1. GENERAL PART

1.1. Garlic (*Allium sativum* L.) and shallot (*Allium cepa* L. *Aggregatum* Group), two important crop species, which need to be maintained vegetatively in plant collections

The genus *Allium* comprises about 800 species. This genus contains important vegetables, spices or medicinal plants and such species, which have potential interest as wild relatives of crops or are envisaged for future use because of their valuable characters.

Garlic is a multipurpose crop of high importance. It is used as vegetable, spice and, in increasing extent, also as medicinal plant (Koch & Lawson, 1996). For the year 2009, production of garlic worldwide was reported to amount to 22.3 million tons, only 773,200 tons of it in Europe (3.5 %) (FAOSTAT, 2011), although Europe maintains the highest diversity of this crop. Garlic production has a long tradition, reports on its culture date back to the antique period (Rabinowitch & Currah, 2002). Spreading out from its centre of origin in Central Asia, the introduction into Europe in ancient times led to a very high diversity of this crop, which is caused by the high extent of geographic diversity on this continent and many different cultures and traditions in its populations. This is reflected by the existence of different subgroups in this species, which are termed by many specific folk names in the various countries. This valuable diversity is very much endangered by invading garlic from China. This country dominates the world production to 80.6 % (FAOSTAT, 2011). The danger of a worldwide production of only a small number of genotypes within a crop species, the monoculture, has been experienced in the history already several times as, e.g., in potato and maize. A safe bank of most representative local garlic germplasm is, therefore, an essential tool to counteract this danger.

Shallot, botanically representing the vegetatively propagated part of the species onion, has also high impact as vegetable and spice worldwide. In the FAOSTAT database (FAOSTAT, 2011), shallot forms a component of the green onion category, thus, the data are not as transparent. In 2009, the world production of this crop group was 3.7 million tons. The production in Europe of 355,800 tons corresponds to 9.5 %. Though not to such an extreme extent, the endangerment of shallot's germplasm is similar to that of garlic, the market is dominated by China (23.7 %), Japan (15.2 %) and South Korea (14.4 %).

The high diversity of both crops is represented by a whole set of garlic and shallot collections in Europe. Europe's *Allium* germplasm maintenance is, like that of all the other crops, supervised and coordinated by the European Cooperative Programme for Plant Genetic Resources, ECPGR, which is structured into working groups within thematic networks. The *Allium* Working Group is one of them. A crop-specific database is coordinated by this group, the European *Allium* Database, EADB. Here all who are interested in passport data and other information about garlic, shallots and other alliums, can get information. A recent survey about vegetatively propagated alliums in Europe is given in the IPGRI Newsletter for Europe, which is downloadable on the homepage of Bioversity International (new name of former IPGRI - Keller & Astley, 2006).

Garlic is a crop which normally does not form seeds. It lost its fertility already long ago in its history. Although there have been some successful attempts to re-store fertility in the last years, and some more or less fertile material was found in Central Asia, the overwhelming part of garlic's germplasm must be maintained vegetatively.

Similar situation is present in shallot, which is also only partly fertile, and the breeding of which is mainly based on clonal material. Therefore, the maintenance of garlic and shallot was performed in the past by means of cultivation gardens. As an example, some impression on IPK's garlic and shallot field collection is given in the pictures below:

In IPK, the most garlic accessions are cultivated in field plots, which are replanted every four years (upper pictures). However, the garlic core collection is replanted annually (pictures below). Shallots are planted in spring and harvested in late summer. The bulbs are stored over winter in cool places under the roofs of the buildings.

However, due to abiotic (unfavourable weather, flood etc.) and biotic factors (mainly virus diseases and fungi), the existence of such field collections is endangered and a certain percentage of more susceptible accessions will disappear year by year. Therefore, more and more genebanks maintain garlic also by means of modern biotechnological methods such as *in vitro* culture and cryopreservation. These methods provide protection of the material against infection; they enable elimination of viruses from the plants and long-term storage of shoot tips without genetic changes. Since intensive studies were performed of all aspects of taxonomy including morphology, anatomy, cytology, phenology and molecular markers, which gives results only when originally collected material is used, the proportion of originally collected material is high in the European genebanks.

