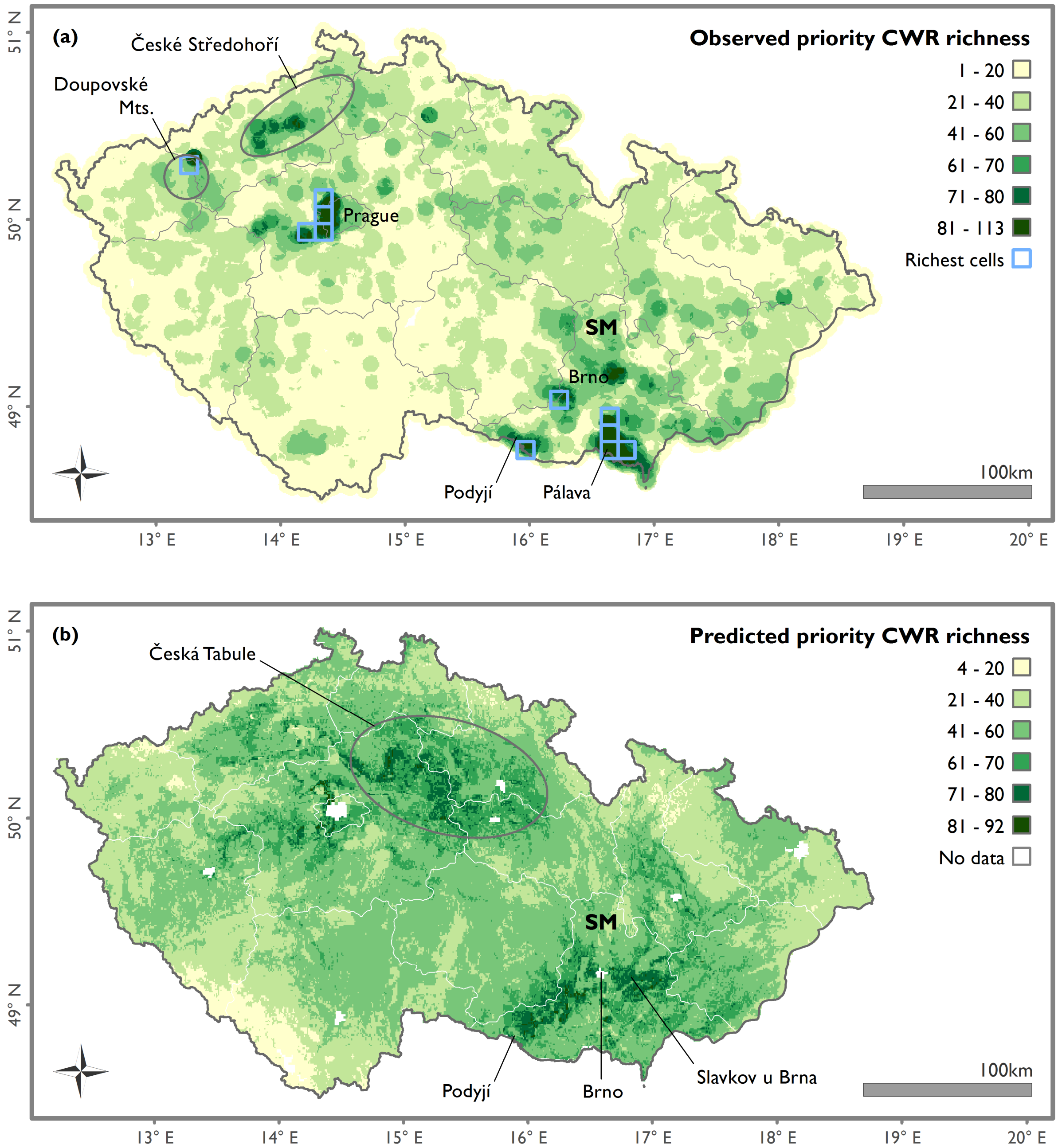

Figure 2 Overall *in situ* conservation strategy for Czech priority CWR. Priority areas are the top 11 cells from complementarity analysis (large green squares), the richest areas of omitted ecogeographic zones (small blue areas) and an additional species-rich area to fill a conspicuous geographic gap (TU, orange; cells with predicted species richness ≥ 57). Black backgrounds added to increase contrast. For complementary cells: numbers outside parentheses refer to the priority rank of complementary cells; first number in parentheses is the number of priority CWR species in each cell not already included in the network; second number in parentheses is the total number of priority CWR species in each cell. PAs overlapping the priority areas are presented: complementary PAs are named in the figure; all ecogeographic PAs are named in Table 5. EcoGeo – ecogeographic; Geo – geographic; PA – protected area; PL – Plzeň (region); SB – South Bohemia (region); TU – Tábor Uplands. PA abbreviations as in Table 4. Projection: Transverse Mercator 33N.

