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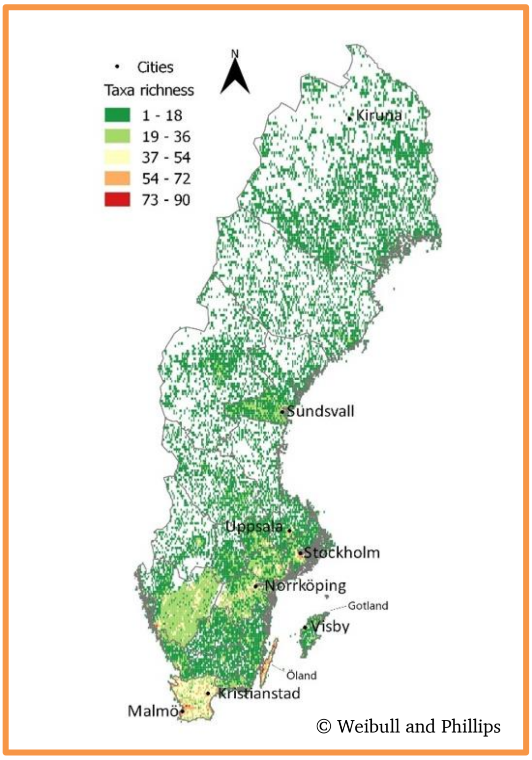

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Focus and Scope of *Genetic Resources*

Genetic Resources is an open access journal disseminating global knowledge and tools used by the community of practitioners of plant, animal and forest genetic resources involved in monitoring, collecting, maintaining, conserving, characterizing and using genetic resources for food, agriculture and forestry. *Genetic Resources* publishes original research as well as methods, strategies, guidelines, case studies or reviews on a variety of topics of interest on the present and future use of genetic resources. These may include the documentation, conservation, management, assessment, characterization and evaluation of genetic resources and their link to broader biodiversity, socioeconomic practices, policy guidelines or similar, serving stakeholders within and across sectors. Occasionally, *Genetic Resources* publishes special issues with a focus on selected topics of interest for the genetic resources community. The journal welcomes contributions from all world regions.

Cover illustration:
The taxon richness of priority crop wild relatives across Sweden, as discussed by Weibull and Phillips on pp. 17-23

GenRes Bridge
Genetic resources for food-secure and forested Europe

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Genetic Resources

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EDITORIAL

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Genetic Resources – a new attempt at serving the community

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Abstract: Through the Horizon 2020 project 'GenRes Bridge' the new journal *Genetic Resources* was conceived to serve as a new cross-cutting platform for stakeholders and practitioners in genetic resources. Its ambition is to provide access to relevant information and tools for the monitoring, conservation, management, characterization and use of genetic resources, and thus to contribute to the FAO global plans of action on genetic resources. Conceived to fill the gaps left by the discontinuation of the journals *Plant Genetic Resources Newsletter* and *Animal Genetic Resources*, it aims at serving the genetic resources community worldwide and across sectors.

Keywords: GenRes Bridge, Plant Gentic Resources Newsletter, Animal Genetic Resources, ECPGR, ERFP, EUFORGEN

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History

Between 1970 and 2009, FAO and IPGRI/Bioversity International were jointly producing the *Plant Genetic Resources Newsletter* (*PGRN*)[1], a peer reviewed journal focusing on "genetic resources of useful plants, resulting from new work, historical study, review and criticism in genetic diversity, ethnobotanical and ecogeographical surveying, herbarium studies, collecting, characterization and evaluation, documentation, conservation, and genebank practice". Paper issues were originally distributed free of charge and eventually the publication was converted into an online open access publication. This magazine covered a specific niche of interest for the plant genetic resources community, publishing information that normally would not be suitable for existing scientific journals. It was open to contributions from all regions and offered a free of charge publication service.

The FAO journal *Animal Genetic Resources*[2], supporting the implementation of the global plan of action for animal genetic resources (GPA-AnGR, (FAO, 2007), had a similar history and was discontinued in 2016. Over a 30-year period, more than 600 papers were published and available in 3 languages (English, French and Spanish). The papers published between 2010 and 2016 are freely available online. A majority of the articles were focused on the ruminant species and about 60% of them were related to the characterization and monitoring of animal genetic resources.

The discontinuation of *PGRN* and *Animal Genetic Resources* was determined by strategy changes and redefinition of priorities of the international organizations that were managing these journals.

In the field of plant genetic resources, at least two peer-reviewed magazines (*Genetic Resources and Crop Evolution* and *Plant Genetic Resources: Characterization and Utilization*) were already in operation at the time of *PGRN* and continue today to offer the opportunity to

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[1] *Plant Genetic Resources Newsletter* archive: https://www.bioversityinternational.org/e-library/library-services/plant-genetic-resources-newsletter/

[2] *Animal Genetic Resources* archive: https://www.cambridge.org/core/journals/animal-genetic-resources-resources-genetiques-animales-recursos-geneticos-animales

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publish scientific contributions. However, the possibility was lost to formally publish 'grey literature' type of contributions, i.e. methods, strategies, guidelines, reports, case studies, etc., with the support of an international institution as a publisher, offering free of charge services. In fact, since the termination of *PGRN*, the Secretariat of the European Cooperative Programme for Plant Genetic Resources (ECPGR) received various inputs from its members, inquiring about the possibility to resurrect the journal and soliciting action.

As for the field of AnGR, several journals are offering the possibility of publishing work related to animal genetic resources, provided that their scientific interest and scientific quality is in scope with the magazines' objectives, however none of them are specifically dedicated to AnGR. In Europe, an alternative was sometimes found through oral or poster presentations during scientific conferences in Europe, such as the European Federation of Animal Science (EAAP) conference. The annual conference of the EAAP organizes a specific session devoted to AnGR every year, coordinated by ERFP, FAO and EAAP.

A new attempt

The opportunity to attempt the re-establishment of a journal that would take on the heritage of *PGRN* and *Animal Genetic Resources* in a new context was provided by the decision of the three European networks on plant (ECPGR), animal (European Regional Focal Point for Animal Genetic Resources, ERFP) and forest (European Forest Genetic Resources Programme, EUFORGEN) genetic resources to join forces within the Horizon 2020 project 'GenRes Bridge - Joining forces for GenRes and biodiversity management'[3], which was approved for funding by the European Commission (EC) for the period 2019-2021.

As part of this project a task dedicated to the creation of the journal *Genetic Resources* was included, thus securing funding for the journal's initial establishment and the production of the first three issues. It is the intention of the three networks to develop a sustainability plan for the continuation of the journal after the end of the project, depending on its success and the feedback received from users, thereby exploring the possibility in the future to share costs and responsibilities as part of their own budgets, as well as seeking support from other possible sponsors.

Prior to the launch of the journal, a survey was carried out of selected audiences about the main gaps that a journal on genetic resources would be expected to fill (Maggioni, 2019). The survey gave an indication of an existing interest for a new journal on genetic resources (91% of respondents). Although agricultural plants (80%), including wild relatives (59%) raised the highest interest, the forestry (24%) and farm animals (9%) domains also registered a significant interest, considering that only a limited number of stakeholders from these domains were included among the survey recipients.

The journal was started under the management of the ECPGR Secretariat and is published by Bioversity International on behalf of the ECPGR. The editorial board includes members of the plant and animal genetic resources community with expertise in different sectors of relevance. The journal's focus is on publishing original research as well as methods, strategies, guidelines, case studies or reviews on topics of interest on the present and future use of genetic resources, thus serving a variety of stakeholders across sectors.

Genetic Resources uses the open-source web publishing platform Open Journal Systems (OJS[4]), and a web-based production software (www.typeset.io), which guarantee that running costs of the publication are limited, allowing the journal to provide real open access publications, which are free to publish and free to read. The journal is a member of Crossref[5], a not-for-profit cooperative effort among publishers to enable persistent cross-publisher citation linking in online academic journals. It also adheres to publication ethics as published by the Committee on Publication Ethics (COPE)[6].

The active support of voluntary reviewers recruited worldwide and particularly within the European networks ensures timely processing of manuscript submissions. The European networks also offer hubs of expertise to support the editorial and language review of articles.

Based in Europe and mainly supported by the European networks, *Genetic Resources* is open to worldwide contributions, offering a public free of charge service also to other regions. This new journal can thus be seen as a contribution of the European region to the implementation of the FAO global plans of action on genetic resources (FAO, 2011, 2014, 2007), particularly in terms of promoting access to communication, data and information exchange.

The importance of genetic resources, the challenges ahead (primarily habitat destruction and climate change) and the existing gaps of knowledge call for intensified actions and global collaboration, as illustrated in the keynote review by the Commission for Genetic Resources for Food and Agriculture (CGRFA) in this first issue (Pilling *et al*, 2020).

This journal has the aspiration to contribute to encouraging a fertile framework of collaboration and exchange of information. It is hoped that the opportunity to use the publication services of *Genetic Resources*, starting with this issue, will be increasingly used and appreciated by contributors and readers. Their feedback will be taken into account and their judgement will eventually determine whether this enterprise deserves to grow and to continue into the future.

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[3] www.genresbridge.eu

[4] https://pkp.sfu.ca/ojs/

[5] www.crossref.org

[6] https://publicationethics.org/

Acknowledgements

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Pilling, D., Belanger, J., Diulgheroff, S., Koskela, J., Leroy, G., Mair, G., and Hoffmann, I. (2020). Global status of genetic resources for food and agriculture: challenges and research needs. *Genetic Resources* 1(1), 4–16. doi: 10.46265/genresj.2020.1.4-16

Genetic Resources

REVIEW

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Global status of genetic resources for food and agriculture: challenges and research needs

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Abstract: Plant, animal, forest, aquatic, micro-organism and invertebrate genetic resources are vital to food security, nutrition, livelihoods and the resilience and adaptability of global agricultural production systems. Despite increasing efforts in recent years, much remains to be done to improve the management of these resources. Many are at risk of extinction or erosion and many have been overlooked in terms of use and development. There is an urgent need to address these deficiencies, both within the individual sectors of food and agriculture and in terms of how genetic resources management can be better integrated across sectors. These efforts will need to include action to address the multiple knowledge gaps that constrain improvements to management. They will also need to include the creation of policy and institutional frameworks that promote collaboration and stakeholder participation and allow sustainable management strategies to be implemented effectively at appropriate scales.

Keywords: genetic resources, food and agriculture, Sustainable Development Goals, global assessments, knowledge gaps

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Genetic resources — the foundation of food and agriculture

Genetic resources for food and agriculture (GRFA) are vital to food security, nutrition, livelihoods and the productivity, resilience and adaptability of production systems in the crop, livestock, forest, fisheries and aquaculture sectors. They are key resources in efforts to achieve the Sustainable Development Goals (SDGs). This paper presents an overview of the state of GRFA and their management, drawing largely on the findings of the monitoring activities overseen by the Commission on Genetic Resources for Food and Agriculture (Commission) of the Food and Agriculture Organization of the United Nations (FAO) (Boxes 1 and 2) and highlighting future management challenges, with an emphasis on knowledge gaps.

Status and trends uneven, with worrying declines

Knowledge of the status and trends of GRFA varies across sectors. The following subsections present short overviews. Selected key facts and figures on the status and trends of genetic resources and their management, at global level, are presented in Table 1.

Plant genetic resources for food and agriculture (PGRFA)

More than 6,000 plant species have been cultivated for food (Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 2020), but today nine species (sugarcane, maize, rice, wheat, potatoes, oil palm, soybean, cassava and sugar beet) provide 67 percent of crop production by weight (FAO, 2020b). The precise status and trends of within-species genetic diversity is difficult to assess.

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Table 1. Selected facts and figures on the state and trends of genetic resources and their management at global level

Categories	Plant genetic resources for food and agriculture	Animal genetic resources for food and agriculture	Forest genetic resources[1]	Aquatic genetic resources for food and agriculture[2]	Micro-organism and invertebrate genetic resources for food and agriculture[3]
Total number of known species	Estimated 391,000 plant species[4]	More than 17,000 avian and mammalian species[5]	More than 60,000 tree species[6]	More than 160,000 aquatic species	Unknown
Number of species and subspecies groups (i.e. varieties, breeds, etc.) used for food and agriculture	6,000 species[7] Unknown number of varieties[8]	Around 40 species[9] Over 8,700 breeds[10]	8,000 species of trees, shrubs, palms and bamboo reported by countries	1,800 species targeted by capture fisheries 694 commercially farmed species items Few well-established improved farmed types	Unknown
Species concentration in food and agricultural production	9 species provide 67% of global crop production[11]	8 species provide 97% of global meat production[11]	2,400 species reported as actively managed for products and services	10 species provide 50% of global aquaculture production[12]	Not applicable
Status and trends of species and within-species genetic diversity	Reported decreases in crop diversity in farmers' fields, but situation variable and complex[8] Many species of crop wild relatives under threat[8,13,14]	28% of local breeds at risk, 10% not at risk, 62% unknown risk status[10]	57% of species (34,204) have a conservation assessment: 38% of these are threatened globally[15] No systematic global monitoring system for within-species diversity Loss of genetic diversity in commercially important species a concern.	Limited information below species level Increase of species diversity in aquaculture, but increased emphasis on production of a few species	Limited information Available evidence indicates widespread declines

Continued on next page

Table 1 continued

Categories	Plant genetic resources for food and agriculture	Animal genetic resources for food and agriculture	Forest genetic resources[1]	Aquatic genetic resources for food and agriculture[2]	Micro-organism and invertebrate genetic resources for food and agriculture[3]
Breeding	443 crop species reported by 28 countries with active public pre-breeding and breeding programmes (81 species reported as being used in private programmes)[16]	Well-organized breeding programmes and use of advanced techniques largely restricted to developed regions and focused on a limited range of mostly temperate breeds[9]	700 species reported to be included in breeding programmes	55% of farmed species reported to be subject to some kind of genetic management	Few invertebrate and micro-organism species are subject to genetic improvement activities
***In situ* conservation**	Among 30,000 *in situ* conservation sites reported in 39 countries, 9% had management plans for crop wild relatives and wild food plants[16]	*In situ* conservation activities widely reported, but many gaps in coverage[9]	Of 8,000 species used for various purposes, about 1,000 are included in *in situ* conservation programmes	Aquatic protected areas and effectively managed fisheries contribute to *in situ* conservation of aquatic genetic resources	Limited action specifically targeting these groups
***Ex situ* conservation**	5.4 million accessions from more than 50,000 species conserved in over 700 genebanks in 103 countries and 17 regional and international research centres[17]	Of 7,760 local breeds (including extinct ones), 258 reported to have genetic material stored in genebanks, 79 of these with sufficient material stored to allow them to be reconstituted[10]	1,800 species reported as being conserved *ex situ* 159,579 accessions reported globally	290 species, almost 200 of which considered threatened at national or international levels, are maintained in 690 *ex situ* collections	791 culture collections, containing over 3 million microbial cultures of 50,875 species and subspecies, in 78 countries and regions, are registered with the WFCC[18]

Sources: [1] FAO (2014b), unless noted otherwise; [2] FAO (2019c), unless noted otherwise;[3] FAO (2019d), unless noted otherwise; [4] Royal Botanic Gardens Kew (2016); [5] BirdLife-International (2018) Burgin *et al* (2018); [6] Beech *et al* (2017); [7] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (2020); [8] FAO (2010); [9] FAO (2015); [10] FAO (2020a); [11] FAO (2020b); [12] FAO (2020c); [13] Magos-Brehm *et al* (2017); [14] Bilz *et al* (2011); [15] Global Tree Assessment (GTA) (2020); [16] FAO (2020e), data refer to reporting period 2012–2014; [17] FAO (2020e), data refer to 2019; [18] World Federation of Culture Collections (http://www.wfcc.info/ccinfo/statistics/).

There are no comprehensive figures available for the status of crop varieties across the world's production systems and there is as yet no standardized way of assessing their risk status. However, the available evidence indicates that, overall, the crop diversity present in farmers' fields has declined (FAO, 2010). Many farmers' varieties and landraces have disappeared or become rarer. The situation is, however, complex, with new varieties sometimes being grown in addition to, rather than in replacement of, traditional ones. The state of genetic vulnerability ("the condition that results when a widely planted crop is uniformly susceptible to a pest, pathogen or environmental hazard as a result of its genetic constitution, thereby creating a potential for widespread crop losses" (FAO, 1997a)) is also difficult to assess. However, many countries have reported significant genetic vulnerability in their production systems (FAO, 2010). Crop wild relatives are key resources in plant breeding and are widely under threat (Bilz *et al*, 2011; FAO, 2010; Magos-Brehm *et al*, 2017).

Threats to domesticated PGRFA include changes to production systems that lead to declines in the use of traditional varieties (FAO, 2010). Crop wild relatives are affected by pressures on their habitats, including those related to climate change and to land-use changes associated, *inter alia*, with agriculture (Magos-Brehm *et al*, 2017; Bilz *et al*, 2011; FAO, 2010).

Animal genetic resources for food and agriculture (AnGR)

Among the more than 17,000 known avian and mammalian species, (Burgin *et al*, 2018; BirdLife-International, 2018), only about 40 have been domesticated for use in food and agriculture (FAO, 2015). Production is very concentrated among a few species, with eight (pig, chicken, cattle, sheep, goat, turkey, duck and buffalo) providing 97 percent of global meat production in 2018; four of these (cattle, buffalo, goat and sheep) accounted for almost 100 percent of global milk production, and chickens alone accounted for 93 percent of egg production (FAO, 2020b). A total of 8,719 livestock breeds are recorded by FAO as of 2020; 26 percent of these are classified as at risk of extinction, 13 percent as not at risk, 6 percent as extinct and 55 percent as being of unknown risk status (FAO, 2020a).

SDG Indicator 2.5.2 is "Proportion of local breeds, classified as being at risk, not-at risk or unknown level of risk of extinction" ("local breeds" are breeds found in only one country). As of 2020, 62 percent of local breeds are classified as being of unknown status, 28 percent as at risk and 10 percent as not at risk (the figures exclude extinct breeds) (FAO, 2020a). In all regions other than Europe and the Caucasus and North America, more than 80 percent of local breeds are of unknown risk status. Improving reporting is thus a major challenge. AnGR are threatened by a range of factors. Immediate threats include breed substitution, poorly managed cross-breeding and the decline of livestock-keeping livelihoods, all driven in turn by a variety of economic, social and environmental factors, exacerbated by weak policies and institutions (FAO, 2015). Acute events such as disease outbreaks can be a threat to small, geographically concentrated breed populations (ibid.).

Forest genetic resources (FGR)

There are over 60,000 tree species in the world (Beech *et al*, 2017). Most of these are wild species that have not been subject to any form of domestication. The country reports submitted for *The State of the World's Forest Genetic Resources* (SoW-FGR) (FAO, 2014b) listed nearly 8,000 species of trees, shrubs, palms and bamboo, of which about 2,400 were being actively managed for the products and/or services they supply and over 700 were included in breeding programmes. Information on the status of tree species remains incomplete. The Global Tree Assessment, which aims to assess the conservation status of all known tree species by 2020, reports as of March 2020 that 34,204 species (57 percent of all tree species) have been assessed and that 12,237 (36 percent of the assessed species) are threatened globally (Global Tree Assessment (GTA), 2020). There is no systematic global monitoring system in place for intraspecific diversity in tree species, but loss of genetic diversity in commercially important species has long been a concern among forest managers (FAO, 2014b).

FGR are threatened, *inter alia*, by land-use change, particularly conversion of forests to cropland and grazing land, overexploitation, selective harvesting and climate change (ibid.). Forests cover 31 percent of the global land area (4,060 million hectares), but they continue to be lost at an alarming rate despite efforts to promote natural regeneration and tree planting (FAO and UNEP, 2020). Between 2015 and 2020, the rate of forest expansion was 5 million hectares per year, while the rate of deforestation was 10 million hectares per year, meaning that the net loss of forests was about 5 million hectares per year (ibid.).

Aquatic genetic resources for food and agriculture (AqGR)

There are more than 160,000 species of fish and aquatic crustaceans, molluscs and plants in the world (FAO, 2019c). Of these, around 1,800 species or species items (a species item is a category of aquatic animal or plant at the species, genus, family or higher taxonomic level) are targeted by capture fisheries (ibid.). The total number of farmed species items recorded in aquaculture production by FAO, as of 2018, was 622, corresponding to 466 individual species, 7 interspecific hybrids of finfish, 92 species groups at genus level, 32 species groups at family level and 25 species groups at order level or higher (FAO, 2020d). However, *The State of the World's Aquatic Genetic Resources for Food and Agriculture* (SoW-AqGR) (FAO, 2019c) indicated that such production figures underestimate the number of cultured species, reporting farming of 694 species or

Box 1. The work of the Commission on Genetic Resources for Food and Agriculture

The Commission, a permanent intergovernmental body currently comprising 178 countries and the European Union, was established in 1983 as the Commission on Plant Genetic Resources for Food and Agriculture. It negotiated the legally binding International Treaty on Plant Genetic Resources for Food and Agriculture, adopted in 2001 (FAO, 2009). In 1995, its mandate was extended to cover all components of biodiversity of relevance to food and agriculture. The Commission regularly oversees country-driven global assessments of particular categories of genetic resources. The first of these, *The State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 1997a), was followed by *The State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007a), *The State of the World's Forest Genetic Resources* (FAO, 2014b) and *The State of the World's Aquatic Genetic Resources for Food and Agriculture* (FAO, 2019c), the latter covering farmed aquatic species and their wild relatives within national jurisdiction. The Commission has also overseen a global assessment covering all components of biodiversity of relevance to food and agriculture, *The State of the World's Biodiversity for Food and Agriculture* (FAO, 2019d). The global assessments are repeated at intervals of approximately ten years, meaning that second reports on plant and animal genetic resources have been published (FAO, 2015, 2010) and that the second assessment on forest and the third on plant genetic resources are ongoing. The first global assessments for plant, animal and forest genetic resources were followed by the adoption of global plans of action for the respective sectors (FAO, 2014a, 2007b, 1997b). In the case of plants, a second global plan of action was adopted in 2011 (FAO, 2011). A global plan of action for aquatic genetic resources is currently under negotiation (FAO, 2019b). The Commission has overseen the development of a number of codes, standards and guidelines to support the implementation of the global plans of action. In 2019, the Commission adopted a Work Plan for the Sustainable Use and Conservation of Micro-organism and Invertebrate Genetic Resources for Food and Agriculture (ibid.).

species items. In Asia, approximately twice as many species are reported farmed as in other continents. The report also records over 200 species that are farmed in countries where they are not native.

Aquaculture is, for the most part, a relatively new activity and the sector has few well-established improved farmed types equivalent to the varieties and breeds of terrestrial crops and livestock (FAO, 2019c). Farmed aquatic organisms are often very similar to their wild counterparts, which are sometimes used as broodstock or seed. Little information is available on the status of AqGR below the species level. As noted in Box 2, FAO is currently developing a prototype registry for these "farmed types".

Micro-organism and invertebrate genetic resources for food and agriculture (MIGR)

Micro-organisms and invertebrates contribute to food and agriculture in a multitude of ways, including in pollination, pest control, nutrient cycling, food processing, and digestion in ruminant animals. The status and trends of micro-organisms and invertebrates, including those that contribute to food and agriculture, are generally less well monitored than those of plants and vertebrate animals. However, at the level of broad taxonomic and functional groups the available evidence indicates worrying declines (e.g. (FAO and ITPS, 2015; FAO, 2019d; IPBES, 2019; IPBES, 2016). The habitats upon which useful micro-organisms and invertebrates depend are often in decline (FAO, 2019d). While the overall number of honeybee colonies worldwide has increased over recent decades, some countries have experienced substantial falls in colony numbers or have required extra efforts on the part of beekeepers to maintain production (FAO, 2019d; IPBES, 2016).

There are big knowledge gaps on the state of soil biodiversity, but there are grounds for serious concern in all regions of the world (FAO, 2019d; FAO and ITPS, 2015). Threats to MIGR include habitat destruction, inappropriate use of pesticides and other agricultural inputs and the effects of climate change (FAO, 2019d).

Management strengthened, but progress patchy

Management of GRFA is taken here to include use and conservation. Each of the three existing global plans of action (GPAs, see Box 1) sets out priorities in each of these areas. Implementation is monitored via periodic rounds of country reporting and via the information systems mentioned in Box 2. The following subsections provide overviews based on these and other sources. It needs to be borne in mind, however, that use and conservation are multifaceted and interlinked fields of activity and that their boundaries are not clearly defined. Definitions and approaches to monitoring vary across sectors, as does the significance of specific management activities (e.g. *in situ* vs. *ex situ* conservation). Space precludes a detailed discussion of the state of the art in management or of the status of implementation of management activities around the world. Readers are directed to the "*State of the World*" reports (Box 1) for additional information.

Plant genetic resources for food and agriculture

Higher level composite indices for the implementation of the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (Second GPA-PGRFA) were calculated for the period 2012 to 2014 based on data provided by 69 countries (FAO, 2020e). Scores for actions related to the sustainable use of PGRFA were generally at a medium level (averaging approximately 4.3 out of a maximum possible 8). A

Box 2. FAO's information systems on genetic resources for food and agriculture

FAO operates global information systems for plant and animal genetic resources for food and agriculture, both of which are used for monitoring progress towards Sustainable Development Goal (SDG) Target 2.5.

The Domestic Animal Diversity Information System (DAD-IS)[a] provides tools that can be used to monitor national breed populations and to support informed decision-making on the management of animal genetic resources for food and agriculture. It provides access to official data for monitoring progress towards the animal component of SDG Target 2.5.

The World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS)[b] provides access to official data for monitoring progress towards the plant component of SDG Target 2.5 and on the implementation of the 18 priority activities of the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture.

In 2019, the Commission on Genetic Resources for Food and Agriculture requested FAO to initiate the development of a new global information system on forest genetic resources (FAO, 2019b). Work is also underway to develop a global information system for aquatic genetic resources for food and agriculture, including a prototype registry of farmed types based on standardized terminology (Mair and Lucente, 2020). In the absence of such a system, AqGR are largely excluded from the monitoring of progress towards SDG 2.5. These new information systems will be fundamental to the implementation and monitoring of the Global Plans of Action in the respective sectors.

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[a] http://www.fao.org/dad-is/en/

[b] http://www.fao.org/wiews/en/

preliminary study conducted on a smaller sample of country reports (FAO, 2016) indicated several positive developments in the field of characterization, evaluation and further development of specific collection subsets to facilitate use, with many genebank accessions reported as having been assessed and distributed for use. In the field of plant breeding, genetic enhancement and base-broadening, again a range of activities were reported, focused mainly on major crop species. International and regional networks of genebanks were reported to be widely involved in the supply of germplasm. About one-third of the reported activities in this field aimed to address constraints relevant to the production systems of small-scale farmers or local communities. Genetic enhancement and pre-breeding activities mainly targeted local cultivars and landraces. Actions promoting diversification of crop production and broadening crop diversity received a relatively low average score. However, several initiatives were reported, including the introduction of a number of new crops or wild species into cultivation. Countries reported a range of laws, policies, programmes and projects promoting the development and commercialization of crop varieties. Actions related to supporting seed production and distribution received the highest average scores, with vegetables and cereals being the crop groups most widely reported to be targeted[1].

The state of *ex situ* conservation for PGRFA is monitored under SDG Target 2.5 (Box 2). Over the past 24 years, the number of PGRFA accessions stored under medium or long-term conditions has steadily increased (by approximately 100,000 accessions per year) reaching 5.4 million – held in over 700 genebanks in 103 countries and 17 regional and international centres – in 2019 (FAO, 2020e). These figures are lower than previously published estimates (e.g. FAO, 2010) as current WIEWS data comply with SDG 2.5.1 prescriptions for avoiding duplication in the reporting of collections and accessions within national inventories. Between 2000 and 2018, the number of species conserved in these collections more than doubled, increasing from about 24,000 to over 51,000 (ibid.). While the highest rate of increase occurred during the first 10 years, on average about 700 new species were added to *ex situ* collections worldwide annually during the period from 2014 to 2018. These increases were the result both of collecting missions and of improved taxonomic classification of already conserved materials.

As of December 2019, 290 genebanks around the world held almost 96,000 samples from over 1,700 species listed in the International Union for Conservation of Nature's categories of major global concern[2] (FAO, 2020e), including relatives of crops particularly important for global and local food security. Despite the progress made, the global response in terms of preserving crop diversity in *ex situ* facilities compliant with genebank standards is likely to be insufficient to respond to the alarming pace of the growth of the threats posed by climate change, particularly of the case for crop wild relatives, wild food plants and neglected and underutilized crop species. Species in these groups continue either to be absent from genebank collections or have their intraspecific diversity poorly represented.

Reporting on the implementation of the Second GPA-PGRFA between 2012 and 2014 indicated that increased attention was being given to the *in situ* conservation of crop wild relatives. Among the 30,000 *in situ* conservation sites reported in 39 countries, 9 percent had management plans addressing crop wild

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[1] All the findings presented here from the preliminary study were confirmed by the analysis of the larger sample of countries (FAO, 2020e).

[2] Extinct in the Wild, Critically Endangered, Endangered, Vulnerable, Near Threatened and Data Deficient (IUCN, 2020).

relatives and wild food plants (FAO, 2020e). However, indicator scores for this area of management were low. Overall, *in situ* conservation and on-farm management (comprising priority activities in the fields of surveying and monitoring, supporting on-farm management and improvement, assisting farmers in disaster situations to restore crop systems, and promoting *in situ* conservation and management of crop wild relatives and wild food plants) underperformed as compared to *ex situ* conservation and other areas of PGRFA management (ibid.).

Animal genetic resources for food and agriculture

The third round of country reporting on the implementation of the Global Plan of Action for Animal Genetic Resources (GPA-AnGR) took place in 2019. Analysis of the 104 country progress reports submitted is ongoing at the time of writing, but broadly speaking they reveal that many countries have continued to strengthen their activities related to sustainable use and development. However, the level of implementation and the extent to which progress has been made since the adoption of the GPA vary greatly both across regions and across countries within regions, with higher levels reported in Europe and the Caucasus and North America than elsewhere. In 2014, when the previous round of country reporting took place, strategic priorities targeting sustainable use and development were at low to medium levels of implementation, with global average scores of between 0.5 and 1 out of a maximum of 2 (FAO, 2014c). Actions related to breeding programmes scored slightly better than those related to ecosystem approaches and support for local and traditional production systems. Sustainable use policies scored lowest, with averages dragged down by the underdeveloped state of access and benefit-sharing policies in many countries (ibid.).

As with PGRFA, the state of *ex situ* conservation of AnGR is monitored under SDG Target 2.5 (Box 2). Out of 7,760 local breeds (including extinct ones), 258 are reported to have some genetic material stored, and 79 are reported with sufficient material stored to allow them to be reconstituted (FAO, 2020a). The 2019 progress reports on the implementation of the GPA-AnGR indicate that conservation actions have continued to be strengthened over recent years in many countries. The previous round of country reporting again indicated low to medium levels of implementation of strategic priorities in this field (FAO, 2014c). *In situ* conservation scored relatively well compared to *ex situ* conservation (ibid.), although it needs to be borne in mind that *in situ* activities and their impacts are difficult to monitor because of a lack of detailed data and differences in the way the term is used in different countries. Country reporting for The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture (FAO, 2015) indicated that at least some *in situ* conservation activities were being implemented in most countries, with a wide variety of different approaches reported, including those related to breeding programmes, to market development and to other forms of support for farmers and herders raising rarer breeds. However, it also clearly indicated that levels of implementation were far below those that countries considered necessary to provide an adequate degree of protection for their AnGR (ibid.). As of May 2020, 223 (11 percent) of the 1,808 local breeds recorded in DAD-IS (Box 2) as “critical” or “endangered” were listed as “maintained”, meaning that “active conservation programmes are in place or populations are maintained by commercial companies or research institutions” (FAO, 2020a).

Forest genetic resources

The first round of country reporting on the implementation of the Global Plan of Action for the Conservation, Sustainable Use and Development of Forest Genetic Resources (GPA-FGR) took place in 2018 (FAO, 2019a). The response rate was quite low (44 countries) and hence it is not possible to draw comprehensive conclusions. Across the GPA-FGR as a whole, reporting countries had on average achieved 67 percent of action points and had initiated efforts to achieve a further 10 percent. Only four had achieved all 15 action points. A total of 1,145 tree and other woody plant species (including hybrids) were included in the 44 country progress reports. With regard to the state of use, a total of 531 tree species were reported to be included in national tree seed programmes. The numbers reported by individual countries varied greatly, from zero in several cases up to 114. A total of 288 species were reported to be included in tree-breeding programmes, with the numbers reported per country ranging from zero to 55. However, many more species are used in forestry; for the SoW-FGR, countries reported about 2,400 species as being actively managed for products or services in forestry and more than 700 as being included in tree improvement programmes (FAO, 2014b).

Information on the status and trends of *in situ* conservation activities – the main approach to FGR conservation – is limited. In 2018, only 568 species were reportedly included in *in situ* conservation programmes and 647 in *ex situ* programmes. However, the country reports submitted for the SoW-FGR listed nearly 8,000 species of which about 1,000 were reportedly conserved *in situ* and 1,800 *ex situ* (FAO, 2014b). Only 625 out of 2,260 priority species listed were reported to be subject to any kind of *ex situ* conservation, with maintenance in field collections, including clone banks and provenance trials, much more frequently reported than storage in seed or *in vitro* collections (ibid.).

Aquatic genetic resources for food and agriculture

As a GPA for the sector has yet to be adopted, AqGR management has no global monitoring system equivalent to those existing in other sectors. However, some relevant data are available. For example, Metian *et al* (2020) report the use of a large and increasing range of species in aquaculture, particularly in Asia, and argue that this enhances the resilience of the sector by improving capacity to adapt to change. While new species are being developed for aquaculture and the list of cultured species continues to expand, global aquaculture production is increasingly dominated by a few key species, with the top ten species accounting for 50 percent of global production (FAO, 2020c), a trend which, if it continues, may erode resilience to challenges such as disease and climate change.

As noted above, genetic improvement activities are relatively underdeveloped in the aquaculture sector. Among the species listed as being farmed in the country reports submitted for the SoW-AqGR only 55 percent were reported to be subject to any kind of genetic management (FAO, 2019c). While studies indicate that there is potential for major gains in productivity via selective breeding of farmed aquatic species (ibid.), 45 percent of countries reported that genetic improvement was yet to have any significant impact on their aquaculture production, and the report identified an important need to increase the adoption of genetic programmes, especially for lower-value species important to food security. The report highlights the need to set an appropriate balance between investment in the diversification of species used in aquaculture and the application of genetic technologies to better adapt existing cultured species to diverse culture environments.

In situ conservation of AqGR relates mainly to the protection of wild species, for example via the establishment of protected areas, management and regulation of fishing and other habitat-protection measures, although “on-farm” conservation to prevent the loss of farmed-type genetic resources is also required. Both aquaculture and capture fisheries have an important role to play and conservation objectives need to be integrated into aquaculture development and fisheries management strategies.

Countries that contributed to the SoW-AqGR generally considered protected areas to be an effective means of conserving the genetic resources of wild relatives of farmed aquatic species (FAO, 2019c). Seventy-five percent of the 92 reporting countries indicated the implementation of *ex situ* conservation activities for aquatic organisms of national relevance falling within the scope of the report. Approximately 290 different species, almost 200 of which were considered to be threatened at national or international levels, were being maintained in a total of 690 *ex situ* collections. Finfish accounted for 90 percent of the species concerned, with the other 10 percent accounted for by macro-invertebrates and aquatic micro-organisms such as rotifers and micro-algae. Most *ex situ* conservation is *in vivo*. About 38 percent of reporting countries indicated the existence of *in vitro* conservation of AqGR (farmed species and wild relatives), involving a total of 133 different species. Because of the difficulty of preserving the eggs and embryos of aquatic organisms, most *in vitro* conservation involves cryopreservation of sperm.

Micro-organism and invertebrate genetic resources for food and agriculture

Many micro-organisms and invertebrates of importance to food and agriculture are not actively managed in any way by producers. However, many approaches that involve introducing them into production systems or managing habitats to encourage their presence, for example in the context of integrated pest management, pollination management or integrated plant nutrition management, are becoming more widely implemented globally (FAO, 2019d). Few species are subject to genetic improvement. However, there are a substantial number of commercial honey-bee breeding companies around the world that implement genetic improvement programmes, with the main goals being higher honey production, greater docility, reduced swarming and, particularly in recent years, better disease tolerance (ibid.). Micro-organisms used in food processing and in agro-industrial processes are subject to a variety of genetic-improvement strategies (Alexandraki *et al*, 2013; Chatzipavlidis *et al*, 2013). Some genetic improvement is also being conducted in micro-organisms used in plant nutrition, biological control and food preservation (FAO, 2019d).

Micro-organisms and invertebrates are conserved *in situ* along with other components of biodiversity in protected areas. They also benefit from the adoption of biodiversity-friendly management practices in the food and agriculture sector and elsewhere. However, the number of species specifically targeted is limited, as is information on the coverage and effectiveness of conservation measures (ibid.). Micro-organisms can be stored under laboratory conditions in a range of different ways. Existing culture collections are, however, far from representing the full range of micro-organisms of relevance to food and agriculture (ibid.). Various invertebrates of importance to food and agriculture are raised in captivity by commercial companies or by research institutes. However, there are few systematic *ex situ* conservation programmes, even for high-profile groups of invertebrates such as pollinators. Some work has been done on the cryoconservation of bee semen, although the technique has not become widely used (ibid.).

Knowledge gaps a key constraint

Knowledge gaps are a major constraint to the effective management of GRFA. As discussed above, population status and trends are inadequately monitored across most categories, hindering the planning of conservation efforts. The following subsections briefly outline key knowledge gaps by sector and related to cross-sectoral integration.

Plant genetic resources for food and agriculture

Monitoring PGRFA diversity *in situ* and on-farm to predict and minimize loss of inter- and intra-specific genetic variation is a major challenge, particularly in vulnerable groups such as crop wild relatives, wild food plants and underutilized crops. National conservation planning would greatly benefit from the development of indicators that could be widely used to quantify genetic erosion and monitor changes in the extent and distribution of individual species and populations at various scales. Research on the characteristics of the above-mentioned vulnerable groups, including on their reproductive biology, agronomic and nutritional properties, traditional and potential uses, and contributions to the health of agro-ecosystems, is vital to efforts to improve their conservation and sustainable use. Knowledge of their geographical distribution also needs to be improved.

Efforts to integrate *in situ* and on-farm management and conservation of PGRFA with the work of national, regional and international genebanks and research institutes need to be documented and widely publicized. Knowledge gaps on recalcitrant seed physiology and behaviour in neglected species, along with a lack of standardized protocols for their *in vitro* conservation and cryopreservation – and a lack of alternative low-cost conservation methods – is often a severe constraint to national *ex situ* conservation programmes. Other key knowledge gaps relate to breeding systems, reproductive biology, dormancy mechanisms and technical problems associated with regeneration practices for “unconventional” species. The use of molecular methods, biochemical assays and high-throughput phenotyping in germplasm characterization and evaluation to identify useful genes, understand their expression and variation, and in particular understand their roles in adapting to climate change, increasing nutritional values and strengthening ecosystem services, has been limited to a few major crops in developed countries. Further work is also needed on development and harmonization of standards for the exchange of data on *in situ* germplasm and the documentation of ethnobotanical information on farmers’ varieties, landraces and underutilized species.

Animal genetic resources for food and agriculture

The genomic revolution has led to impressive progress both in terms of improving knowledge of AnGR and in terms of genetic improvement. However, it has also widened gaps between developed and developing countries and between the relatively few international transboundary breeds that increasingly dominate high-input production systems globally and the mass of breeds adapted to more extensive systems. There are clear knowledge gaps in terms of the characterization of phenotypes (especially functional and adaptive traits) and their relations to production environments. As characterization is a prerequisite for effective implementation of genetic improvement programmes (Leroy *et al*, 2016), these knowledge gaps are to some extent hindering the realization of the opportunities offered by genomics.

One of the most important challenges in AnGR management relates to the difficulty of developing governance systems that fully integrate livestock keepers from developing regions (Leroy *et al*, 2017). Systems of this kind are vital to the implementation of characterization studies, breeding programmes and market development (Gowane *et al*, 2019). Experiences in this field need to be documented and publicized, although success will also depend on the provision of adequate institutional, technical and financial support over the long term (Mueller *et al*, 2015).

Forest genetic resources

Priorities in the field of FGR management include improving knowledge of the amount and distribution of genetic diversity in forest trees and of how well current efforts to conserve FGR *in situ* are maintaining this diversity in the long term (FAO, 2014b). There is also a need to enhance the production of seed and other forest reproductive material, especially for many native tropical and subtropical tree species, to meet demand for restoration and for establishing new forests and tree-based production systems (FAO, 2014b; FAO and UNEP, 2020). Furthermore, recent advances in forest genomics need to be translated into practical applications for conserving and using FGR and for increasing our understanding of the adaptation of forest trees to climate change (e.g. Holliday *et al*, 2017).

Aquatic genetic resources for food and agriculture

Characterization and monitoring of AqGR suffers from a lack of knowledge of genetic resources below the level of species and a lack of standardization and harmonization of terminology and nomenclature. The prototype registry being developed by FAO for farmed types (Box 2) will help address this issue by promoting the collection and sharing of key information on the availability and properties of AqGR. A variety of genetic technologies can be used to develop and improve farmed types for use in aquaculture. However, a clear understanding of the risks and benefits of these technologies is often lacking. Aquaculture stands to benefit greatly from effective implementation and uptake of well-managed breeding programmes, with a focus on selective breeding. Many governments consider this a role for the public sector, but such programmes often fail to deliver tangible and long-term increases in production. There is a need to identify mechanisms for effective engagement of the private sector in such programmes, for example through public–private partnerships. Finally, cryopreservation clearly has a role to play in *ex situ* conservation of AqGR, but further research is needed into methods for cryopreservation of eggs and embryos.

Micro-organism and invertebrate genetic resources for food and agriculture

There are enormous knowledge gaps related to MIGR. In every taxonomic and functional group, many species remain to be identified and characterized. The roles of MIGR in the supply of ecosystem services, how they are affected by environmental changes and how they can be managed to support food and agricultural production need to be much better understood. Knowledge of the significance of micro-organism and invertebrate diversity at within-species level to food and agriculture is very limited.

Integrated management

Integrated use of the various "sectoral" categories of genetic resources can give rise to a range of synergies and complementarities that can help increase productivity in a sustainable way and make production systems more resilient (Dawson *et al*, 2018; Duval *et al*, 2018; FAO, 2019d). There is a need for research into how integrated management can be made more effective at a range of scales, from the individual plot to the landscape. This needs to include research into how genetic resources management can contribute, for example via appropriate choice of combinations of species, varieties, breeds, etc. for use in particular integrated systems and via appropriate genetic improvement strategies.

Time to step up action

Despite some positive developments in various aspects of GRFA management, much remains to be improved. Progress towards SDG 2.5 has been minimal overall. Action clearly needs to be urgently stepped up across all sectors. However, there is also a vital need to improve cross-sectoral cooperation. For example, many drivers of loss of GRFA affect more than one sector of food and agriculture and in many cases also affect species and ecosystems that are priorities for the nature conservation sector. Habitat destruction is a major driver of loss of forest, aquatic, invertebrate and micro-organism genetic resources, as well as of wild relatives of crops and livestock and of biodiversity in general. Climate change is a severe threat across all categories of GRFA. Threats of this kind need to be addressed in a comprehensive and cross-sectoral way, with the food and agriculture sector recognizing its role as a major contributor to biodiversity loss.

For domesticated plants and animals, changes in consumption patterns and production systems that lead to declines in the use of diverse GRFA are a major threat. This threat can to some extent be addressed by ensuring that diversity is utilized as fully as possible in the interests of livelihoods and food security, for example via the production benefits of raising species, breeds, varieties and farmed types that are well adapted to local conditions, the nutritional significance of diversity in the food supply and the marketing opportunities associated with unique products provided by specific GRFA. However, there is a need to recognize that the maintenance of genetic resources for the long term is a public good and that interventions specifically aimed at supporting producers in this role will, in some cases, be necessary. The challenge is to maximize synergies and manage trade-offs among the various demands placed on production systems in terms of supporting and improving local livelihoods and in terms of the reliable supply of a broad range of ecosystem services, including genetic resources conservation. Within a given landscape or seascape, this may require cooperation among stakeholders from the crop, livestock, forest, aquaculture, fisheries and nature-conservation sectors (among others).

Approaches that effectively combine *ex situ* conservation with *in situ* conservation, and conservation with sustainable use, need to be promoted. These activities need to ensure that they target a sufficiently wide range of genetic resources to meet the needs of producers and other stakeholders across a range of diverse and changing production systems and, in the longer term, the needs of future generations. In this regard, there is a need to increase efforts to raise awareness among policy-makers (and other stakeholders, including consumers) of the importance of neglected and underutilized GRFA. More generally, awareness raising with respect to the significance of all types of GRFA and the need to manage them sustainably remains a key priority.

Across all sectors (including in the context of integrated management), the numerous knowledge gaps that constrain effective management of GRFA need to be urgently addressed. Where research is concerned, there is again a need to ensure that activities are sufficiently broad based in terms of the genetic resources and production systems targeted. Attention needs to be given to how new technologies and existing good practices can be scaled up and adapted for implementation in different contexts.

Enabling policy, legal and institutional frameworks for sustainable management need to be put in place at all levels, including mechanisms for ensuring active and equitable stakeholder participation and collaboration. Stakeholder organizations and networks of various kinds have important roles to play, and their establishment or strengthening should be promoted where necessary. Although not a topic focused on in this paper, problems with the implementation of access and benefit-sharing mechanisms also remain to be addressed in many countries.