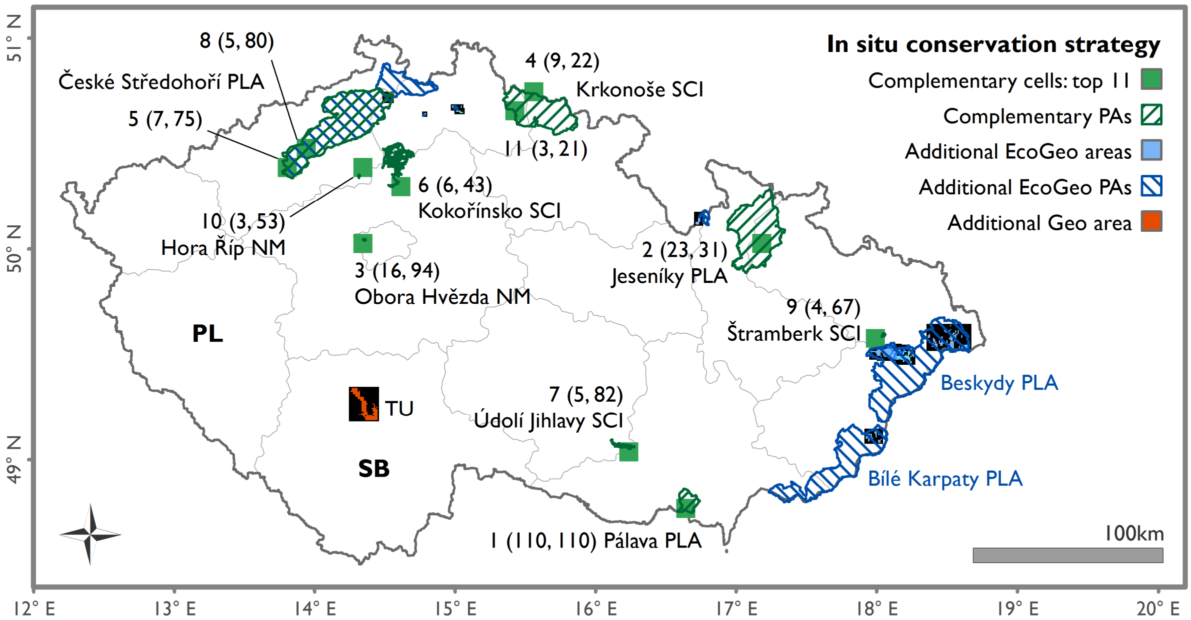

Figure 3 (a) Geographic and (b) Ecogeographic coverage of germplasm accessions for 66 priority CWR with georeferenced accessions. Each point represents one species. Points are grouped according to representativeness: blue filled circles – low (GR ≤ 1% or ER ≤ 5%); black open circles – moderate (GR or ER ≤ median); green triangles – high (GR or ER > median). Solid grey lines represent 100% representativeness (GR or ER); dashed grey lines represent 30% representativeness. Total coverage was derived from SDMs and accession coverage from georeferenced accessions. In panel (a), outlier at (96.1,23.2) is *Lactuca serriola*. In panel (b), points are jittered slightly on both axes for clarity.

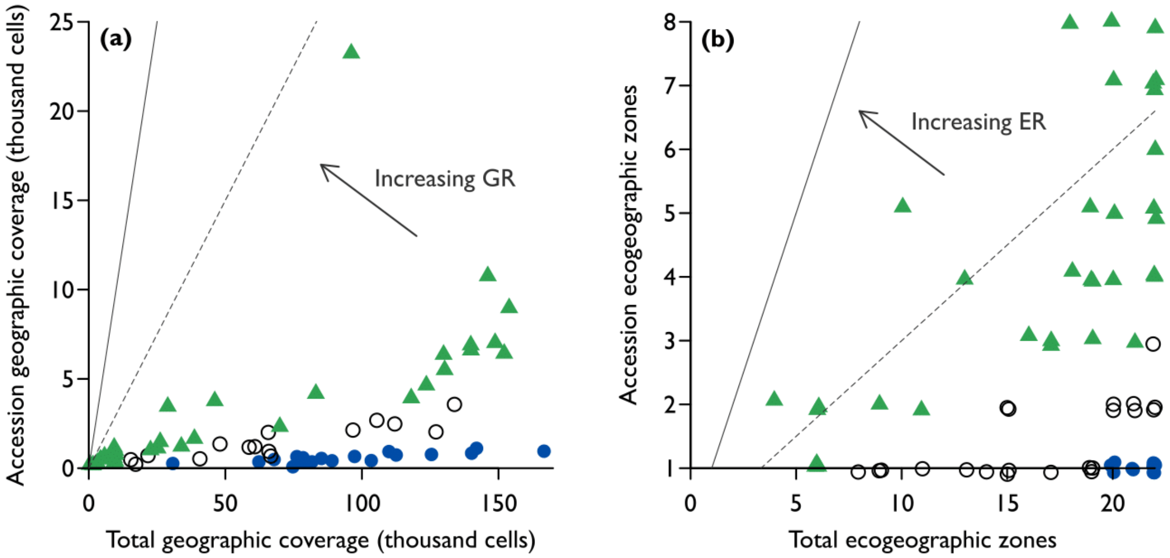

Figure 4 Efficient collecting strategy to fill gaps in *ex situ* collections of Czech priority CWR. Expedition locations identified through complementarity analysis (green squares; first number in parentheses is the number of priority CWR species without accessions that are not included in previous complementary cells; second number is the total number of priority CWR species without accessions in each cell), through overlap of geographic gaps (blue boxes) or as the most species-rich areas (green ovals) of ecogeographic zones with the most ecogeographic gaps (EGZs 12, 20 and 21). CB – Central Bohemia (region); EcoGeo – ecogeographic; Geo – geographic; L – Liberec (region); Mts. – mountains; SB – South Bohemia (region); SM – South Moravia (region). Projection: Transverse Mercator 33N.