At global scale, the existing GPAs have provided a valuable framework for planning and monitoring actions across the various fields of GRFA management, helped to raise awareness and promoted international cooperation. Over the coming period, the Commission will be working to finalize a global plan of action for AqGR and a global plan of action or other policy response for biodiversity for food and agriculture as a whole. The Convention on Biological Diversity is in the process of developing a global framework for all biodiversity for the post 2020 period (Convention on Biological Diversity, 2018). There is an urgent need for the international community to engage fully in these processes and in the implementation of their outcomes and those of the existing GPAs. Research has an essential role to play in informing both policy development and the implementation of agreed actions.

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Author contributions

D.P. and J.B. contributed to the conception and design of the submitted manuscript. D.P. drafted the manuscript. All authors contributed to data gathering and analysis and to the drafting, revision and final approval of the submitted manuscript.

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Swedish Crop Wild Relatives: towards a national strategy for *in situ* conservation of CWR

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Abstract: In 2015, the Nordic countries (Sweden, Denmark, Finland, Norway and Iceland) initiated a project to help strengthen the efforts of conservation and use of crop wild relatives (CWR) across the region. Policy recommendations that were put forward included creating national strategies for each Nordic country and adopting and implementing complementary *in situ* conservation as the main approach for safeguarding CWR across the region. The present work explores in greater detail the situation for Sweden. Taxa rich areas and areas where potential data bias may be prevalent are located. An eco-geographic map is constructed to help determine how genetic diversity may be portioned across the country within populations of taxa. An *in situ* complementarity analysis accounting for taxa richness, eco-geographic richness and the protected area network in the country is also presented. Possible reasons for diverging results, as compared to the regional analysis, are discussed. The document serves as a starting point for further in-depth research on CWR distribution, conservation and use within Sweden.

Keywords: crop wild relatives, protected areas, ELC-analysis, Sweden

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Introduction

Already back in 1979, the Nordic countries established what was probably the first regional genebank for the *ex situ* conservation of seeds of agricultural and horticultural plants. For many years, the (then) Nordic Gene Bank stood as an example of foresight regarding long-term conservation and use of plant genetic resources. Although occasional attempts were made to raise the issue of *in situ* conservation at the Nordic level (Blixt *et al*, 1992), concrete work and activities never took off. Decades later, during the period 2015-2019, the Nordic countries (Sweden, Denmark, Finland, Norway and Iceland) joined forces and initiated two subsequent projects to help strengthen the efforts of conservation and use of crop wild relatives (CWR) across the region. Whereas the first project focused on reviewing and revising previously published compilations of CWR taxa, and their prioritisation, the second one put more emphasis on developing guidelines. As a result, policy recommendations were put forward that included creating national strategies for each Nordic country, and adopting and implementing complementary *in situ* conservation as the main approach for safeguarding CWR across the region (Weibull *et al*, 2016).

A central activity of the second project (Wild genetic resources – a tool to meet climate change) included an Eco-geographic Land Characterisation (ELC) analysis. Using eco-geographic diversity as a proxy for genetic diversity is a well-known technique (Parra-Quijano *et al*, 2012) that has been employed for certain Nordic countries (Phillips *et al*, 2016), but not previously for the entire Nordic region. Based on more than 971,000 occurrence records, and using ELC and so-called Complementary Conservation Analysis (Rebelo, 1994), Fitzgerald *et al* (2019) were able to single out those protected areas (PAs) in the region harbouring the largest number of priority CWR. The number one complementary PA site was in Aalborg Commune in

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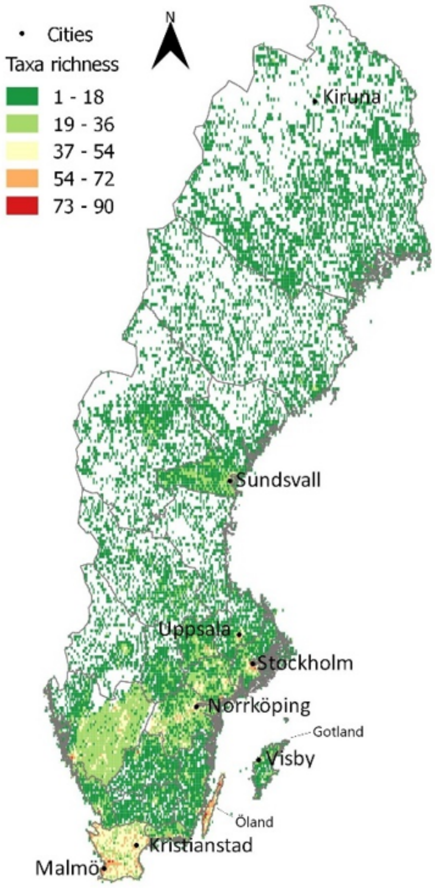

Figure 1. The taxon richness of priority CWR across Sweden.

Denmark covering 88 target species in two ELC zones. The surprising fact that the first complementary PA site in Sweden firstly appeared as number 13 on the Nordic list and, secondly, was represented by a PA site in the mountain region close to Norway called for an extended analysis.

The work presented below takes a specific Swedish perspective and aims to answer the following questions: (1) How common are Nordic priority taxa in Sweden and how are they distributed over the country? (2) Will a targeted ELC analysis provide an eco-geographic map of higher resolution? (3) How well does taxon diversity and genetic proxy diversity coincide with the existing distribution of PAs? (4) Will we be able to pinpoint specific sites in Sweden where active *in situ* conservation of CWR may begin? We began by locating taxa rich areas and areas where potential data bias might be prevalent and continued by constructing an eco-geographic map to help determine how genetic diversity could be portioned across the country within populations of taxa. An *in situ* complementarity analysis accounting for taxa richness, eco-geographic richness and the PA network in the country was also performed. We see this work as a starting point for further in-depth research on CWR distribution, conservation and use within Sweden.

Methods and Results

Priority CWR in Sweden

In line with the Nordic level approach, priority CWR for Sweden were identified from the regional list of priority CWR (Fitzgerald *et al*, 2018). Therefore, the Swedish priority list contained 121 naturalized and indigenous taxa. Data on the taxa distribution was gathered from Swedish LifeWatch (https://www.analysisportal.se/) and limited to data gathered between the years 1990-2018. Distribution data was combined for duplicated taxa from the initial list, e.g. *Barbarea vulgaris* and *Barbarea vulgaris* var. *vulgaris*, to limit duplication of results. In total, 102 priority taxa were used for Sweden that altogether consisted of 617 320 occurrence points. Number of occurrences per taxon ranged from 29 646 (*Vaccinium myrtillus* L.) to less than 100 (*Brassica nigra* (L.) W.D.J. Koch, *Lactuca quercina* L., *Rubus allegheniensis* Porter, *Trifolium alpestre* L. and *Trifolium pratense* var. *maritimum* Zabel). The taxa with fewer than 100 occurrences should be considered for further research and surveying efforts to confirm their distribution and levels of vulnerability.

Analysis of species richness and bias

To identify areas of species richness and data bias the TomBio Tool in QGIS software QGIS (2020) was utilised. Analysis of taxon richness (Figure 1) shows clearly that the south and east of Sweden, including the island Öland in the Baltic Sea, are the areas containing the highest number of different taxa.

The areas in the north of Sweden appear to be the least rich in priority taxa, however these areas also have the lowest number of recorded taxon occurrences (Figure 2).

This is to be expected since these areas cover two thirds of the country and to a very high degree overlap with the three boreal zones (southern, middle and northern) and the alpine zone$, i.e. bio-geographical zones characterised by lower winter temperatures, shorter vegetation periods and lower habitat diversity. An exception to this general picture includes the coastal area along the Bothnian Gulf all the way up to the Swedish-Finnish border at Haparanda which is characterised by slightly more favourable climate and, thus, growing conditions.

Although there are more CWR occurrence data in the areas in the southern third of the country, especially around large cities, this pattern is not completely reflected within the taxon richness map. Historically, occurrence data tend to be collected on an ad hoc, non-systematic, basis and closer to cities due to ease of access (Chapman, 2005). The pattern of CWR occurrences in Figure 2 also mirrors the demography of Sweden[1] and, as an additional effect, the location of main educational centres (universities, colleges). Therefore, any potential bias this may cause in the

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[1] See e.g. https://sedac.ciesin.columbia.edu/data/set/grump-v1-population-density/maps/2?facets=region:europe

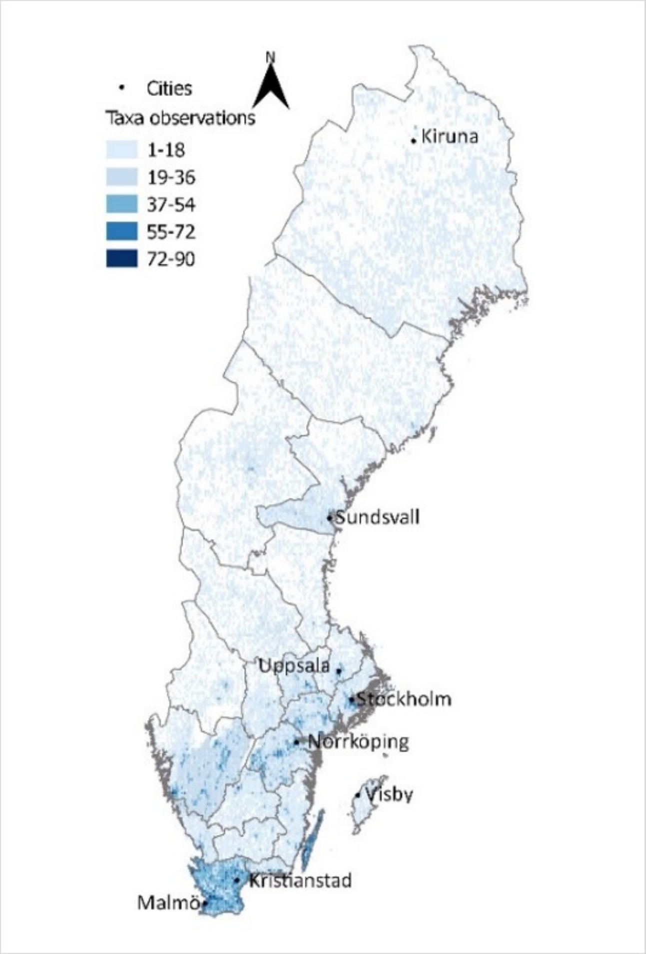

Figure 2. The number of occurrences of the priority taxa across Sweden.

results should be acknowledged. This also shows a need to further survey those areas showing gaps in occurrence data to limit any biased results in future work. More surveying in the northern and western boreal and alpine regions of Sweden will help to fill in gaps in our knowledge on CWR distribution and increase the accuracy of predictive analyses using Geographic Information Systems.

Developing an Eco-geographic Land Characterization map

Eco-geographic maps take account of environmental variables that combined create unique adaptive scenarios for plant species. A combination of geophysical, edaphic and bioclimatic variables that have the greatest influence on abiotic adaptation of the species are then used to create an Eco-geographic Land Characterization map (ELC map). The resulting ELC zones can be used as a substitute to represent genetic diversity (Parra-Quijano *et al*, 2012). Thus, if populations are conserved both *in situ* and *ex situ* across their eco-geographic range (i.e. within all their ELC zones), this will ensure that the full range of genetic diversity is protected.

The eco-geographic map for Sweden was created with the CAPFITOGEN software (Parra-Quijano *et al*, 2016) using the following environmental variables: isothermality (average temperature range/annual temperature range), elevation, aspect of slope, 'northness', 'eastness', topsoil organic carbon content, topsoil pH, and topsoil depth[2]. Figure 3 (left) shows the ELC map comprising 25 ELC zones at a resolution of 1 km^2 cells. The large-scale pattern of ELC zones agrees reasonably well with the dominating land use classes of the country (Figure 3, right), which indicates that the ELC analysis does provide a useful estimate of vegetation characteristics and habitat diversity.

Complementarity analysis

The complementarity analysis is an important concept for ensuring efficient conservation of resources. As described by Rebelo and Siegfried (1990), the analysis uses an iterative selection approach in which the cell, or PA, with the highest taxon number is selected first. These taxa are subsequently excluded from the analysis and the location with the next highest number of different taxa is selected, upon which the procedure is being repeated until all taxa are conserved across a network of reserve locations. The complementarity analyses were created using CAPFITOGEN software (Parra-Quijano *et al*, 2016). Our complementarity analysis of the priority CWR within the network of PAs identified eight complementary areas that altogether conserve 101 (99%) of the priority taxa (Figure 4).

The PA complementary network ensures that the largest number of different taxa are protected. In Sweden, the majority of suitable PAs, as regards priority CWR, were found to be located in coastal zones of Southern Sweden. The number one priority reserve, Kristianstad Vattenrike – a UNESCO-MAB Biosphere Reserve – is the number one priority location as it contains the highest number of unique taxa (85 of 102 taxa; Table 1). With the addition of the two following PAs – Stora Alvaret, a Birds Directive PA, and Tjålmejaure-Laisdalen, a Ramsar Site in Lapland – 93% of the unique taxa on the Swedish priority list are covered.

Using the eco-geographic map, we can determine which ELC zones are within each of the complementary PAs. This will help to determine how well represented the eco-geographic zones are within the proposed network, which may then help to determine the range of genetic diversity among populations that is captured within the network. In our study, 13 of the 24 ELC categories, or 54 %, are represented within the proposed PA complementary network.

Grid cell complementarity analysis

The grid cell complementary network takes account of the number of taxa across the whole of Sweden (not

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[2] The dataset used in this publication was made available by the Swedish Forest Soil Inventory, with responsibility in the Department of Soil and Environment, SLU. The authors are solely responsible for the interpretation of data. url: https://www.slu.se/miljoanalys/statistik-och-miljodata/miljodata/webbtjanster-miljoanalys/markinfo/markinfo/kartor/ (accessed 2020-04-23)

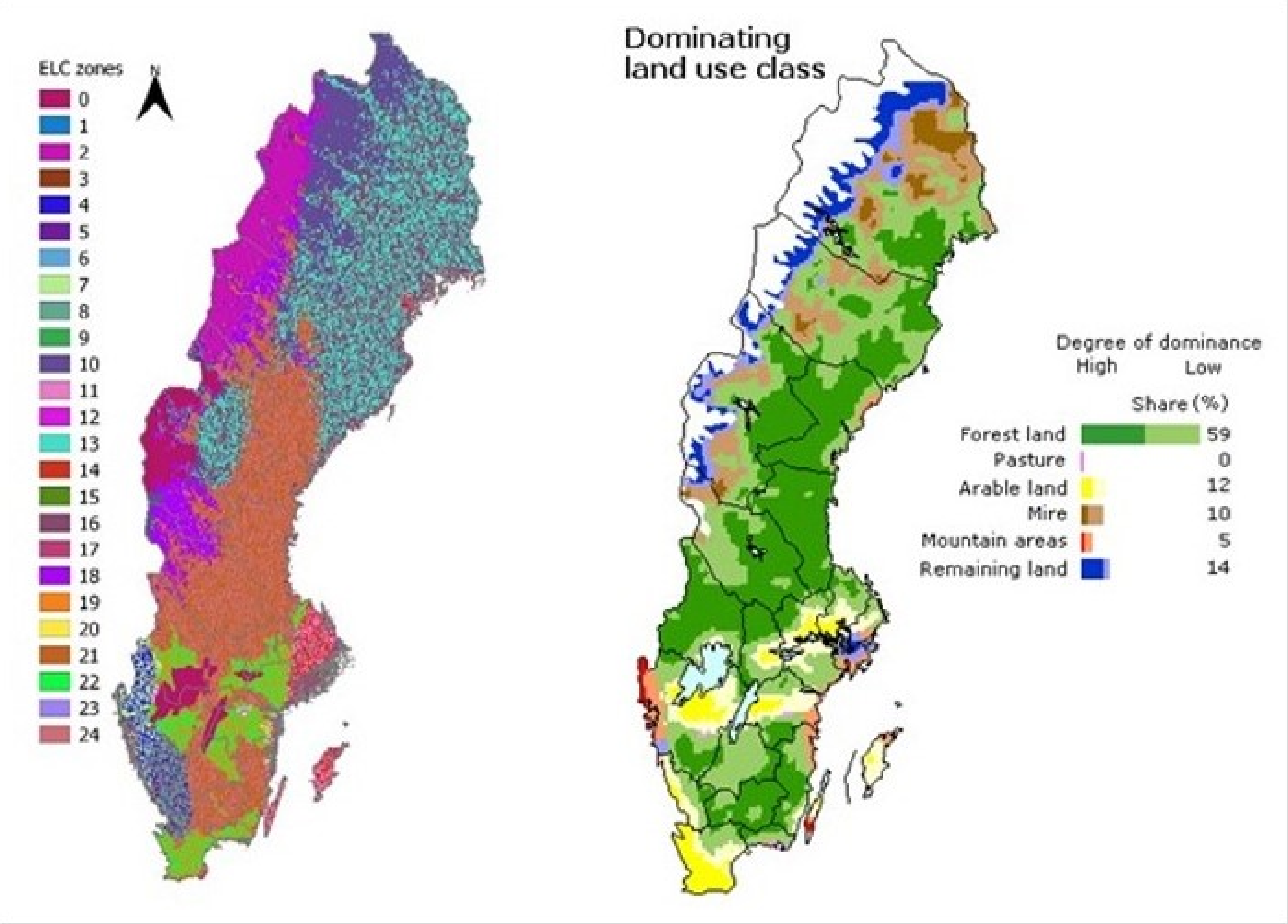

Figure 3. The Swedish Eco-geographic Land Characterization map at a 1 km^2 resolution (left), and a schematic view of dominating land use classes in Sweden (right).

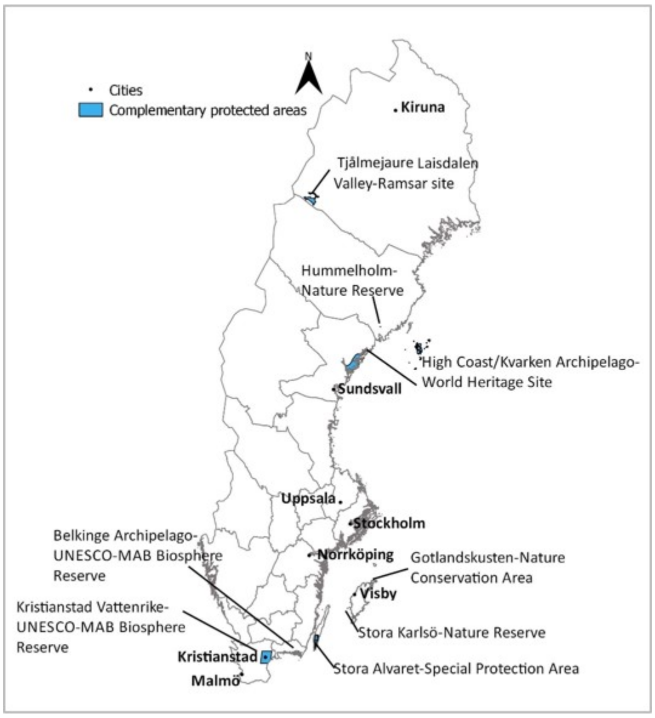

Figure 4. The eight protected areas needed to conserve 101 priority CWR taxa.

just within PAs). The grid cell complementarity analysis revealed that to protect the same 101 priority taxa, altogether 10 (5 km^2) locations are required (Figure 5). The majority of these are found in the south of Sweden along the coast and in the east of Sweden around Stockholm and Uppsala. While the number one grid cell, located near Stockholm, includes 76 different taxa the three top cells in Sweden protects close to 90 % of the priority taxa.

Overlaying both the PA complementary and grid cell complementary networks shows where locations overlap. This serves to help identify which locations to investigate further for potential *in situ* protection of CWR. In Sweden, it would be most efficient to focus initial *in situ* conservation efforts within those PAs that are located in the south such as Kristianstad Vattenrike, Stora Alvaret and Gotlandskusten. These PAs are also close to priority grid cell complementary locations.

Discussion

The rationale for carrying out this extended analysis was the fact that Sweden came out rather poorly in the study by Fitzgerald *et al* (2019). The southern

Table 1. Protected Area Complementarity Analysis. The 'number of taxa' is the total number of different taxa in the protected area. The 'number of additional taxa' is the number of unique taxa within that protected area (i.e. these taxa are not found in any of the previous protected areas).

Protected area	Designation	Number of taxa	Number of additional taxa	Priority	Cumulative %
Kristianstad Vattenrike	UNESCO-MAB Biosphere Reserve	85	85	1	84,2%
Stora Alvaret	Special Protection Area (Birds Directive)	71	6	2	90,1%
Tjålmejaure-Laisdalen	Ramsar Site, Wetland of International Importance	19	3	3	93,1%
Blekinge arkipelag	UNESCO-MAB Biosphere Reserve	77	2	4	95,0%
Gotlandskusten	Nature Conservation Area	66	2	5	97,0%
Stora Karlsö	Nature Reserve	43	1	6	98,0%
Höga kusten/ Kvarkens arkipelag	World Heritage Site	42	1	7	99,0%
Hummelholm	Nature Reserve	14	1	8	100,0%
		Total	**101**		

parts of the country, known to have been repeatedly inventoried since the mid-1800s and whose flora is very well mapped (e.g. Weimarck and Weimarck, 1985; Sterner, 1986; Genberg, 1992; Rydberg and Wanntorp, 2001; Fröberg, 2006; Edqvist and Karlsson, 2007; Johansson *et al*, 2016; Johansson and Petersson, 2016), were surprisingly underrepresented as compared to the findings of other countries. When comparing our results with those of Fitzgerald *et al* (2019), we observe some immediate differences. Whereas both studies have three sites in common – Höga kusten (World Heritage Site), Gotlandskusten (Nature Conservation Area) and Hummelholm (Nature Reserve) – all other locations differ. In particular, we note that our two top locations – Kristianstad Vattenrike (UNESCO-MAB Biosphere Reserve) and Stora Alvaret (Special Protection Area - Birds Directive) – were not even included in the joint Nordic analysis.

There may be several reasons for this, but we suggest that a main cause could be the background data upon which the analysis is based. The datasets provided by the UN Environment Programme World Conservation Monitoring Centre (WCMC-UNEP) contain the entire spectrum of PAs, ranging from areas with 'strict' protection such as national parks, nature reserves, habitat protection areas, and wildlife and plant sanctuaries via so-called natural monuments (e.g. individual and unique trees) to World Heritage Sites and UNESCO-MAB Biosphere Reserves. In our analysis for Sweden, we deselected sites that could give bias to our analysis including, e.g., those representing different habitats or purposes of protection such as HELCOM areas (Baltic Sea PAs), OSPAR (Marine PAs), and RAMSAR sites. In addition, natural monuments that commonly represent individual objects were also removed. In our view, these measures provide a better subset of PAs on which to draw conclusions.

Another aspect relates to the analysis of occurrence data. While Fitzgerald *et al* (2019) used 971,633 data points in their analysis of the entire Nordic region, we based our results on 617,320 data points from Sweden only (time frame 1990-2018). We argue that data robustness is absolutely essential to be able to draw proper conclusions from analyses at a higher level of resolution. The risk of bias when using large data sets of distribution records, such as those available from the Global Biodiversity Information Facility (GBIF), has been shown earlier (Beck *et al*, 2014). We certainly acknowledge the value of the broad Nordic analysis, but, as shown in this study, care should be taken when drawing generic conclusions to describe the situation 'on ground'.

The large differences in number of ELC zones found in the regional vs. the national analysis, respectively, may at first seem surprising. What could the reason(s) be that we observed 25 ELC zones while Fitzgerald *et al* (2019) only described 8-10 in their analysis? The fact that an analysis covering the entire Nordic Region *per se* implies a much larger geographical scale also means that the ELC variables used should try to capture the landscape over a wider range of eco-geographic 'niches'. Given that the diversity of zones vary greatly from Northern

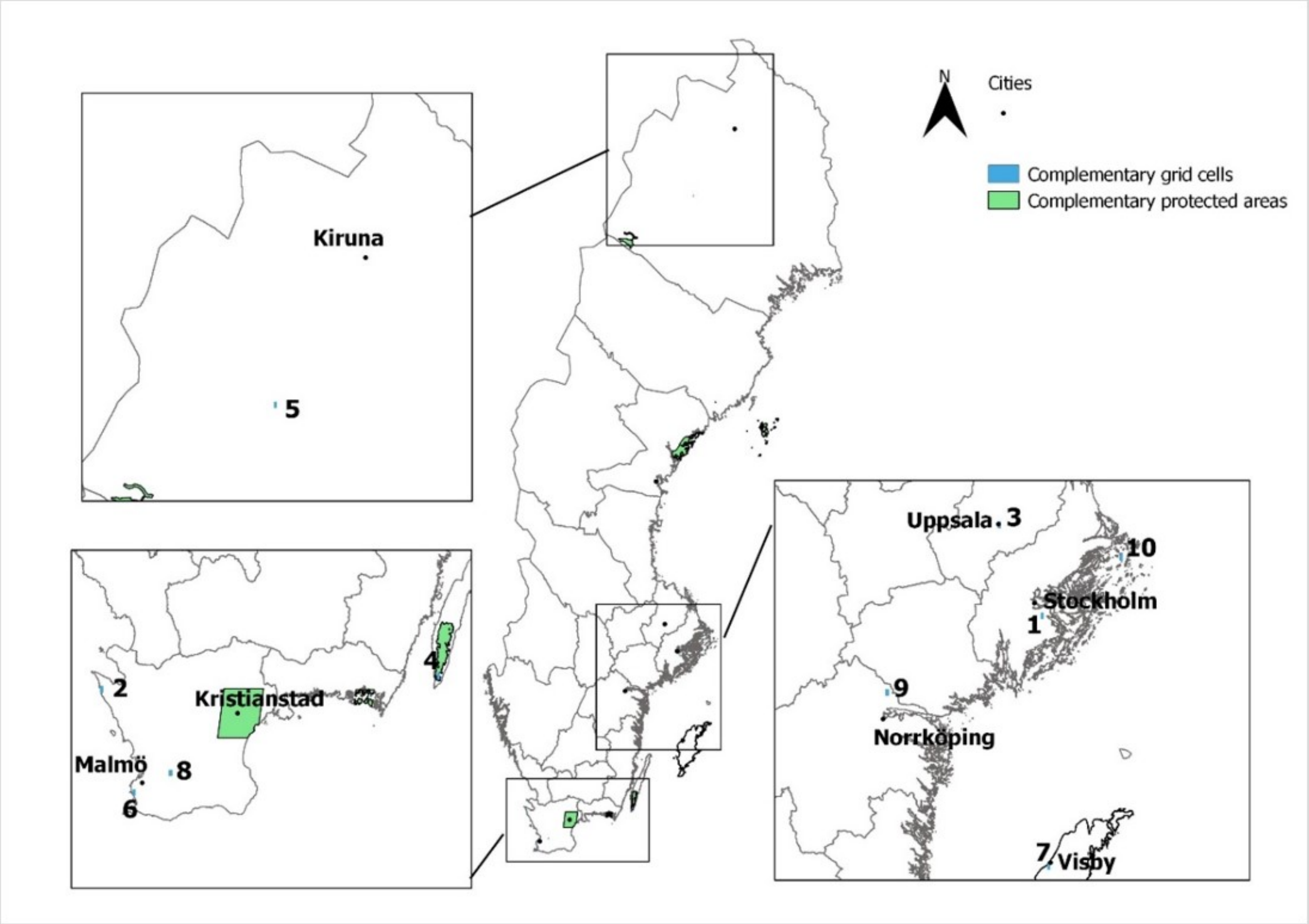

Figure 5. The protected area and grid cell complementary networks. The large black numbers on the map represent the priority grid cell locations and the blue areas are the complementary protected areas.

Iceland to South Denmark and Eastern Finland, it should be expected that Sweden – not sharing all the same niches – would only be described by a share of all the zones. While the regional analysis is important from the point of developing joint approaches, this observation highlights the importance of also looking at domestic eco-geographic variability as a basis for selecting key PAs for CWR *in situ* conservation. Finally, the observation by Fitzgerald *et al* (2019) that 58 % of the identified important PAs for CWR conservation were situated in Norway raises the notion of possible data bias, as well as the procedure by which sites for CWR diversity are being identified. Firstly, while the total number of PAs in Norway is only 27 % and 50 % of that of Finland and Sweden, respectively, the vast majority (80.6 %) are classified as Strict Nature Reserves (IUCN PA category Ia). Finland, on the other hand, is characterised by a large proportion of category VI PAs (89.5 %). The fact that such areas are "[. . .] often established to protect particular species or habitats rather than the specific ecological aims of category Ia" (IUCN, 2020) points to the possibility that CWR diversity is higher in category Ia areas and it is for this reason that Norway takes a lead in the Nordic regional comparison. Secondly, the finding that well-known and diversity-rich sites in several of the countries (e.g. Åland archipelago in Finland and Öland in Sweden) did not appear in the regional analysis calls for a careful evaluation of how data points and variables are used in the analysis. Fitzgerald (personal communication) noted a general problem with coastline taxa that, "depending on the coordinate points and country map boundaries [. . .] in some cases end[ed] up in the sea and therefore [had to] be removed from the analysis." From a national perspective, where priorities need to be made, it is essential that those sites comprising the widest taxon and eco-geographic diversity are selected.

Conclusion

Our extended analysis of occurrence data of Swedish CWR has helped us to identify three major PAs where *in situ* conservation could take off. Initial steps are now being taken to proceed with concrete measures within the UNESCO-MAB Biosphere Reserve Kristianstad Vattenrike. Further work is needed, however, to ensure the long-term robustness of any CWR conservation strategy within Sweden. Such planned activities are framed within the established Nordic CWR network that is led by NordGen, and include:

- An *ex situ* conservation analysis to identify any gaps in the collection of material for conservation and use outside of PAs, on the assumption that seed management of CWR is technically and economically feasible;
- A predicted distribution analysis of how populations may move under the current climate and to help identify collecting and data bias gaps across the country; and
- A climate change analysis to determine if, how and when taxa may shift their distributions as the climate changes. This will be vital in determining which *in situ* PAs will be the most effective in the long-term conservation of Swedish CWR.

Finally, from a European perspective, it would be worthwhile in the future to foster synergies with other genetic resource domains (e.g. forestry, animal) in terms of identifying conservation sites and needs. Such an approach may help to strengthen an *in situ* conservation network for CWR by adding “value” to proposed *in situ* sites.

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Author contributions

JP carried out most of the PA and ELC complementary data analysis during 2019, and JW re-edited the report into the present format.

Conflict of interest statement

The authors declare no conflicts of interest.

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Developing a Methodology to Balance Benefit-Sharing: Application in the Context of Biodiversity Beyond National Jurisdiction

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Abstract: The effectiveness and success of benefit-sharing measures to date, particularly in contributing towards the conservation and sustainable use of biodiversity, has been questionable. This is likely related to the degree of beneficial impacts versus burden on the users and regulatory authorities in terms of administrative complexities. It is, therefore, timely to reconsider which forms of benefit-sharing may most favourably balance the associated beneficial and burdensome aspects. The aim of this paper is to develop and demonstrate a benefit-sharing balance methodology which can be used as a tool to help decision-makers to select options in an objective and transparent manner. Application in the biodiversity beyond national jurisdiction context provides a useful example of how this tool can be used. Results suggest that sharing of genetic sequence data and research results provide the most favourable balance in terms of non-monetary benefit sharing, whilst the most favourable monetary benefit-sharing options were associated with research funding and salaries. The benefit-sharing balance methodology presented here provides a useful tool and starting point, which can be built upon in the future, to include more detailed information gathered from expert groups to consolidate the perceived balance of beneficial impacts versus burden. In addition, the equation can be tailored according to different policy settings where different benefit-sharing factors may be more appropriate. Ultimately, use of this tool could help to enhance implementation of benefit-sharing policies/legislation with greater potential to balance beneficial impacts with associated burden, thereby enhancing workability of the access and benefit-sharing system as a whole.

Keywords: benefit-sharing, methodology, genetic resources, marine, areas beyond national jurisdiction

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Introduction

According to Article 1 of the Convention on Biological Diversity (CBD, 1992), the objective of the Convention is '*the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources*'[1]. Article 15.7 of the Convention takes a step further by stating that Parties shall take measures to share benefits such as results of research and development (R&D) and benefits arising from commercial and other utilization of genetic resources (CBD, 1992). However,

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[1] Article 1, (CBD, 1992): '*The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.*'

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despite attempts made by authors such as Morgera (2014), there is as yet no specific, internationally agreed legal definition of the term 'benefit-sharing' in the context of utilising genetic resources (Schroeder, 2007; Morgera and Tsioumani, 2010; Morgera, 2014; Parks, 2019). In addition, the terms 'fair' and 'equitable' are also not clearly defined (de Jonge, 2011; Morgera, 2014). This can lead to difficulties between stakeholder groups in terms of different interpreted definitions and requirements associated with benefit-sharing, as well as differences in motivation with respect to the notion (de Jonge, 2009, 2011). Nonetheless, the Nagoya Protocol and other genetic resource frameworks, such as the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and the WHO Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits, provide suggestions of what benefit-sharing could entail (FAO, 2001; Nagoya Protocol, 2011; World Health Organization, 2011). A list of types of non-monetary and monetary benefits that can be shared are listed in the Annex of the Nagoya Protocol (2011).

Negotiations for a new international legally binding instrument (ILBI) under the United Nations Convention on the Law of the Sea (UNCLOS, 1982) for the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction (BBNJ) began in 2018. Negotiations address a 'package' of four elements and cross-cutting issues. The four elements include: marine genetic resources (MGR), including questions on the sharing of benefits; measures such as area-based management tools (ABMT), including marine protected areas (MPAs); environmental impact assessments (EIA); and capacity-building and transfer of marine technology (UNGA Res. 72/249, UN Doc. A/Res/72.249, 24 December 2017, para. 2.). In order to govern MGR in areas beyond national jurisdiction (ABNJ) and to answer questions on the sharing of benefits, it is important to consider which benefit-sharing options may be most appropriate in this particular context.

Under the Nagoya Protocol, benefits should be shared by the users of genetic resources with the provider state, in accordance with domestic legislation (Art 5. Nagoya Protocol 2011). However, in the BBNJ context, although the types of users may be similar, there will be no provider of the genetic resources, since MGR exist outside the borders of national jurisdiction. It has not yet been agreed who the beneficiaries will be, but could involve 'developing states Parties, in particular least developed countries, landlocked developing countries, geographically disadvantaged states, small island developing states, coastal African states and developing middle-income countries' (Art 11, BBNJ draft text, 2019a). In addition, since the Nagoya Protocol applies only to areas within national jurisdiction, the same access and benefit-sharing (ABS) provisions do not apply to the utilisation of genetic resources from ABNJ. As such, new benefit-sharing options are under consideration for adoption as part of the new BBNJ agreement (BBNJ draft text, 2019a)[2].

Whilst a list of potential benefit-sharing options can be found in the Annex of the Nagoya Protocol (2011), the effectiveness and success of these measures, and the ABS system as a whole, has been questioned by stakeholders (Fedder, 2013; Pauchard, 2017; Ruiz-Muller, 2018). Between 1996 and 2017, a total of 217 ABS agreements for commercial research and 248 for non-commercial research were concluded (Pauchard, 2017). To date, there is a lack of evidence to support the assumption that benefit-sharing leads to effective conservation of biodiversity (Suneetha and Pisupati, 2009; Pisupati and Bavikatte, 2014). In addition, the burden on the users (such as universities and private companies) and regulatory authorities in terms of administrative complexity when complying with ABS legislation and conducting benefit-sharing can act as a disincentive for utilisation of genetic resources, potentially limiting the benefits derived and shared (Richerzhagen and Holm-Mueller, 2005; Tvedt, 2013). Further challenges exist with regards to achieving fair and equitable benefit-sharing and sustainable development (Louafi, 2013; Tsioumani, 2018). It is timely and appropriate, therefore, to re-evaluate how the success of implementing benefit-sharing options are measured and reconsider which forms may most fairly balance the positive and burdensome associated aspects (Ruiz-Muller, 2018). In order to understand this balance, key factors may be used to assess the overall positive influence of a benefit-sharing option, such as the number of beneficiaries receiving benefits, the effect on biodiversity goals and the long-term impacts and significance of benefit-sharing, as well as the overall burdensome aspects, such as the burden on the user of genetic resources and the burden on the regulator (Tvedt, 2013; Correa, 2017; Morgera, 2018a; Harden-Davies and Gjerde, 2019).

Consideration of the benefit-sharing options for adoption as part of the new BBNJ agreement provides a good opportunity to identify benefit-sharing factors and assess the overall positive versus burdensome balance of different benefit-sharing options. It has been noted that of the four elements under consideration as part of the BBNJ package, MGR including questions on the sharing of benefits remains the most challenging and immature element, with few detailed solutions suggested to date (Voigt-Hanssen, 2018). This is to be expected given the different stakeholder perspectives, goals and concerns Collins *et al* (2020). However, review of non-monetary and monetary benefit-sharing options in light of benefit-sharing factors and understanding of which measures may provide the most fair and balanced outcome could provide a useful platform on which negotiatons can progress (BBNJ, 2019a; Sirakaya, 2019; Collins *et al*, 2020).

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[2] See Part II, Article 11 '[Fair and equitable] sharing of benefits', Draft BBNJ Agreement, 2019a.

The first aim of this paper, therefore, is to develop a benefit-sharing methodology which can be used as a tool to understand the balance of beneficial versus burdensome aspects associated with different benefit-sharing options. This will involve making use of a multi-criteria analysis technique (MCA) to help decision-makers to select options in an objective and transparent manner (de Brucker *et al*, 2013). The main objective of the MCA technique is to overcome challenges that human decision-makers experience when handling large amounts of complicated information in a consistent manner (Dodgson *et al*, 2009). The second aim is to demonstrate how the methodology can work by applying it to the BBNJ context, to review the different types of non-monetary and monetary benefit-sharing options with potential for adoption under the new agreement, in order to determine which forms may provide more balanced outcomes. The BBNJ context was selected for demonstration of the methodology because these negotiations represent a relevant, current opportunity which may directly benefit from such an exercise with regards to making informed decisions in terms of the benefit-sharing options to be adopted in the treaty text. This objective was achieved by identifying the key benefit-sharing factors to consider, as well as their relative importance to stakeholders. The benefit-sharing factors were then applied to different benefit-sharing options through the use of the new equation, to reveal the balance of beneficial impacts versus burden associated with the different options.

The authors acknowledge the limited number of interviewees involved and suggest that further interviews with a larger number of participants would be needed to draw significant conclusions in the context of ongoing BBNJ negotiations, as well as also in other genetic resource ABS circumstances. In addition, the authors wish to highlight the broad scope of the current paper and general nature of the factors considered. Future research is needed to build on the results gathered in this study and to include more comprehensive literature review as well as more detailed information gathered from various expert groups. This would help to consolidate the perceived balance of beneficial impacts versus burden associated with benefit-sharing options.

Development of a Benefit-Sharing Tool

Materials and Methods

The study began with a scoping literature review to identify benefit-sharing factors that may enable analysis of the positive versus burdensome aspects of benefit-sharing options. The benefit-sharing options which could be considered for a potential governance (ABS) system for MGR from ABNJ were also identified (FAO, 2001; Nagoya Protocol, 2011; World Health Organization, 2011; BBNJ, 2019b; Collins *et al*, 2020). The literature review involved searches through Pubmed, Embase, EurLex, the United Nations Convention on the Law of the Sea, the United Nations BBNJ website[3], the Convention on Biological Diversity (CBD) and the Nagoya Protocol (UNCLOS, 1982; CBD, 1992; Nagoya Protocol, 2011). Search keywords included: benefit-sharing, ABS, genetic resources, MGR, ABNJ, and capacity building.

Results

Benefit-Sharing Factors

As a result of the authors' own experience in terms of how benefit-sharing works in practice, specifically the modalities of drafting and negotiating benefit-sharing contracts, coupled with literature review, five factors were identified as being the main objectives and considerations of benefit-sharing associated with utilisation of genetic resources (referred to in this paper as 'benefit-sharing factors', Young and Tvedt, 2017). Listed below and in Table 1 are descriptions of the benefit-sharing factors considered in this study.

1. **Biodiversity goals:** Different goals and objectives, as defined by legal biodiversity acts or treaty's, may be attained through benefit-sharing. BBNJ context: relevant goals include contributing towards conservation of marine biological diversity of ABNJ, promoting sustainable use of MGR from ABNJ, fostering scientific R&D and promoting fair and equitable benefit-sharing (Art 140, UNCLOS, 1982; CBD, 1992; Harden-Davies and Gjerde, 2019; Collins *et al*, 2020).
2. **Direct beneficiaries:** The number of individuals receiving benefits will vary according to the type of benefit that is being shared. BBNJ context: a greater number of individuals may have access to MGR data if this is shared, such as via online databases, compared to the number of people who may receive funding to conduct PhD projects on topics related to BBNJ.
3. **Benefit-sharing significance:** The impact associated with different forms of benefit-sharing could be approximately determined in terms of the significance, value and the duration of the impact. For example, impacts could include enhanced employment and contribution towards scientific knowledge. Duration of impacts may vary from weeks to months or years and could give an indication of whether future generations may be positively influenced by the benefit-sharing or not (Harden-Davies and Gjerde, 2019). BBNJ context: training courses as part of capacity building initiatives may lead to longer-term positive impacts on a group of people, perhaps linked to enhanced employability, than would be the case for access to MGR data.
4. **Burden on the user:** Users could include any organisations or individual researchers from developed or developing states. If benefit-sharing is a requirement, these users may encounter a degree of burden linked to the process of sharing. This burden may take the form of monetary cost to

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[3] https://www.un.org/bbnj/ - accessed 08 March 2020

generate the so-called 'benefits' in the first place, such as tailored training courses or a monetary payment, but also the transaction cost of sharing benefits. In addition, the generation and sharing of benefits will involve human labour costs, for example sending experts to beneficiary locations to conduct capacity-building or training initiatives. Time may represent another burdensome aspect. Whilst some types of benefit-sharing require only one simple transaction, other forms may require repeated actions over time. BBNJ context: sharing of benefits in the BBNJ context may involve burdens and costs to the user, for example to set up and organise databases in which MGR data can be stored and accessed, or sending of laboratory equipment as a form of technology transfer.

5. **Burden on the regulator:** A regulator may encounter a degree of burden linked to the process of benefit-sharing, as well as related to the need to build capacity in the context of novel regulation. This may involve raising awareness of and enforcing compliance with applicable legislation. These burdens may be both in terms of monetary costs and human labour costs, similar to those described for the users above. However, for the regulator, these costs may be linked to monitoring and checking compliance of users with the benefit-sharing requirements. BBNJ context: at present, it remains unclear whether there will be a regulator for ABNJ, or who this could be (Mohammed, 2017; BBNJ, 2019b). However, in the event that there is a regulator/regulatory body charged with regulating ABS linked to MGR from ABNJ, and benefit-sharing is a requirement, then the regulator may face burdens associated with overseeing the process of benefit-sharing, particularly in terms of monetary costs, complexity and time.

Rationale for Selecting These Five Benefit-Sharing Factors

The reasoning behind the five factors on benefit-sharing is threefold. The first reason relates to the concept of establishing a balanced ABS system as referred to in our previous research (Sirakaya, 2019, 2020). For an ABS system to successfully establish balance between the user and the provider (in bilateral genetic resources frameworks), so as to fairly distribute benefits arising from utilisation of genetic resources between the users of genetic resources and beneficiaries, it is crucial to ensure that such a system attends to the international biodiversity goals. The authors here specifically explore benefit-sharing options and therefore the factors pay due regard to the international biodiversity goals as identified under our current research as well as the previous research conducted on the matter (Sirakaya, 2020).

Secondly, the five factors were selected since they clearly define and influence the modalities of benefit-sharing and can be negotiated when drafting agreements (Young and Tvedt, 2017). The authors believe that these factors, coupled with agreement between users and providers, can promote an objective balance in benefit-sharing.

Multiple stakeholder groups are involved within the BBNJ context and it is of utmost importance that an instrument is developed that attains the needs of all of these groups to the greatest extent possible. A stakeholder-driven MCA is the best available method to achieve this (de Brucker *et al*, 2013) (Sirakaya and De Brucker, personal communication). A recent study conducted by Sirakaya and De Brucker (under review) demonstrates how MCA can been applied to design regulatory frameworks for access to genetic resources in cases which involve multiple stakeholders. As such, the third reason for the selection of the criteria refers to the importance of stakeholder consultation under the MCA. According to Freeman (1984), stakeholders are defined as 'any individual or group who can affect an organisation's performance or who is affected by the achievement of this organisation's objectives.' Looking into regulatory issues related to benefit-sharing in ABNJ, taking into account Freeman's definition, five key stakeholder groups were identified: developing states, developed states, civil society, the scientific research community and the private sector (Collins *et al*, 2020). Although questions remain regarding whether there will be any form of regulatory body in the BBNJ context, this could potentially involve members from both developing and developed states working together to fulfill the role. The scientific research community and private sector represent the potential users of MGR. Developing states and civil society are the likely beneficiary groups who would receive benefits shared from utilisation of MGR from ABNJ.

Stakeholders' perceptions play a crucial role in MCA conducted on ABS frameworks. This is due to the fact that a balanced ABS system can only be established if there is a reasonable ABS framework that the users would be incentivised to adhere to. The same principle applies to a potential benefit-sharing system for ABNJ. The users (i.e. the scientific research community and private sector) would only be incentivised if there is a foreseeable balance between the impact of being a part of the system and the burden thereof. Likewise, the system would only function as intended if the cost borne to the regulator (including members from developing and developed states) of establishing and maintaining the system is considerably lower than the benefits generated through the system to be shared with beneficiaries (developing states and civil society). In line with this, Harden-Davies and Gjerde (2019) stipulate the 'need to strike a balance between the right to use and the responsibility to share'. Therefore, by reviewing the balance of potential beneficial impacts (private, social and environmental aspects) versus burden, negotiators may be in a better position to make informed decisions regarding which benefit-sharing options may be most

Table 1. Description of the five benefit-sharing factors considered in this study.

Benefit-Sharing Factors	Description	References
Biodiversity goals (G)	i. Contributing towards **conservation** of marine biological diversity of ABNJ ii. Promoting **sustainable** use of MGR from ABNJ iii. Fostering scientific R&D iv. Promoting **fair and equitable benefit-sharing** v. **Inclusivity of developing states** in access to and utilisation of MGR of ABNJ	CBD (1992); Collins *et al* (2020); BBNJ (2019a); Harden-Davies and Gjerde (2019); Morgera (2018a); UNCLOS (1982)
Direct beneficiaries (#)	i. **Number of individuals impacted**/receiving the benefits	Morgera (2014)
Benefit-sharing significance (S)	Size of impact, for example: i. **Duration** (months or years – gives an indication of whether the impact will be beneficial to current and future generations) ii. **Enhanced employability/employment rates** iii. **Meaningfulness**	Harden-Davies and Gjerde (2019)
Burden on the users of (M)GR (U)	i. **Monetary cost and human labour** to generate and share the benefits, including costs to transfer to and distribute amongst beneficiaries. ii. **Opportunity cost** iii. **Time value**	Harden-Davies and Gjerde (2019)
Burden on the regulator (R)	i. **Monetary cost and human labour** to monitor benefit- sharing ii. **Complexity** iii. **Time value**	Pisupati and Bavikatte (2014)

appropriate to consider including as part of a new benefit-sharing framework for ABNJ.

In sum, these five benefit-sharing factors are crucial in establishing a balanced benefit-sharing framework for ABNJ with governance options that attain international biodiversity objectives, as outlined by legal acts and treaty's, and pay due regard to stakeholder preferences (Collins *et al*, 2020).

Benefit-Sharing Balance

In order to objectively evaluate different benefit-sharing options in terms of the ratio of potential positive impacts versus associated burden in a fair and consistent manner, an equation was proposed, taking into consideration the five benefit-sharing factors (see Figure 1). Weighted values were used in this study to incorporate consideration of the fact that some benefit-sharing factors may be perceived as more important than other factors and should therefore contribute more to the final score. Different methods can be used to generate the weighting values for different factors. For example, an analytic hierarchy process, case-based reasoning, simple multi-attribute rating technique, mathematical programming or interview approaches could be used (von Winterfeldt and Edwards, 1986; Ho *et al*, 2010; Nerini *et al*, 2014). In this study, the values of weightings were obtained through an interview process. Benefit-sharing factors (G, #, S, U, R) were first each multiplied by an average weighting (a-e) assigned to each factor by interviewees. The three positive, weighted benefit-sharing factor scores are multiplied together, as are the two burdensome, weighted factor scores. The positive aspects are then divided by the burdensome aspects to give a benefit-sharing balance value for each benefit-sharing option.

Applying the Benefit-Sharing Tool to the BBNJ Context

Materials and Methods

After development of the benefit-sharing methodology (see Figure 1), a semi-structured, qualitative interview was prepared (see Supplemental File 1). Interviews were conducted with ten experts. Availability and willingness to participate in the interview represented a controlling factor in the recruitment of participants, as well as the variety of stakeholder groups, and contributed to the limited numbers.

$$\boxed{\frac{(aG \times b\# \times cS)}{(dU \times eR)}}\;\begin{matrix}\left.\vphantom{X}\right\}\text{Positive aspects}\\[1em]\left.\vphantom{X}\right\}\text{Burdensome aspects}\end{matrix}$$

Figure 1. Equation to determine the balance of potential positive impact versus burden associated with different forms of benefit-sharing. Letters a-e represent the average weight assigned by interviewees to the five benefit-sharing factors. Benefit-sharing factors: G= biodiversity goals; # = direct beneficiaries; S = benefit-sharing significance; U = burden on the user, and; R = burden on the regulator.

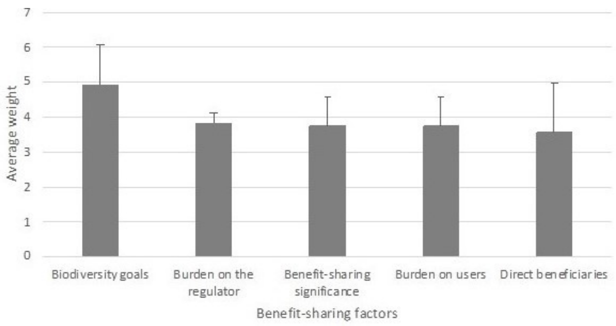

Figure 2. Average weight assigned by interviewees to the five different benefit-sharing factors considered. Error bars indicate standard deviation of the mean.

These experts are based in different locations around the world (four from developing and six from developed states) and representative of different stakeholder groups: two from the scientific research community (one from a developed and one from a developing state), three from developing states delegations, three from developed states delegations, and two from civil society (both from developed states). Whilst effort was made to include private sector stakeholders in the interview process, and indeed representatives from one organisation did provide helpful feedback on some of the questions, time constraints and availability of representatives rendered this not possible in the scope of the current study. Interviews were conducted during the third session of the intergovernmental conference (IGC3) for BBNJ in New York (between 19-28 August 2019).

The interviews followed a pre-defined guide list of questions (see Supplemental File 1), which started by requesting participants to rank five benefit-sharing factors (see Table 1) on a scale from 1 to 5 in terms of perceived relative importance (see Figure 2). Participants were then asked to score a variety of non-monetary and monetary benefit-sharing options in terms of potential impact of these on the five benefit-sharing factors (from 0 = no impact, to 5 = very high impact), according to their perspective, and to give short reasons for their decisions. These scores were used to perform a MCA, whereby scores were multiplied by the average weighting assigned to each factor and inserted into an equation (see Figure 1), to determine the balance of potential positive impact versus burden associated with each different form of benefit-sharing (see Figure 3 and Figure 4). This enabled comparison of the ratio of potential positive impacts versus burden between different benefit-sharing options, taking into account the relative importance of different key factors. Interviews were audio-recorded and transcribed. Microsoft Excel software was used as a means to store the data. A thematic analysis of the transcripts was conducted to identify common themes in responses. All data were anonymised by grouping results into stakeholder groups. Written informed consent forms were signed by all of the interviewees in this study.

Results

Weighting the Benefit-Sharing Factors

According to the average stakeholder ranking, biodiversity goals were considered as the most important factor to be taken into consideration when assessing how balanced different benefit-sharing options are. The other four benefit-sharing factors were considered approximately equal in terms of importance (see Figure 2). The range of ranking given by interviewees was greatest for the direct beneficiaries factor, as indicated by the larger error bar in Figure 2. Reasons given by interviewees for these rankings are described below.

Biodiversity Goals. The majority of interviewees stated that the biodiversity goals are the most critical factor from their perspective. Whilst many different goals could fall under this category, interviewees most frequently referred to conservation of biodiversity as the aspect that they consider most important. This is because the goals of ‘conservation and sustainable use of marine biological diversity’ are the key overarching goals of the new agreement as a whole (BBNJ, 2019b). Collins *et al* (2020) present further detailed information regarding stakeholder goals for a potential new ABS mechanism for ABNJ. Other goals, such as promoting marine scientific research, are viewed as necessary to achieve the conservation objectives. They also indicated that biodiversity goals are more important than any of the other four benefit-sharing factors considered in this study. One interviewee noted that biodiversity goals are important for all stakeholders involved, not just for the beneficiaries of benefit-sharing.

Direct Beneficiaries. Most interviewees indicated the benefits should be shared amongst as many people as possible, and that the greater the number of people who are positively affected the better. However, three interviewees also noted that the importance of beneficiaries depends on the definitions, whether it is only the people who are directly affected, or also those who indirectly benefit. Sometimes only a few people may immediately and directly benefit from a benefit-sharing initiative, such as collaborative or joint venture projects, but such an effort may indirectly have a large impact on many more people and other factors, perhaps over time. For example, sharing of MGR samples and data for scientific research may only initially affect a moderately low number of people, but if this leads to development of new pharmaceutical products to treat human illness or to maintaining the health of the ocean through conservation measures, then a far larger number of beneficiaries will be encountered.

Benefit-Sharing Significance. The majority of interviewees assigned this factor a moderately high score. Two interviewees stated that the duration of benefit-sharing significance is vital. However, two other interviewees gave this a moderately low score and suggested that, from their perspective and in the current context, this factor was not as important as the others considered in this study.

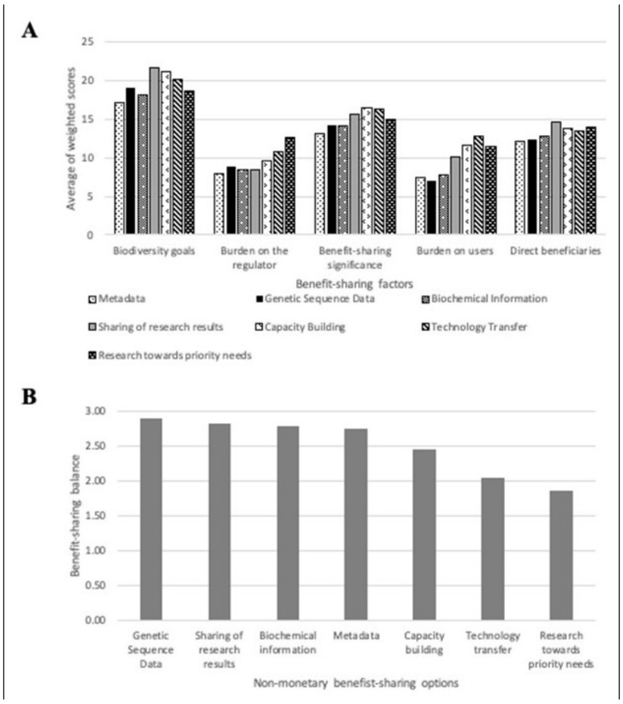

Figure 3. A) Average weighted scores for the seven non-monetary benefit-sharing options according to the five benefit-sharing factors, and B) scores according the benefit-sharing balance equation for the seven non-monetary benefit-sharing options.

Burden on the Users of (M)GR. Burden on the user was considered moderately important by over half of interview participants. One interviewee raised the question of whether there might be the possibility to charge a ‘handling fee’ for certain types of benefits (such as sharing of material). The degree of burden on the user may be affected by whether there is the possibility to charge a handling fee (or similar) to disseminate MGR samples and other benefits. It was suggested that sharing of benefits cannot all be for free.

Burden on the Regulator. The majority of interviewees assigned this factor a high score. Whilst it remains unclear whether there might be or who might constitute the regulatory authority in the BBNJ context, it is possible that such an institution will be established. Interviewees acknowledged that this is an important factor because if burden on the regulator is not taken into careful consideration, then the whole benefit-sharing system, whether non-monetary or monetary, could fail. For example, if the system is overly expensive, then any potential monetary benefits may be used to fund running of the system itself rather than accruing for the benefit of beneficiaries (Morgera and Tsioumani, 2010; Morgera, 2014; Tsioumani, 2018). In addition, burden on the regulator could result in lengthy decision-making processes, thereby indirectly affecting potential users.

Non-Monetary Benefit-Sharing Options

To gather perspectives on benefit-sharing, interviewees were asked to consider the potential influence of different benefit-sharing options on the five benefit-sharing factors (Table 1). Analysis of the President’s aid to negotiations (2019) and a review of the literature lead to identification of seven non-monetary benefit-sharing options which could be considered in the context of governing the utilisation of MGR from ABNJ (see Figure 3). A description of the options considered in this study, and their significance, can be found in Sirakaya (2019) and Collins *et al* (2020). Non-monetary benefit-sharing options considered here include:

1. Sharing of Raw Data:
    (a) Metadata;
    (b) Genetic Sequence Data (GSD)[4],[5],[6]
    (c) Biochemical Information
2. Sharing of Research Results
3. Capacity Building
4. Technology Transfer
5. Research Directed Towards Priority Needs

The benefit-sharing balance, as calculated using the equation described above, indicated the most favourable balance of beneficial impacts versus burden associated with sharing of GSD and sharing of research results. Sharing of biochemical information, metadata and capacity building received a similar, but slightly lower score. Technology transfer, and in particular research directed towards priority needs, received less favourable balances (see Figure 3B). Reasons given by interviewees for potential positive impacts versus burden associated with different non-monetary benefit-sharing options are described below.

Sharing of Raw Data (Metadata, GSD and Biochemical Information). For some participants, all three types of raw data sharing are viewed together as a package. It was suggested that if raw data were to be made publicly available online, this could have a positive impact on a relatively large number of beneficiaries (Figure 3A). This is because many people would then be able to access the data easily, quickly and possibly for free. However, two interviewees also acknowledged that many people, particularly in developing states, may not be able to make use of the raw data since they lack the capacity to work on it. As such, unless accompanied by capacity building, the number of beneficiaries impacted by sharing of raw data may not actually be very high.

Interviewees suggested that sharing of raw data may not immediately have a large effect on the number of

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[4] GSD is the term most commonly used in the BBNJ context to refer to data/information which is described as Digital Sequence Information (DSI) under the auspices of the CBD. According to the Consortium of European Taxonomic Facilities (CETAF) and the Society for the Preservation of Natural History Collections (SPHNC), the term GSD is in line with the concept of DSI. However, despite efforts to define DSI/GSD, there is currently no official, internationally accepted definition of the term.

[5] https://www.cbd.int/abs/DSI-views/2019/CETAF-DSI.pdf - accessed 09 March 2020

[6] https://www.cbd.int/abs/DSI-views/2019/SPNHC-DSI.pdf - accessed 09 March 2020

direct beneficiaries, the biodiversity goals or benefit-sharing significance. However, by building up large data sets, the beneficial impact of sharing raw data on these benefit-sharing factors may grow and could become relatively high over time. This could also be in part due to the long-lived nature of data; once data is curated and stored, it can be made available and accessed for a very long time. One interviewee suggested that sharing of raw data could be one of the best things we could do to have a positive influence on the benefit-sharing significance.

One interviewee explained that a lot more work is required to generate biochemical information than for generation of metadata and GSD, because the process is comparably less straightforward. In addition, one interviewee suggested that whilst it is already best practice to share metadata and GSD, they were unsure whether it is yet best practice to share all biochemical information. Therefore, the burden on the user associated with sharing of biochemical information, in terms of opportunity cost, may be higher than that associated with the other forms of raw data sharing. In addition, the burden on the users related to sharing of data may depend on the stakeholder group in question and the type of data that is required to be shared. For some users, such as those in the private sector, this is likely to be very burdensome and possibly even a deterrent, particularly if mandatory and involves the obligation to share commercially important data. This is because mandatory sharing of raw data would likely change the incentive to invest. Scientists in the private sector routinely conduct novel scientific research and publish results in scientific journals. They also disclose scientific information in the form of patents. However, from a commercial viewpoint, if sharing of data beyond current practice is mandatory, this may have broad implications for protection of intellectual property (IP) and for maintaining competitive market advantage. As such, mandatory sharing of valuable data may disincentivise investments in private sector projects involving MGR from ABNJ.

Interviewees indicated that the level of burden on the users and on the regulators would depend on the way in which benefit-sharing is done. Under the assumption that a new system is developed and must be used to share benefits, for example sharing of GSD through a clearing house mechanism or other form of database, this could lead to significant burden on the users and regulators. One interviewee noted that in the draft treaty text (BBNJ, 2019a), reference is made to a new system whereby benefits will need to be shared through a clearing house[7] and raises the question of timing. According to the interviewee, the issue of when and how benefits will be shared could have huge impacts on the degree of burden experienced. If benefit-sharing is left according to current practices, particularly in terms of where raw data are stored, then this could have very little or even zero impact on the burden felt by users or the new regulator. However, if we proceed with a new system, requiring a change in the way that data are shared, burden may be encountered by users in terms of requiring conversion of data into a particular format to fit into a specific database. As such, if benefit-sharing were to become mandatory and involves new requirements and procedures, the burden on the users will be higher than at present.

The burden on the regulator could be relatively straightforward, if all they have to do is verify that data is being shared with a database, but the degree of burden will depend on how much information they need. One interviewee also noted that it may be important to consider the burden on the beneficiaries accepting raw data. This may involve requirements to develop infrastructure to receive and make use of data.

Sharing of Research Results. Interviewees indicated that a relatively large number of beneficiaries could be impacted by the sharing of research results (Figure 3A). However, one interviewee suggested that research results, such as a research paper, may be read by fewer people compared to the number of people who could access or use raw MGR data.

Sharing of research results could have a large positive effect on the overall impact of benefit-sharing, and also on the biodiversity goals such as conservation. It was suggested that this could be partly due to the long-lived nature of research results.

Interviewees suggested that the impact of sharing research results on the burden experienced by users would be moderate, since writing of research articles inherently involves a degree of human labour costs. However, it was also noted that this could be less burdensome for users than sharing of raw data, because this would involve a different system which may remain more similar to current practice. It was suggested that the burden on the regulator may also be moderate, but would depend on whether this was mandatory or voluntary. If the regulator was required to track that sharing of research results has been done, then this could lead to quite a heavy burden.

Capacity Building. It was suggested that the influence of capacity building on biodiversity goals and significance would depend on how sustainable the capacity building initiatives are. For example, if scientists are trained as part of a capacity building effort and then decide to leave the country, this cannot be considered as sustainable. According to one interviewee, this is unfortunately the case when reviewing current capacity building activity, and represents a challenge faced in many developing countries. The key question here is how to ensure the sustainability of capacity building. In an ideal

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[7] 'Samples, data and related information shall be made available in open access [through the clearing-house mechanism [upon access] [after [. . .] years]]' and 'States Parties shall publish and communicate the reports of the results of the assessments in accordance with [articles 204 to 206] [article 205] of the Convention [, including through the clearing-house mechanism]' (President's aid to negotiations, 2019).

world capacity building would be sustainable, so that effects are long-lasting.

The burden on the users of MGR may be greater in association with capacity building than some of the other options (Figure 3A). This is because it requires a combination of time, money and specific expertise to execute such an initiative.

Technology Transfer. It was suggested that the number of beneficiaries who could benefit from technology transfer would be moderately low. It was proposed that this may be because only a few people will be able to make use of the technology, particularly if the technology is very sophisticated and the absorptive capacity is low. One interviewee suggested that the impact of technology transfer on the biodiversity goals will be less than that associated with other benefit-sharing options (Figure 3A). As with capacity building, the influence of technology transfer on the benefit-sharing significance will depend on the sustainability. Interviewees were of the opinion that technology transfer may have an impact for a short amount of time, but were uncertain whether this would have long-lasting effects.

The burden on the users related to technology transfer may depend on the stakeholder group in question, the type of technology that is required to be shared, who pays for the transfer of technology and the means by which this is done. It was suggested that for some users, such as the scientific research community, this may be less burdensome than sharing of raw data. However, for other uses, such as those in the private sector, this is likely to be very burdensome, particularly if mandatory. This is because investors in the private sector aim to recoup investments, and mandatory, free transfer of technology would possibly discourage further investment. Alternatively, one interviewee suggested that if technology transfer were to be conducted on commercial terms, this could represent less burden for the private sector.

Research Directed Towards Priority Needs. One interviewee stated that, although research directed towards priority needs has the potential to reach a lot of people and could have a very high impact on attending to the biodiversity goals, particularly contributing towards conservation and sustainable use, they simply did not think that it was going to happen. This is because the interviewee felt that such a system of focusing research on priority needs would be unfeasible and unworkable. As such, the anticipated number of beneficiaries and influence on biodiversity goals and significance was scored relatively low (Figure 3A). In addition, it was noted that the impact of research directed towards priority needs on the benefit-sharing factors would depend on what exactly the priority needs are.

The burden on the user was thought to be moderately high, unless the users are already conducting research in the priority needs area. One interviewee suggested that such priority needs could be linked to research with socially beneficial uses, including 'health and food security' as described in Article 8 of the Nagoya Protocol (2011). This is because forcing scientists to change their research from one field of work to a different one, associated with the identified priority needs, would require significant monetary costs. Similarly, the burden on the regulator would likely also be high. It was suggested that the regulator may experience burden associated with understanding who the developing states are, identifying their priority needs and deciding how this should be regulated. One interviewee noted that it would likely be very difficult for a regulator to monitor this, and could be very challenging if it involves a new system in which to direct their regulation towards this specific purpose.

Monetary Benefit-Sharing Options

Analysis of the President's aid to negotiations (2019) and a review of the literature lead to identification of eight monetary benefit-sharing options which could be considered in the BBNJ context (see Figure 4). A description of the options considered in this study, and why they might be important, can be found in Sirakaya (2019) and Collins *et al* (2020). Monetary benefit-sharing options considered here include:

1. Research Funding
2. Salaries
3. Joint Ventures[8]
4. Joint IP Rights
5. Royalties
6. Milestone Payments
7. License Fee
8. Access Fee

Interviewees were asked to consider the potential impact of different monetary benefit-sharing options on the five benefit-sharing factors. According the benefit-sharing balance calculation, the most favourable monetary benefit-sharing option, in terms of balancing beneficial impacts versus burden, was research funding followed closely by salaries (see Figure 4B). Joint ventures were considered the next most favourable option, followed by joint IP rights. Access fee, milestone payments, license fee and royalties were viewed as similar in terms of balancing the potential positive impacts versus burden. Reasons given by interviewees for potential positive impacts versus burden associated with different monetary benefit-sharing options are described below.

Interviewees noted that their answers in this section were given under the assumption that commercialisation of MGR is successful and that there will be money to share. This is crucial, because four interviewees out of the ten indicated that they do not believe that there will be any financial profits derived from R&D on MGR, and therefore no money to share.

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[8] In this paper, the term 'joint ventures' is used in a general, broad sense to describe a collaborative project/initiative undertaken jointly by two or more entities which otherwise retain their distinct identities. The term is not used here in the corporate or legal manner.

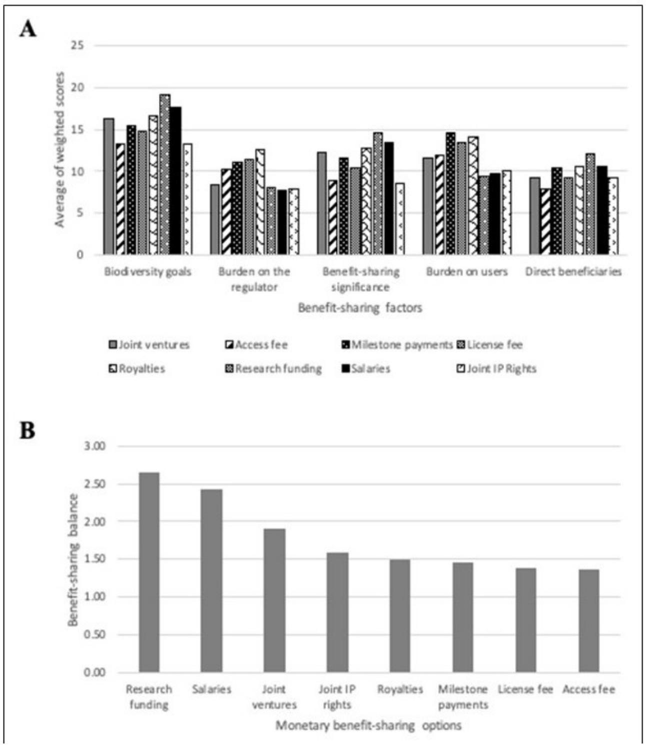

Figure 4. A) Average weighted scores for the eight monetary benefit-sharing options according to the five benefit-sharing factors, and B) scores according to the benefit-sharing balance equation for the eight monetary benefit-sharing options.

Research Funding and Salaries. Five of the ten interviewees indicated that they view research funding and salaries as similar in terms of the potential positive impacts versus burden. Interviewees indicated that research funding and salaries could have a greater positive influence on the biodiversity goals and benefit-sharing significance than the other forms of monetary benefit-sharing, and may have more long-term positive effects (see Figure 4A). For example, research funding could help to build capacity in developing states, potentially contributing towards enhanced employability, and could also be directly focused on long-term conservation and sustainable use of biodiversity.

One interviewee noted that the level of burden on the user associated with research funding and salaries would depend on who is paying for it. The burden on the users would be low if this is to be paid by the regulator, and the burden on the regulator may be relatively high. However, vice versa may be true if the users are required to pay this. It was also suggested that since research funding is something that is already done by the scientific community, then the burden on the regulator would likely be less for research funding than other monetary benefit-sharing options, if the regulator is only required to make sure that the money has been channeled appropriately.

Joint Ventures. Most interviewees agreed that joint ventures would influence a relatively low number of beneficiaries (Figure 4A). One participant stated that joint ventures will require money which cannot be shared amongst many people, and therefore the number of people affected will likely be low. The influence of joint ventures on the biodiversity goals and significance was also thought to be low, and would likely depend on the conditions.

It was suggested that the level of burden experienced by the user and the regulator would be moderate, but could be low if joint ventures were considered on a voluntary rather than a mandatory basis. This is because if joint ventures were required on a mandatory basis, then perhaps users/ organisations who are not equipped with the appropriate capacity would struggle to establish such projects.

Joint IP Rights. The majority of interviewees felt that joint IP rights (IPR) would impact a low number of beneficiaries (Figure 4A). One interviewee suggested that perhaps it was possible for this number to grow over time. Participants were also of the view that the influence of joint IPR on biodiversity goals and benefit-sharing significance would be low, possibly with no long-term positive effects. With regards to burden on the user, interviewees suggested that this would depend on the conditions associated with the joint IPR, but was likely to be moderate to high. The burden on the regulator is likely to be high, and participants noted that they were unsure how this form of benefit-sharing could be regulated.

One interviewee indicated that they did not see joint IPR as a feasible option, as it would either not be relevant or appropriate in most cases. The interviewee stated that only inventors or co-inventors can claim IPR and requesting joint ownership with entities who do not represent this would lead to an inability to fulfill the required criteria to claim IPR. The only situation where such an approach has been considered previously is in the context of traditional knowledge, where indigenous knowledge may be included in an invention. However, according the interviewee, even then there are much better approaches to follow than joint IPR in order to share benefits. Nonetheless, if scientists from developing countries are included in a research project and at some point in time a new invention is developed, then joint IPR could be appropriate. It was also noted that publications have copyrights involved, which could also be considered as a form of joint IPR if multiple co-authors are involved in the effort. As such, the feasibility of these options will depend on the details regarding how this is done.

Milestone Payments, License Fee and Royalties. Most interviewees considered that milestone payments, license fees and royalties would lead to similar impacts on the benefit-sharing factors (see Figure 4A). One reason given for this is that people find it very difficult to distinguish between the three. It was suggested that the milestone payments could have similar positive impacts on the benefit-sharing factors as an access fee (see below). As with the access fee, questions were raised regarding whether users of MGR would be able to pay the milestone payments, license fees or royalties. One interviewee stated that they did not believe that a license fee would actually be workable.

Interviewees indicated that license fees and royalties may be more burdensome on the user and regulator than the milestone payments, but that all three would likely be more burdensome than an access fee. This is because the access fee could take the form of a one-off payment (with no subsequent costs), of a preset and limited amount, whereas the others may incur more than one fee and may be payable at times which are less clearly defined than at the point of access (Collins *et al*, 2020).

Access Fee. The majority of interviewees were of the opinion that an access fee would impact only a low number of beneficiaries (Figure 4A). It was suggested that this may be in part because relatively little money would be generated and few users would be able to pay this if it was expensive, although research institutions could potentially seek scholarships to cover this (see Collins *et al,* 2020) for more detail). Questions were raised regarding to whom this fee would be paid.

The influence of an access fee on the biodiversity goals and significance was also considered low. One interviewee noted that the influence would depend what this fee is used for, but was sceptical that it would have a positive effect on these factors. Another interviewee stated that an access fee system could be attractive because it would ensure that some income is generated when MGR are collected from the high seas which could be channelled into benefit-sharing. However, the same person also acknowledged that they did not think an access fee would be feasible, because it would inflict on the 'Freedom of the High Seas' principle. Therefore, from a negotiation point of view, this would likely be very difficult to implement.

Discussion

A Benefit-Sharing Tool

Development of a Benefit-Sharing Methodology

Benefit-sharing in the context of existing genetic resource frameworks under the Nagoya Protocol (conducted in a bilateral manner) is often considered to lack transparency, clarity and accountability, particularly for monetary benefit-sharing options (Fedder, 2013; Pauchard, 2017; Ruiz-Muller, 2018). For example, when dealing with ABS under the Nagoya Protocol, and since ABS contracts are private and users are not obliged to report what is in the contract, governments do not have access to information regarding the amount of money that flows out as a result of ABS. Making use of a methodology, such as that described in this study, could help to achieve a more favourable balance by negating the issue of transparency, by clarifying the key factors and helping negotiators to make informed decisions. In addition, lessons learnt from the ITPGRFA in terms of aspects to consider as part of an operating, multilateral ABS system may also be useful here (Louafi, 2013; Stannard and Moeller, 2013; Tsioumani, 2018). Ultimately, use of this tool could help to enhance understanding and implementation of benefit-sharing policies/ legislation with greater potential to balance beneficial impacts with associated burden, thereby enhancing workability of the ABS system as a whole. However, challenges with the methodology have been identified during application in this study (as described in the section below) which should be addressed in order to develop a more robust tool for application in a variety of benefit-sharing contexts.

Lessons Learnt from Application of the Methodology to the BBNJ Context

Accurately determining the influence of different benefit-sharing options on benefit-sharing factors, such as the significance, are difficult to measure. Indeed, interviewees found it challenging to give scores for some options, partly because participants may not have fully understood or known how to measure and give an accurate answer. This is a clear aspect of the tool which could be improved in the future through consultation with a greater number of experts on that particular aspect. In addition, given the fact that these questions were asked in the context of ongoing negotiations, it is expected that some participants will have responded with strategic rather than objective answers. This may be the reason why a small minority of interviewees indicated that salaries as a form of monetary benefit-sharing would have a large positive influence on benefit-sharing factors, whilst the authors expected the impact to be moderately low compared to other options. In addition, the authors do not suggest that results collected in this study represent a comprehensive review of stakeholder perspectives on this subject matter. Further interviews with a much larger number of participants would be needed to draw significant conclusion for the BBNJ context. However, it is suggested that the benefit-sharing equation described in this article provides a useful tool and starting point, which can be built upon by decision makers in the future, to include more detailed information gathered from various expert groups, such as likely impacts on potential beneficiaries, to consolidate the perceived balance of beneficial impacts versus burden. In addition, the equation can be tailored according to different policy settings where different benefit-sharing factors and additional nuance may be more appropriate.

Benefit-Sharing in the BBNJ Context

Benefit-Sharing Factors

The most important benefit-sharing factor, according to average weights allocated by interviewees (see Figure 1), was the influence on biodiversity goals. This is likely because these goals, in particular conservation and sustainable use of biodiversity, are the primary objective of existing genetic resource frameworks, such as the Nagoya Protocol[9] and the Plant Treaty, and as such set the precedence for similar frameworks in the future (CBD, 1992; FAO, 2001; Nagoya Protocol, 2011; World Health Organization, 2011; BBNJ, 2019a). In the BBNJ context, it has already been suggested that fair and equitable benefit-sharing related to MGR from ABNJ can enable the international community to address global challenges (Morgera, 2018a). These global challenges may be related to environmental protection, social objectives and private economic goals (de Brucker *et al*, 2013; Mohammed, 2017; Morgera, 2018a). The particular goals of greatest importance to stakeholders are different in the BBNJ context compared to areas within national jurisdiction, where aspects such as legal certainty are considered most vital (Collins *et al*, 2020; Sirakaya, 2020). Legal certainty is perceived to be of particularly high importance for private sector users of genetic resources (in order to promote investment), who at present appear to view genetic resources in areas within national jurisdiction as more relevant to them than MGR in ABNJ (Sirakaya, 2019; Collins *et al*, 2020). As such, the benefit-sharing factors involved in the methodology, and the weighting assigned to each, are likely to vary according to the circumstances.

Non-Monetary Benefit-Sharing Options and Balance of Potential Positive Aspects vs Burden

Interviewee results indicate that sharing of GSD and research results provide the most favourable balance in terms of beneficial impacts versus burden. Technology transfer, and in particular research directed towards priority needs, received less favourable balances (see Figure 3). It has been noted by the Ad Hoc Technical Expert Group on DSI on Genetic Resources under the CBD[10] that DSI '*plays an important role in deepening knowledge about biodiversity, identifying and mitigating risks to threatened species, enhancing our ability to track illegal trade, identifying species and the geographic origins of products, and assisting with biodiversity planning and conservation management*'. As such, the sharing of this type of information in the context of BBNJ is likely to have a positive influence on the biodiversity goals, in particular the conservation and sustainable use of BBNJ. Sharing of GSD appears to be a relatively straightforward procedure, and it is considered best practice amongst the scientific research community (Devi and Pisupati, 2018). Under the assumption that the BBNJ agreement describes requirements to share GSD in keeping with current practices, through the same current channels (and not through other systems), then this form of benefit-sharing may not incur additional burden on the users of MGR. However, the authors acknowledge the contentious nature of discussions regarding GSD/DSI in multiple fora, including the CBD and the Plant Treaty. Whilst sharing of GSD/DSI itself may be relatively uncontroversial, the potential requirement for monetary benefit-sharing associated with utilisation of GSD/DSI is highly contentious (Kobayashi, 2019). Sharing of research results is thought to have a similar positive effect to the sharing of GSD on enhancing the potential for conservation of marine biodiversity, by helping to build an enabling environment in which MSR can flourish (Harden-Davies and Gjerde, 2019).

Technology transfer represented a relatively unfavourable balance. This may be associated with a combination of relatively high expected burden on the users of genetic resources and on the regulators, together with limited potential beneficial impacts. The potential burdens on different stakeholder groups associated with technology transfer are described in Collins *et al* (2020). It is suggested that the degree of burden, in terms of financial cost and administration, will depend on the type of technology, the conditions, how it is funded and how the transfer is managed, but on the whole, these will be more significant than for other forms of non-monetary benefit-sharing. For example, it is generally expected that the funding required for technology transfer will be greater than for other non-monetary benefit-sharing options (Collins *et al*, 2020). In addition, the owners of technology are often private companies or individuals (Prip *et al*, 2015). As such, transfer of this technology will need to involve consideration of commercial aspects, such as the inherent requirement for private sector entities to generate a return on investment and maintain market competitiveness. Related factors will include appropriate intellectual property arrangements and/ or private economic incentives or public funding, particularly in the circumstance of private ownership of technology (Prip *et al*, 2015). Moreover, the beneficial impacts linked to technology transfer may be limited by absorptive capacity (or enabling factors) in the recipient state. According to Prip *et al* (2015), successful technology transfer depends on three factors: the existence of relevant technology to address particular (environmental) challenges, the relevant dissemination of technology in a manner which makes it available to entities which need this, and the application of technology in a manner which is conducive to solving the challenges in mind.

As noted by interviewees involved in a study conducted by Sirakaya (2019), research directed towards priority needs would involve a degree of burden associated with making sure that the research precisely benefits the provider country (or in the context of BBNJ, the beneficiary state(s)), because this would require a considerable amount of time, effort and resources. This potential burden is likely to contribute to the less favourable balance for research directed towards priority needs.

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[9] For example, according to Article 9 of the Nagoya Protocol (2011), '*The Parties shall encourage users and providers to direct benefits arising from the utilization of genetic resources towards the conservation of biological diversity and the sustainable use of its components*'.

[10] https://www.cbd.int/doc/c/079f/2dc5/2d20217d1cdacac787524d8e/dsi-ahteg-2018-01-03-en.pdf - accessed 09 March 2020

Monetary Benefit-Sharing Options and Balance of Potential Positive Aspects vs Burden

According to interviewee results, research funding, followed closely by salaries, were selected as the monetary benefit-sharing options which provide the most favourable balance regarding beneficial impacts versus burden. Access fees, milestone payments, license fees and royalties received less favourable balances (see Figure 4).

The favourable benefit-sharing balance indicated for research funding and salaries may be because there is a greater degree of perceived transparency involved in these approaches, compared to other forms of monetary benefit-sharing, in terms of where the financial resources are directed and how 'beneficial' this could be (Altman and Simera, 2010). This transparency with regards to research funding is promoted by funding organisations[11,12], science foundations[13], journals and international initiatives, such as the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) network, which aim to encourage transparent reporting of research, research funding processes and the use of reporting guidelines[14]. In addition, salaries and in particular research funding can directly support R&D on MGR, with potential to enhance conservation and sustainable use of BBNJ.

The less favourable balance indicated for access fees, milestone payments, license fees and royalties may, therefore, be associated with a lack of clarity and transparency in terms of how financial resources are used and how much of a beneficial impact this might have (Altman and Simera, 2010). Indeed, interviewees involved in this study raised questions and uncertainty regarding how money could be utilised in the scope of monetary benefit-sharing options to promote biodiversity goals, such as conservation and sustainable use of biodiversity. This uncertainty may also be due to the complicated nature of the factors explored. Further research, building on the current study, should be more specific in nature and would likely be simpler for interviewees to respond to, yielding increasingly insightful results. For example, it was suggested that the balance for monetary benefit-sharing options could be more favourable than currently perceived if money could clearly be linked to supporting these goals. Whilst some monetary benefit-sharing options may at some stage create incentives for biodiversity conservation, this would depend on how and where financial resources are allocated. This is currently not as clear or as transparent as processes associated with research funding and salaries (Altman and Simera, 2010). In addition, the potential impact of benefit-sharing options on bioidiversity goals and the overall benefit-sharing balance may be affected by the approach and language adopted in the new agreement. For example, the Nagoya Protocol (2011) text refers mostly to 'encouraging' the flow of benefits towards biodiversity conservation, but there is no clear obligation[15]. The Benefit-Sharing Fund of the Plant Treaty provides a different example, whereby the Contracting Parties decide for themselves the contributions that they will make (Tsioumani, 2018). In order to reach a more favourable balance, it will be necessary to include language in the BBNJ agreement which clearly ties benefit-sharing options to the objectives. This is an important point which should be kept in mind during the negotiation process.

In this study, the authors investigated benefit-sharing options in the manner in which they are currently being addressed in accordance with existing ABS frameworks under the Nagoya Protocol, Plant Treaty and in the draft BBNJ text. When investigating details regarding monetary benefit-sharing options with a view to developing a methodology to balance these with various factors, it became apparent that these options are pooled together under the 'monetary benefit-sharing' term without making the distinction between those which accumulate (such as royalties, access fees and milestone payments) and those which distribute funding (such as research funding and salaries). Given that monetary benefit-sharing is already a contentious item in the realm of benefit-sharing, the fact that it is dealt with in this confusing manner is not useful and could prove problematic when translated into practice. This point represents an important finding in the present study. Whilst out of scope of this paper, further research should be conducted in the future to adjust and tailor the formula according to whether the monetary benefit-sharing options generate or distribute funding.

A further factor which might contribute towards the less favourable balance of monetary benefit-sharing options compared to the non-monetary options is the fact that the benefits will need to be shared amongst large numbers of beneficiaries. Whilst sharing of data or information with many people does not dilute or divide the benefits received by each individual/ entity (each can receive the same package of data/ information), the sharing of monetary benefits amongst many beneficiaries will likely result in limited and small amounts of money reaching the beneficiaries. This aspect may contribute towards the perception held by many delegates that non-monetary benefit-sharing could represent more predictable and more significant options than forms of monetary benefit-sharing (Morgera, 2018b). By focusing on goals and identified capacity requirements, non-monetary benefit-sharing can have a more immediate and tangible impact

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[11] https://www.nihr.ac.uk/blog/how-do-you-make-research-funding-transparent-and-fair/10991 - accessed 02 March 2020

[12] https://www.ukri.org/about-us/policies-and-standards/transparency/ - accessed 09 March 2020

[13] https://www.nsf.gov/od/transparency/transparency.jsp - accessed 21 March 2020

[14] https://www.equator-network.org/- accessed 09 March 2020

[15] Article 9, Nagoya Protocol, 2011: *'The Parties shall encourage users and providers to direct benefits arising from the utilization of genetic resources towards the conservation of biological diversity and the sustainable use of its components.'*

on bridging the equity gaps related to R&D on MGR. This approach has been considered the principal success of the Plant Treaty (Stannard and Moeller, 2013; Tsioumani, 2018).

Conclusion

To date, benefit-sharing related to the utilisation of genetic resources has not been as effective as was hoped (Ruiz-Muller, 2018; Pauchard, 2017). This is at least partially related to the lack of focus associated with benefit-sharing options on conservation and sustainable use of biodiversity, possible hampering of scientific research as well as the burden placed on the regulator and users of genetic resources. This has in some cases led to a loss of incentive to conduct R&D on genetic resources, with potential to further limit conservation and sustainable use of biodiversity (Tvedt, 2013). The authors acknowledge that there are many other political and legal issues, in addition to the technical aspects referred to in this study, which contribute towards the challenges currently experienced with existing benefit-sharing systems. These issues include, for example, the lack of objectivity in terms of approaches to agreeing appropriate benefit-sharing measures.

A tool, such as the methodology presented in this study (Figure 1), provides a useful means to assess the balance of different benefit-sharing options in terms of the potential beneficial and burdensome impacts, taking into account the varying relative importance of different factors. This would help policy-makers in the future when deciding which forms of benefit-sharing may be most appropriate to adopt, or when improving existing ABS measures, with greater potential for meeting proposed objectives, such as conservation and sustainable use of biodiversity. Use of this methodology would also likely promote transparency, objectivity, clarity and workability of the benefit-sharing system, possibly leading to enhanced generation, accrual and sharing of benefits (Lindhjem *et al*, 2010).

Results in this study indicate that in terms of non-monetary benefit-sharing options under consideration for the new BBNJ agreement, sharing of GSD and research results may provide the most favourable balance. Should monetary benefit-sharing be included in the agreement, research funding and salaries may represent the most fairly balanced options. In addition, in order to reach a favourable balance, it may be necessary to include language in the BBNJ agreement which clearly ties benefit-sharing options to the objectives. However, further interviews with a larger number of participants would be needed to draw significant conclusion for the BBNJ context. Nonetheless, the benefit-sharing equation described and demonstrated in this article provides a useful tool and starting point, which can be tailored according to different policy settings where consideration of different benefit-sharing factors may be more appropriate. In addition, the methodology can be developed to include more detailed information gathered from various expert groups to consolidate the perceived balance of beneficial impacts versus burden associated with benefit-sharing options.

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Supplemental data

Supplemental File 1: Outline for interviews for ”Developing a Methodology to Balance Benefit-Sharing: Application in the Context of Biodiversity Beyond National Jurisdiction”.

Author contributions

JC and TV jointly conceived the idea for the study and manuscript. JC developed the research methodology, performed data collection, data analysis and led writing and editing of the text. AS assisted with developing the research methodology and co-wrote and edited the manuscript. TV also assisted with developing the research methodology and edited and reviewed the manuscript. IH edited and reviewed the manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

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ECPGR recommended Simple Sequence Repeat loci for analyses of European plum (*Prunus domestica*) collections

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Abstract: A set of nine Simple Sequence Repeat (SSR) loci, approved by the ECPGR *Prunus* working group, are proposed as a standard set for genotyping European plum accessions. These loci show sufficient reliability in spite of problems caused by hexaploidy. Polymorphism in the loci is high and enables differentiation between unique plum accessions as well as analyses of genetic grouping and overall genetic structure. A set of seven reference accessions are described. A compiled dataset with allelic information for 165 accessions is presented. Genetic structure reveals three different K-values (2, 4 and 9) demonstrating a major dichotomy between *Prunus insititia*-related accessions and cultivars belonging to *Prunus domestica* sensu stricto, as well as differentiation among minor subgroups defined by pomological traits and geographical origin.

Keywords: DNA fingerprint, genetic resources, molecular markers

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Introduction

Simple Sequence Repeats (SSR), also known as microsatellites, were introduced in plant research almost three decades ago (Akkaya *et al*, 1992). Due to their abundance, reproducibility, and polymorphism, SSRs have proven highly useful for confirming identity of accessions in plant collections and in quantifying their relatedness. In clonally propagated crops, which include most of the fruit and berry crops, each cultivar originates from a single recombination event and all cultivated plants of a particular cultivar would therefore be expected to have the same SSR profile (Nybom and Weising, 2010). Sports, e.g. mutants that differ in fruit colour, usually differ so little from the original cultivar that they cannot be distinguished with SSRs.

The ability to correctly identify plant material from different cultivars has economic importance (e.g. regarding infringement on plant variety ownership) as well as forms a basis for management of plant collections and their utilization in plant breeding and research. Compared to some of the more recently developed approaches like Single Nucleotide Polymorphisms (SNP) and Next Generation Sequencing (NGS), SSRs are comparatively easy to apply and interpret, and can be very cost-effective for developing smaller datasets. This means that specific questions asked by growers, plant nursery owners, amateur pomologists and genebank curators can be solved by analysing just a few plant samples and comparing their SSR profiles with

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previously obtained SSR profiles for other samples in a large database.

The possibility of adding new SSR-based data to an already existing database, even when developed in a different laboratory, is thus regarded as a major asset. However, this option is dependent on the application of the same set of SSR markers and suitable standardisation procedures including the appointment of closely defined control or reference genotypes. In crops like apple (*Malus x domestica*), where the standardization of loci and procedures has taken very long to achieve, use of separate sets of SSR loci has resulted in many datasets that cannot be compared across different research groups (Sehic *et al*, 2013). This situation may have been improved recently since several apple studies are now based on the same set of loci as used in the international research project FruitBreedomics (Urrestarazu *et al*, 2016).

The European Cooperative Programme for Plant Genetic Resources (ECPGR; www.ecpgr.cgiar.org) aims to ensure long-term conservation of important germplasm in Europe as well as facilitate increased utilization of this germplasm, e.g. in plant breeding. An important task is to select unique and well-documented accessions with valuable traits, of European origin or important to Europe, for the establishment of a decentralized European Collection under the rules of AEGIS (A European Genebank Integrated System; www.ecpgr.cgiar.org/aegis). A second task is to assess variability among the accessions and investigate possible subgroupings due to, e.g. geographic differentiation. In fruit tree crops like pears (*Pyrus communis*) and sweet cherries (*Prunus avium*), sets of recommended SSR loci have been appointed by ECPGR and published (Clarke and Tobutt, 2009; Evans *et al*, 2009). Since then, numerous research groups have used these SSR loci for analyses of genetic identity and relatedness in these crops, and, in the case of cherry, also for sour cherries (*Prunus cerasus*).

Until recently, relatively few SSR-based studies have been undertaken on genetic diversity in European plums, a major reason being that this is a hexaploid ($x = 7$; $2n = 6x = 42$) crop and therefore less amenable to molecular marker-based analyses. European plums are usually treated as *Prunus domestica*, although small-fruited primitive cultivars and landraces are sometimes referred to a separate mostly wild species *Prunus insititia* or to a subspecies *P. domestica* subsp. *insititia*. *P. domestica* is generally thought to result from hybridization between the diploid cherry plum (*Prunus cerasifera*), the tetraploid sloe (*Prunus spinosa*) and potentially an additional species (Reales *et al*, 2010).

Results and Discussion

Selected SSR loci

Most of the hitherto published studies on SSR diversity in European plums are based on differing sets of loci (Horvath *et al*, 2011; Xuan *et al*, 2011; Öz *et al*, 2013; Gharbi *et al*, 2014; Halapija Kazija *et al*, 2014; Makovics-Zsohár *et al*, 2017; Merkouropoulos *et al*, 2017; Pop *et al*, 2018; Abdallah *et al*, 2019; Manco *et al*, 2019; Urrestarazu *et al*, 2018). In order to facilitate harmonization between future studies, we propose a standard set of nine SSR loci, approved by the ECPGR *Prunus* working group, for European plum (Table 1). These loci have already been used for investigations of genetic diversity, first in plum cultivars and landraces from Norway and Sweden (Sehic *et al*, 2015) and subsequently in two ECPGR-funded projects with accessions sampled in a total of 14 European countries: 'PRUNDOC' (Sehic *et al*, 2019) and 'Prunus Alignment' (Gaši *et al*, 2020); reporting data from both ECPGR studies).

Seven of the nine chosen SSR loci were developed from genomic DNA of peach (*Prunus persica):* BPPCT007, BPPCT014, BPPCT034, BPPCT039 and BPPCT040 (Dirlewanger *et al*, 2002), and UDP96-005 and UDP98-407 (Cipriani *et al*, 1999). One locus, PacA33, is an Expressed Sequence Tag-SSR (EST-SSR) from apricot (*Prunus dulcis)*(Decroocq *et al*, 2003), while CPSCT026 originates from genomic DNA of Japanese plum (*Prunus salicina)*(Mnejja *et al*, 2004). In addition to studies based on eight or nine of these loci (Sehic *et al*, 2015; Gaši *et al*, 2020), five loci, BPPCT034, BPPCT039, BPCT040, PacA33 and UDP96-005, have also been used by Halapija Kazija *et al* (2013, 2014) for screening of plum accessions from Croatia and neighbouring countries.

Marker reliability is critical for producing cultivar profiles to be used in shared databases. Hexaploid plum accessions are considerably more difficult to genotype compared to diploid genotypes since each locus may have up to six alleles. Overlooking a true but faint band is entirely plausible, as well as is mistakenly scoring an artefactual band as an allele (Gaši *et al*, 2020). Although 15 loci remained as serious candidates among a wider set of SSR loci screened initially, six of these had to be discarded due to unreliable amplification (PacA18, PacA49, PacB22, PacB26, PacB35, PacC13) (Decroocq *et al*, 2003), thus leaving the nine chosen loci. Very high reliability was shown recently when pollinizer success could be determined using seven of the chosen SSR loci for analysis of plum embryos harvested after open pollination (Meland *et al*, 2020). The need for very accurate SSR allele scoring is objectively higher in trials that use the obtained DNA profile database for paternity analyses compared to standard diversity studies.

Detected polymorphisms were very high for the nine chosen SSR loci when a joint biostatistical analysis was performed for plum accessions previously genotyped in the above-mentioned studies (Sehic *et al*, 2015; Gaši *et al*, 2020). Although the study by Gaši *et al* (2020) was based on only eight of the nine recommended SSRs, all plum accessions from that study had also been genotyped for the remaining microsatellite locus (BPPCT039). Number of alleles ranged from 18 to 48, and Nei's gene diversity ranged from 0.88 to 0.93 (Table 2).

Table 1. SSR loci, DNA sequences, references and annealing temperature of the nine primer pairs selected by the ECPGR *Prunus* working group for assessment of plum accessions.

Locus	DNA sequence	Reference	Annealing temp. °C
CPSCT026	3'-TCTCACACGCTTTCGTCAAC-5' 3'-AAAAAGCCAAAAGGGGTTGT-5'	Mnejja *et al* (2004)	46
BPPCT034	3'-CTACCTGAAATAAGCAGAGCC AT-5' 3'-CAATGGAGAATGGGGTGC-5'	Dirlewanger *et al* (2002)	56
UDP96-005	3'-GTAACGCTCGCTACCACAAA-5' 3'-CCTGCATATCACCACCCAG-5'	Cipriani *et al* (1999)	56
BPPCT014	3'-TTGTCTGCCTCTCATCTTAACC-5' 3'-CATCGCAGAGAACTGAGAGC-5'	Dirlewanger *et al* (2002)	58
BPPCT039	3'-ATTACGTACCCTAAAGCTTCTGC-5' 3'-GATGTCATGAAGATTGGAGAGG-5'	Dirlewanger *et al* (2002)	58
BPPCT040	3'-ATGAGGACGTGTCTGAATGG-5' 3'-AGCCAAACCCCTCTTATACG-5'	Dirlewanger *et al* (2002)	58
UDP98-407	3'-AGCGGCAGGCTAAATATCAA-5' 3'-AATCGCCGATCAAAGCAAC-5'	Cipriani *et al* (1999)	58
PacA33	3'-TCAGTCTCATCCTGCATACG-5' 3'-CATGTGGCTCAAGGATCAAA-5'	Decroocq *et al* (2003)	58
BPPCT007	3'-TCATTGCTCGTCATCAGC-5' 3'-CAGATTTCTGAAGTTAGCGGTA-5'	Dirlewanger *et al* (2002)	60

Laboratory procedures

All amplifications were performed according to Dirlewanger *et al* (2002) with the minor changes of an increase to 1 U *Taq* polymerase (Thermo Fischer Scientific, Surrey, UK) and the introduction of four different annealing temperatures (Table 1). Diluted PCR products were mixed with Hi-Di formamide (Applied Biosystems, Beverly, MA, USA) and an in-house prepared size standard, after which the amplified fragments were separated on an ABI 3130xl Genetic Analyser (Applied Biosystems).

Since errors due to competitive amplification are more difficult to spot in samples of hexaploid organisms compared to diploid ones, we recommend that all amplifications are performed in simplex. Whether to pool the amplification products from two (or three) loci together before allele sizing in, e.g., an automated gene sequencer, is less critical and therefore a matter of what is regarded as most convenient in each laboratory.

Reference genotypes

A set of seven reference accessions has been appointed: the large-fruited Canadian eggplum 'Valor' (cross between 'Imperial Epineuse and 'Grand Duke'), the small-fruited German eggplums 'Hanita' ('President' x 'Auerbacher') and 'Topfirst' ('Čačanska Najbolja' x 'Ruth Gerstetter'), the French greengage 'Reine Claude Violette' (offspring of 'Reine Claude Verte'), the large-fruited American prune 'Stanley' ('d'Ente Double' x 'Grand Duke'), the French mirabelle 'Mirabelle de Nancy', and the East European small-fruited prune 'Bistrica'. Allele sizes of these references (Supplementary Table 1) can be used as a basis for determination of the size adjustment needed to render data from other laboratories comparable. Leaves of these genotypes can

Table 2. Allele size range, number of alleles and gene diversity (Nei, 1978) for 9 SSR loci, calculated among 175 plum accessions investigated by Gaši *et al* (2020) and by Sehic *et al* (2015), as well as among 7 reference cultivars.

Locus code	Size range (bp)	No. alleles	Gene diversity	Size range (bp)	No. alleles	Gene diversity
	Plum accessions (n=175)			**Reference cv. (n=7)**		
UDP 98-407	156/231	29	0.8825	164/203	10	0.8995
Pac A 33	169/254	37	0.9275	169/252	15	0.8907
CPSCT 026	165/216	22	0.9118	165/208	13	0.8989
BPPCT 040	113/154	18	0.8811	120/146	8	0.8640
BPPCT 007	121/163	19	0.9121	123/147	10	0.8872
BPPCT 014	186/294	48	0.9319	186/258	16	0.9298
BPPCT 034	213/277	25	0.9117	215/259	12	0.9013
UDP 96-005	92/169	34	0.9218	100/165	11	0.8462
BPPCT039	113/187	33	0.9255	126/179	17	0.9339
Mean		29.4	0.9118		12.4	0.8946

be obtained from the Institute of Pomology (Croatian Centre for Agriculture, Food, and Rural Affairs), located in Donja Zelina, Croatia. Alternatively, replicate samples could be used from trees already present in many plum collections following an initial DNA-based confirmation of their identity against the original reference genotypes.

Evaluation of band profiles

In previous ECPGR-recommended sets of SSR markers (Clarke and Tobutt, 2009; Evans *et al*, 2009), 16 loci were selected for both pear and sweet cherries, although only six loci were defined as first priority in cherries followed by three loci as 2^{nd} priority, three loci as 3^{rd} priority and four loci as 4^{th} priority. In these diploid crops, the maximum number of alleles scored would thus reach 32 although the number is usually lower due to the presence of two copies of the same allele in several loci.

Since all European plums are hexaploid, up to six different alleles can be expected in each SSR locus, amounting to a maximum of 54 alleles for nine loci. However, the total number of alleles in a plum sample analysed with the proposed nine loci has only reached 35 on average (Sehic *et al*, 2015). The discrepancy here is also probably due, at least in part, to multiple copies of the same allele in some of the locus/genotype combinations.

In a study of 78 presumably hexaploid plum genotypes screened with all nine loci, 59 accessions revealed six alleles in one to three loci, while the remaining 19 accessions revealed a maximum of five different alleles in any locus (Sehic *et al*, 2015). In another study of 110 accessions analysed with 8 of the proposed SSR loci, 85 accessions revealed six alleles in at least one locus, while 23 accessions revealed five alleles and two accessions revealed a maximum of only four alleles (Gaši *et al*, 2020). Counting the total number of bands for each genotype is thus recommended, since an unusually low number of alleles can be an indicator of poor amplification.

Analyses of genetic diversity

Multilocus SSR profiles are generally scored as 'allelic phenotypes' based on the presence of alleles but not their frequencies. Since the likelihood of actually overlooking alleles is much higher in hexaploid samples compared to samples of lower ploidy, the threshold for determination of whether two (or more) samples are identical, has to be relatively low. In a study of European plums, all pairwise comparisons with the fraction of shared bands, S_{xy} [$= 2n_{xy}/(n_x + n_y)$], reaching 0.88 or higher, were interpreted as resulting from the same recombination event and thus being genetically identical except for possible minor somatic mutations (Gaši *et al*, 2020).

Availability of biostatistical software which can be used on genotyping data of allopolyploid accessions, such as the hexaploid plum, is significantly restricted compared to software solutions for diploid accessions. However, some programs provide options to overcome the challenges of allele dosage ambiguity. Population genetics software SPAGeDI 1.3 (Hardy and Vekemans, 2002) and POLYSAT (Clark and Jasieniuk, 2011), an R package for polyploid microsatellite analysis, enable the replacement of "missing alleles" in loci where fewer than six different alleles (de facto maximum in hexaploid plum) are scored, with the average allele size. This enables the calculation of allele frequency, as well as gene diversity (Nei, 1978) and F statistics (Weir and Cockerham, 1984). Additionally, population structure can be investigated using the Bayesian model-based cluster procedure within Structure version 2.2.3 (Pritchard *et al*, 2000).

Genetic differentiation among groups of genotypes (based on various criteria such as geographical origin, morphological or taxonomic traits, breeding status, or obtained as reconstructed panmictic populations in Structure), can be examined using the GenoType/GenoDive package (Meirmans and Tienderen, 2004) which enables analyses of molecular variance (AMOVA) (Excoffier *et al*, 1992) among polyploids, as well as among a mix of genotypes with varying levels of ploidy.

Although the visualization of relationships among polyploid genotypes is easily accomplished through hierarchical clustering, such as UPGMA dendrograms using a matrix with pairwise comparisons based on the Jaccard similarity coefficient, the use of factorial correspondence analysis (FCA) on SSR data of diploid crops has become increasingly common. This multivariate analysis can be conducted on a matrix of binary microsatellite allele presence/absence data using the "dudi.coa" routine in R 2.15.2 (R Core Team, 2012) as suggested by Muller and McCusker (2009). Construction of the mentioned matrix is quite simple and appropriate for polyploid genotypes. A graphical display of the FCA results can then be achieved with the rgl package version 0.93.945 (Adler and Murdoch, 2013) in the same statistical software.

Compiled dataset and genetic structure

A joint dataset covering 165 accessions (subsequent to removing the duplicates between studies) was compiled from the two datasets (Sehic *et al*, 2015; Gaši *et al*, 2020). The 8 loci from the original study by Gaši *et al* (2020) were complemented by the genotyping of BPPCT039, resulting in 9 loci scored for all accessions. Most of these accessions had never been genotyped before and they were chosen so as to represent both the major coverage of cultivars in plum-growing countries in Europe as well as the whole range of material in germplasm collections, from local and landrace accessions to commercial cultivars produced in modern breeding programs. This dataset is available in Supplementary Table 1 and can be regarded as a starting point for a shared international dataset to be used by different research groups.

Genetic structure among accessions in this compiled dataset was investigated with a Bayesian model-based cluster procedure using Structure version

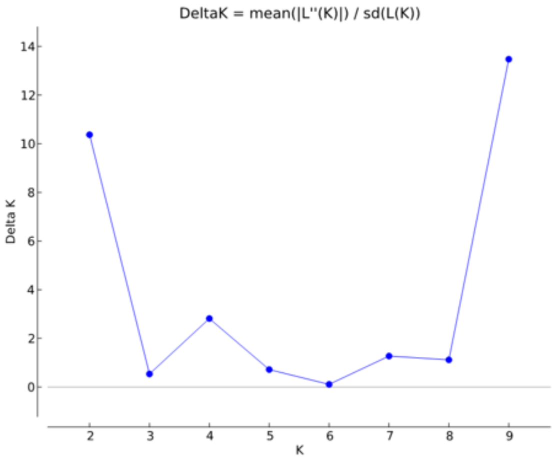

Figure 1. Plot of deltaK values from the Bayesian genetic structure analyses of 165 plum accessions.

2.2.3 (Pritchard *et al*, 2000). For individuals with fewer than six allelic variants per locus, absent alleles were treated as missing data. K (unknown) reconstructed panmictic populations (RPPs) were computed on individuals, testing K (log-likelihood) = 1–10 for all samples, assuming that the sampled accessions were from unknown origin. Ten independent runs were conducted for each K. A burn-in period of 200,000 and 500,000 iterations was applied. Structure Harvester version 0.6.1 (Earl and Holdt, 2011), which implements the Evanno method (Evanno *et al*, 2005), was used to estimate K values for the analysed data (Figure 1). K = 2, 4 and 9 were used to assign individuals to specific clusters. All input files were compiled using MADC version 1.2 (Grahić and Grahić, unpublished data).

Genetic structure analyses

For K = 2, accessions were divided into two approximately equal RPPs (mostly red or mostly green) and a large number of admixed genotypes (Figure 2). Among the green-coloured samples (RPP2:1) were *P. insititia* cultivar no. 81 'Kozlienka' from Slovakia, the feral *P. insititia* accession no. 82 'Krikon' from Sweden, the Hungarian plums no. 106 'Potyó fehér' and no. 107 'Potyó szilva', and the Central–Eastern European prunes also known as zwetschen (e.g., no. 108 'Požegača'). Red-coloured samples (RPP2:2) instead included the French prunes of 'd'Agen' type (no. 36 and 37), most of the greengages (no. 118–121, 123 and 124) and large-fruited cultivars grown across Europe as dessert plums (e.g. no. 153 'Victoria').

For K = 4, the previous RPP2:1 (≈ *P. insititia*) was split into one large (green, RPP4:1) and one smaller (red, RPP4:2) RPP (Figure 3). RPP4:1 contained the above-mentioned 'Kozlienka', 'Krikon', 'Potyó fehér', 'Potyó szilva' and 'Požegača', as well as small-fruited plums of the damson or bullace type like the Italian 'Ramassin' (no. 115–117). RPP4:2 instead comprised several Norwegian landrace plums but also e.g. 'Spilling' (no. 135) collected in Denmark but most likely of German origin. The previous RPP2:2 (*P. domestica* s.s.) was split into one larger RPP (yellow, RPP4:3) with 'Victoria' and many other large-fruited dessert plums, and one smaller (blue RPP4:4) with greengages like 'Reine Claude Bålnäs' (no. 118) and 'Reine Claude grande verte' (no. 124) as well as the French prunes.

For K = 9, most samples showed an admixed genotype (Figure 4). The previous RPP4:1 was divided into three RPPs with the largest (dusty pink, RPP9:1) containing small-fruited accessions like 'Cariadoggia' (no. 33) and 'Muninca' (no. 101) from Italy, 'Karsavas' (no. 73) from Latvia and 'Moravka' (no. 98) from Serbia. The zwetschen (e.g. 'Požegača') were found in the second (orange, RPP9:2), while two putatively diploid samples (no. 2 and no. 51) and the Greek 'Asvestochoriou' (no. 10) made up the third (red, RPP9:3). The previous RPP4:2 was split into two RPPs containing mainly Norwegian landraces (green, RPP9:4, and brown, RPP9:5, respectively). The previous RPP4:3 was divided mainly into two RPPs with the German 'Gräfin Cosel' (no. 49) and 'Ruth Gerstetter' (no. 127) in RPP9:6 (dark blue) and some other large-fruited cultivars in RPP9:7 (pale blue). Several well-known cultivars like 'Victoria' were denoted as an admixture of these two RPPs. The previous RPP4:4 corresponded relatively closely to RPP9:8 (yellow) and contained mainly greengages. The mirabelles (no. 95–97, 161 and possibly also no. 52) formed a RPP of their own (purple, RPP9:9) in spite of having admixed genotypes at lower K-values.

Conclusions

In this contribution we present, for the first time, a set of nine SSR loci recommended by the ECPGR *Prunus* working group for use in genotyping of European plums, and for analyses of genetic variation and structure. A set of seven reference cultivars is proposed. A compiled dataset with allelic information for 165 accessions is presented as a resource to allow comparison of further datasets. Genotyping of these 165 accessions showed that all loci produce highly polymorphic genetic profiles, while analysis of genetic structure revealed a major dichotomy between *P. insititia*-related accessions and cultivars belonging to *P. domestica sensu stricto*, as well as differentiation among minor subgroups defined by pomological traits and geographical origin. By adding genetic profiles for new samples into this dataset, researchers can easily check whether they are synonymous with any of the present 165 accessions and also check for parent-offspring relations. In addition, performance of genetic structure analyses with all available samples is likely to provide valuable information about pomological grouping of the new samples in genetic collections as well as in plant breeding programs.

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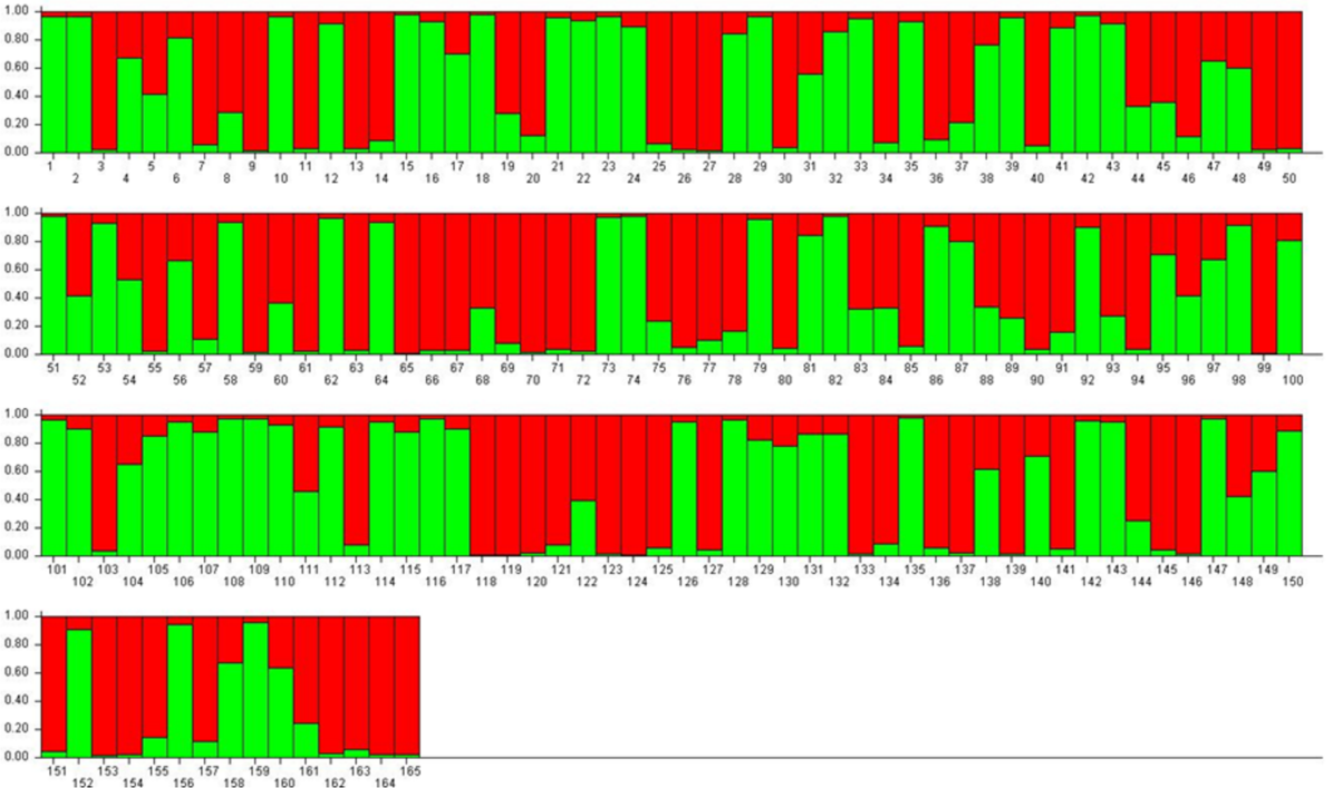

Figure 2. Bar plot of the results from a Bayesian genetic structure analysis with K = 2, green RPP2:1, red RPP2:2. For accession names, see Supplementary Table 1.

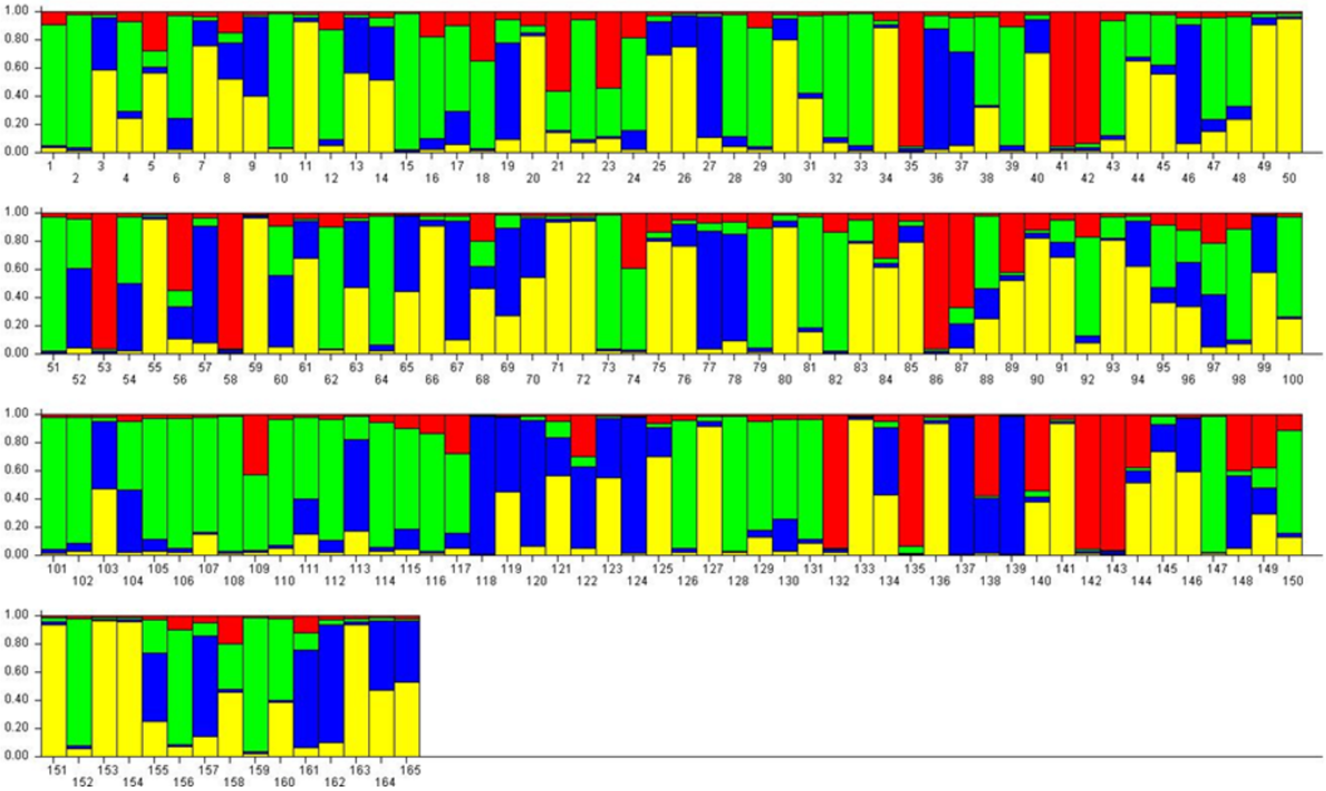

Figure 3. Bar plot of the results from a Bayesian genetic structure analysis with K = 4, green RPP4:1, red RPP4:2, yellow RPP4:3, blue RPP4:4. For accession names, see Supplementary Table 1.

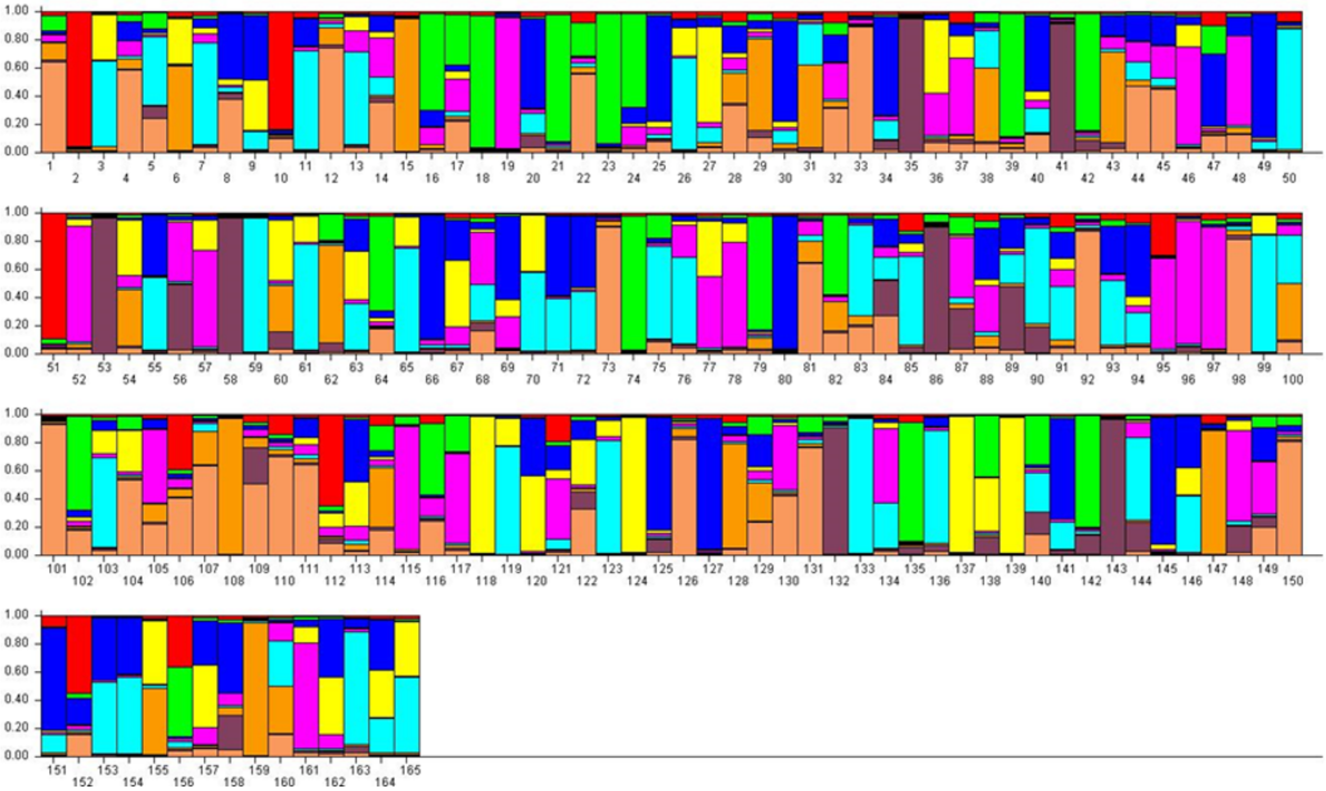

Figure 4. Bar plot of the results from a Bayesian genetic structure analysis with K = 9, dusty pink RPP9:1, orange RPP9:2, red RPP9:3, green RPP9:4, brown RPP9:5, dark blue RPP9:6, pale blue RPP9:7, yellow RPP9:8, purple RPP9:9. For accession names, see Supplementary Table 1.

for the appointed SSR loci. We wish to acknowledge the ECPGR for providing both the suitable international framework of collaboration and the necessary funds to bring this work to completion.

Supplemental data

Supplementary Table 1. SSR profiles for 165 European plum accessions. Sample numbers are the same as used in the Bayesian genetic structure analyses (Figures 2, 3 and 4), while Study = 1 refers to accessions analysed in Gaši *et al* (2020) and Study = 2 refers to accessions analysed in Sehic *et al* (2015). Reference accessions (Ref) are given at the end of the table. Profiles for each SSR are indicated as allele sizes in base pairs.

Author contributions

HN managed projects where the proposed set of SSR loci were used, wrote a major part of the manuscript. DG is the chair of ECPGR *Prunus* Working Group, assisted in organizing projects where the markers were used, assisted with writing the manuscript. MO is the coordinator of the 'Prunus alignment' project where the SSR loci were used, assisted with writing the manuscript. SHH is the coordinator of the 'PRUNDOC' project where the SSR loci were used, assisted with writing the manuscript. JG performed statistical evaluations. FG supervised evaluation of amplification products, performed some statistical evaluations, assisted with writing the manuscript.

Conflict of interest statement

The authors declare that no conflict of interest exists.

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Replenishment and rationalization of seed collections of pumpkin, vegetable marrow and summer squash for ex situ conservation and use for breeding in Armenia

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Abstract: The conservation and sustainable use of crop genetic resources are crucial for food security and economic development, and diverse genetic resources are a critical input in the agricultural production process. To conserve the gene pool of vegetable crops of Armenia, the Scientific Centre of Vegetable and Industrial Crops of the Ministry of Economy of the Republic of Armenia carried out activities to upgrade storage facilities and optimize the existing seed collections. Newly created long-term and medium-term seed collections of pumpkin, vegetable marrow and summer squash reliably conserve genetic resources of these crops and create a base for undertaking genetic enhancement and base-broadening efforts. A database and application of barcodes and QR-code labels facilitate the search of required accessions, making the stored genetic materials more accessible for users and promoting their wider use in breeding.

Keywords: Cucurbitaceae, base collection, active collection, breeding

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Introduction

Vegetable production is one of the leading sectors in Armenian agriculture. Variation in agro-ecological conditions, climate and altitude in Armenia, as well as long-time traditions in multipurpose use of vegetables have led to a large diversity of vegetables grown by farmers. Among vegetables species of the Cucurbitaceae family, the following are of specific interest due to their palatability, nutritional value and dietary qualities: (1) pumpkin (*Cucurbita maxima* Duchesne, *Cucurbita maxima* var. *turbaniformis* (M.Roem.) L.H.Bailey, *Cucurbita moschata* Duchesne, *Cucurbita pepo* L.); (2) vegetable marrow (*Cucurbita pepo* L. subsp. *pepo* var. *pepo* L.); and (3) summer squash (*Cucurbita pepo* L. subsp. *ovifera* (L.) D.S.Decker). Dietary benefits of pumpkin and vegetable marrow are associated with a high content of vitamins C, B1, B2, B6, PP, E and carotenoids (α- and β-carotene, lutein and zeaxanthin), a favorable ratio of potassium and sodium, and low calorie content. These crops are given a great importance in the production of baby food (Piskunova and Muteva, 2016). Summer squash is an excellent source of manganese, copper, folate, magnesium, potassium, and fiber, whereas vitamins C and A act as antioxidants, which may help to protect against hardening of blood vessels (Hashash *et al*, 2017). The nutritional value of pumpkin, vegetable marrow and summer squash makes these crops attractive both for large farms providing vegetables for canneries and for small landholders producing these crops in response to market demand. Moreover, ongoing state programmes on subsidizing agriculture and providing preferential loans and investments that are being put into the modernization of Armenia's greenhouse sector

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create an enabling environment for the introduction and cultivation of different vegetables, both in open fields and in greenhouses.

The Scientific Centre of Vegetable and Industrial Crops (SCVIC) has engaged in breeding and seed production of vegetable crops and provides growers with seeds of different varieties of pumpkin, vegetable marrow and summer squash that have been bred at the Centre and released in the country. So far, the numbers of locally bred varieties of the above-mentioned crops are not many: three varieties of vegetable marrow, four of pumpkin and one of summer squash. To meet grower and consumer demands in both production level and varietal diversity of cucurbitaceous vegetable crops there is a need for strengthening breeding programmes and undertaking genetic enhancement efforts. For this purpose, genetic resources of cucurbitaceous species are required as inputs into the continuing process of enhancement through selective breeding. To conserve and provide breeders with germplasm for further breeding programmes, the seed collection that over many years has been created and maintained at the SCVIC under room conditions was significantly improved by the purchase of freezers, the acquisition of new and regeneration of old accessions, and the rationalization of the existing ex situ collection (Sackville-Hamilton *et al*, 2003) .

Materials and methods

Base and active collections were established both from newly introduced and regenerated germplasm as well as from accessions of working/breeding collections that were collected and maintained in paper bags over a period of about twenty years under room conditions without the possibility to control temperature and humidity. Accessions of foreign varieties have been received in the frameworks of collaboration projects with other countries with the purpose of testing under local conditions. Accessions of research material were received from the World Vegetable Center with the aim of multiplication and use in breeding programmes. Seeds of landraces were collected from local markets and farms. The process of acquisition of germplasm, seed drying, regeneration and storage of base and active seed collections was carried out in line with internationally accepted standards (FAO, 2014). To maintain the genetic integrity of original accessions during regeneration in open fields and avoid risk of outcrossing, plants were planted in blocks, not in rows, and 1,000 m isolation distance between different varieties was maintained. Seeds were collected from plants in the center of each block. In addition, samples with limited numbers of seeds were regenerated in individual polyethylene film greenhouses using hand-pollination.

Selected seed samples were dried to appropriate moisture content using a drying chamber. Before storing, initial seed viability tests were conducted. Dried seeds were placed in laminated aluminum foil bags of 11 μm thickness and hermetically sealed. All seed packages were labeled with printed barcode and QR-code, which includes information on accession name, acquisition date, origin and biological status, reducing the possibility of errors and facilitating search of requested accessions. Accessions of the base collection are stored under long-term conditions at a temperature of –18°C. Material placed under medium-term conditions (i.e. active collection) is stored under refrigeration at 5°C.

Passport data for each accession are fully documented according to the FAO/Bioversity multi-crop passport descriptors (Alercia *et al*, 2015). The database for accession records is developed in Excel format compatible with EURISCO.

Results and Discussion

Purchasing the appropriate seed storage deep freezers and refrigerators, drying chamber, germination boxes and aluminum foil bags provided the possibility for establishing base and active seed collections, each with a specific target and coverage.

The active collection of pumpkin, vegetable marrow and summer squash was created to provide breeders, farmers, and researchers with seed material for study and use in breeding, research and educational programmes, as well as for seed material exchange. All Armenian varieties of pumpkin, vegetable marrow and summer squash released in the country since 1991 are included in the active collection regardless of their present cultivation area and market demand. The active collection includes 11 foreign varieties, which are in demand on the local market due to their economically important traits, such as early maturing and high productivity. Research material that, based on preliminary studies, has potential interest for breeders is also included in the active collection.

The base collection serves as safety material for the active collection and mainly includes varieties bred in Armenia, traditional farmers' varieties, as well as the most valuable breeding/research material of both local and foreign origin. Accessions were incorporated into the base collection based on the following criteria defined by a Scientific Council of the SCVIC:

- ***Strategic importance of a locally bred variety for the country's food production and sustainable agriculture.***Widely cultivated varieties contributing to a country's food security are included in the base collection.
- ***Prevalence of local farmers' variety in the country***. Landraces that are disappearing from markets and farmers' fields as a result of the spread of more productive modern varieties are placed under long-term storage conditions.
- ***Significance of a foreign variety for food security***. Although the majority of the varieties of *Cucurbitaceae* cultivated in Armenia are of Armenian origin, in specific agro-climatic zones some foreign varieties ensure higher yields compared

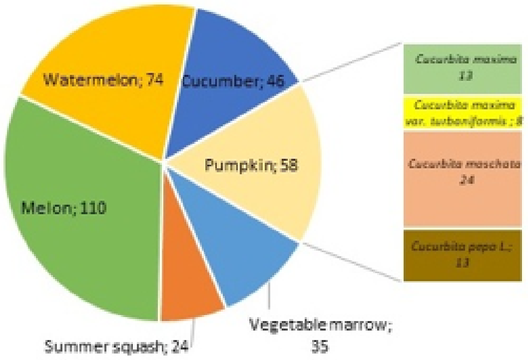

Figure 1. Structure of the base and active seed collections of the Cucurbitaceae family at the Scientific Centre of Vegetable and Industrial Crops, Armenia.

with local ones, and such varieties were added to the base collection.

- ***Value of less common variety in terms of a potential for a niche market***. Varieties that are of specific interest for farms providing products for restaurants or high-end specialty stores were included in the base collection.
- ***High mean value for major breeding traits***. Considering priority areas of breeding, research materials that could serve as a source of important traits, such as resistance to powdery mildew or early ripeness were incorporated into the base collection.

The SCVIC maintains a breeding collection of 88 accessions of pumpkin, vegetable marrow and summer squash, which is stored for a short period (from one to three years, depending on the species) under room conditions. Such collections are intended for breeding research conducted in the short term. Some accessions of the breeding collection have low initial germination or insufficient number of seeds, and must be regenerated, processed and stored in priority order.

The existing *ex situ* collections of pumpkin, vegetable marrow and summer squash were expanded by including obsolete varieties, landraces, research lines, samples obtained from partners in the framework of collaborative projects and cooperation with the World Vegetable Center. Accessions were requested according to the top priority areas of breeding of Cucurbitaceae species in the country, in particular high productivity, early ripeness, bushy growth habit and resistance to powdery mildew. At the same time, duplicate accessions and accessions with low seed variability were eliminated.

The enlarged seed collections of pumpkin, vegetable marrow and summer squash are not big compared with the collections of other species of the Cucurbitaceae family (Figure 1): they are new and contain valuable accessions of varieties and hybrids selected or bred in Armenia with viable seeds. In total, the collection (base and active) includes 117 accessions of six species and covers all available varieties, hybrids, breeding lines and landraces of Armenian origin.

The ratios of breeding varieties, research material and landraces vary depending on the crop. Thus, about 72% of the seed collection of pumpkin are represented by modern varieties. Among accessions of vegetable marrow, the research material represented by breeder's lines, hybrids, and lines of individual selections from hybrid populations exceeds the number of accessions of modern breeding varieties (Figure 2). The summer squash collection is represented in equal quantity by varieties and research/breeding material.

In the base collections of pumpkin, vegetable marrow and summer squash, breeding varieties prevail, while most of the accessions of the active collections are represented by breeding and research material (Figure 2). Although there is a limited number of landraces of the gourd family existing in the country, the inventory indicated gaps in the collection in terms of farmers' varieties of pumpkin and vegetable marrow, which are being gradually displaced by commercially developed modern varieties. As for summer squash, this crop is a relatively new one in cultivation, so landraces do not exist.

Varieties of Armenian origin prevail over foreign-introduced varieties of pumpkin, vegetable marrow and summer squash in both collections. The number of accessions of introduced breeding/research material exceeds those of local origin, triggered by the need for diverse germplasm from outside Armenia in order to minimize genetic uniformity of new varieties.

Conclusion

As a result of establishing base and active collections of pumpkin, vegetable marrow and summer squash at the SCVIC the conservation of the genetic resources of these crops is ensured under conditions that meet internationally accepted standards. Due to the enlargement and rationalization of the *ex situ* collection, a resource base for genetic enhancement of these crops to produce substantial economic benefits was created. It can serve for breeding new varieties that meet requirements of local agricultural production, such as early maturity, high yield, resistance to pests and diseases, tolerance to abiotic stresses and other characteristics dictated by producers and consumers. The conserved genetic resources are available for users and can be provided upon request. As soon as the work on upgrading the collection of the remaining species of Cucurbitaceae, particularly bottle gourd (*Lagenaria siceraria* (Molina) Standl.) and loofah (*Luffa aegyptiaca* (L.) Roxb.) is finished, the passport data on all Cucurbitaceae accessions will be recorded in EURISCO to make the information on available accessions accessible for users at national and international levels.

The work on inventory of the collections, seeds viability tests and placing for long-term and mid-term storage has started with crop species of the Cucurbitaceae family. This is the first step in upgrading the vegetable crops genetic resources collection and establishing properly managed seed collections. The future activities will be focused on further enlargement

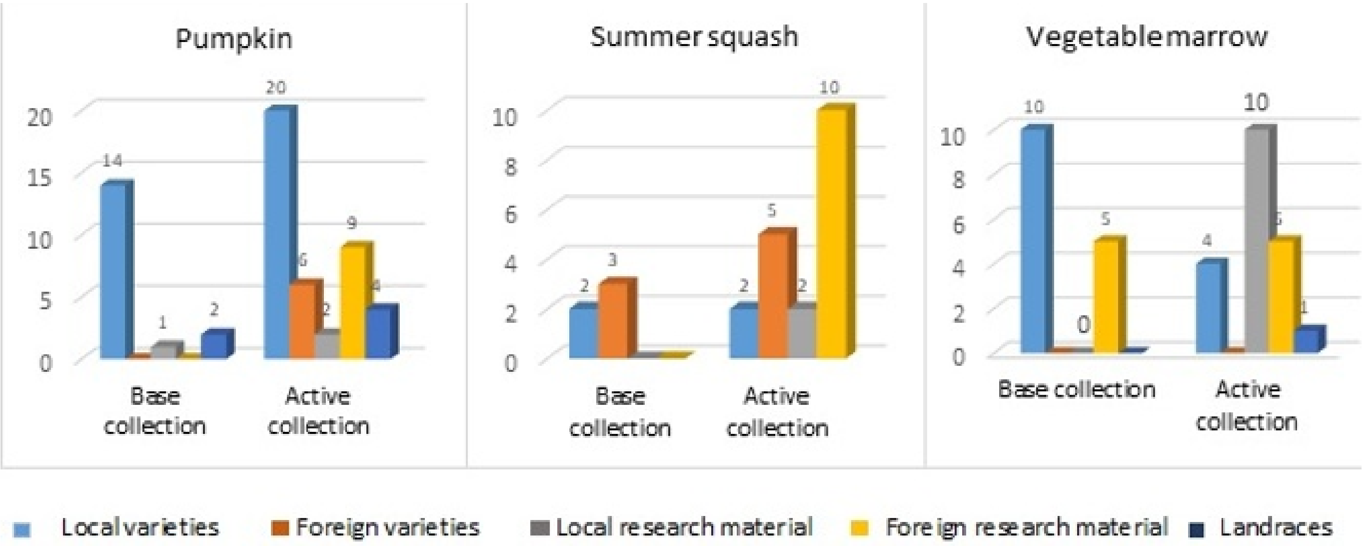

Figure 2. Numbers of accessions of local and foreign origin in base and active collections of pumpkin, summer squash and vegetable marrow at the Scientific Centre of Vegetable and Industrial Crops, Armenia.

of the collection through acquisition of genetic resources with genes linked to important agronomic traits such as disease resistance, stress tolerance and high nutritional value to expand the genetic spectrum of germplasm used in pre-breeding and breeding, and on efficient management of germplasm collections and relevant data through installation of an open-source program. The work of upgrading and rationalizing seed collections will continue for tomato, pepper and eggplant collections. It is planned to initiate the process of safety duplication of varieties of Armenian origin in the Svalbard Global Seed Vault upon finishing the upgrading and rationalization process for the entire vegetables collection.

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Supplemental data

Supplemental File 1: Abstract in Russian

Author contributions

AA established base and active collections, analyzed database information, wrote the manuscript; GS supervised the project on ex situ conservation, contributed to acquisition of germplasm; RB performed viability tests, selected seeds for *ex situ* conservation; TL collected seeds for *ex situ* conservation.

Conflict of interest statement

The Authors declare that no conflict of interest exists.

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