Because of the existence of a large collection in the IPK genebank, garlic's diversity was very well documented here. One of the main approaches has been endeavoured in IPK by Maaß and Klaas in 1995. They used isozymes and RAPD's and summarized that in dendrograms. Good correlation was found between the molecular grouping and morphological characters. The main results of the taxonomical research on garlic in IPK are:

1. One of the main morphological characters of garlic is the gradual reduction of the generative part of the plant, beginning with the loss of fertility within the still existing flowers until to the total reduction of the scape, which corresponds to, spoken in agricultural terms, the loss of bolting.

2. The garlic diversity consists of five main groups, which correlate with the geographical distribution of garlic's diversity.
3. The most original group, situated in the presumable centre of origin, is the *Longicuspis* group. This group is based on the former separate species *Allium longicuspis* Regel, which is clearly a part of the garlic germplasm, and the botanical name at species level is, therefore, no longer valid. This group has the highest degree of molecular diversity
4. The groups can be distinguished by morphological and molecular markers, the correlation of which is sufficiently high.

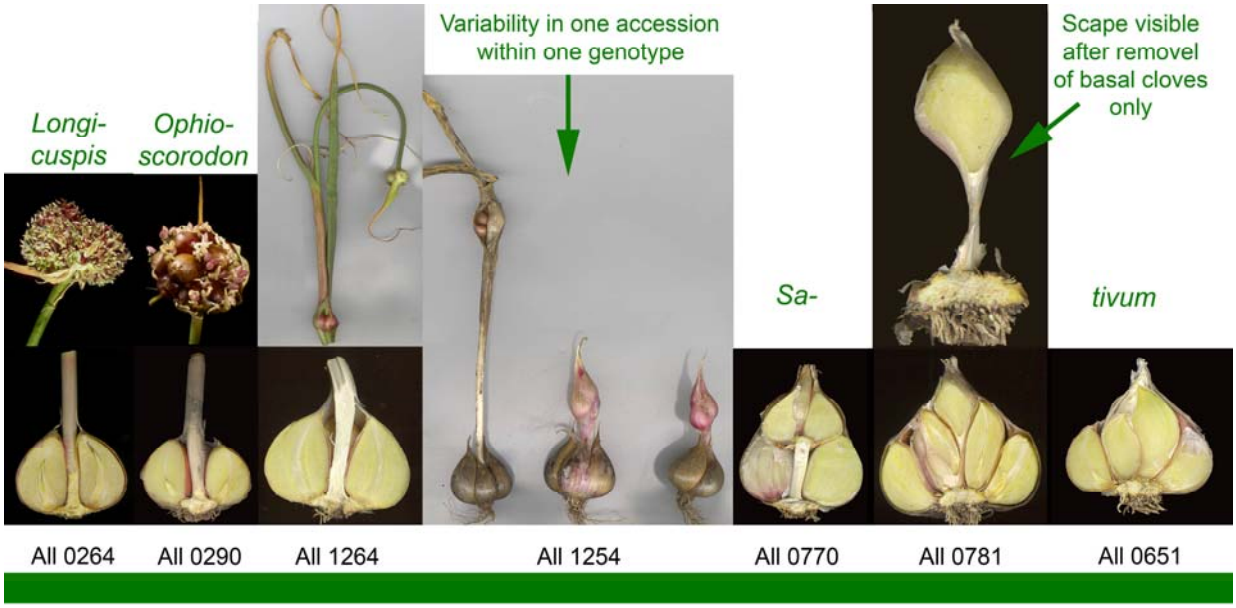
The classification of Maaß and Klaas (1995) revealed a good correlation with the geographical distribution of the groups. This grouping is searchable in this catalogue in all accessions which had been involved in the analyses. The terminology of the infraspecific taxa follows the approach of informal groups. Thus, the following groups are listed in the databases:
- *Longicuspis* group (Maaß & Klaas Groups I and IV) - Central and East Asia, where a special morphologically distinguishable subgroup, the *Pekinense* subgroup (Maaß & Klaas Group IV b p.p.) exists,
- *Ophioscorodon* group (Maaß & Klaas Group III) - from East to Central Europe
- *Sativum* group (Maaß & Klaas Group II) - from Near East through the whole Mediterranean Area (two subgroups exist according to the bolting behaviour, which is an important agronomical character)
- Subtropical group (Maaß & Klaas Group V) - mainly in South and Southeast Asia and of lower relevance for the European collections.

Besides the molecular markers, morphological characters are still the main indicators for the infraspecific diversity. This is also true for garlic. The garlic specialists took part in the re-formulation of the descriptors of garlic, which are summarized, like for many other crop species, in a descriptor list, which is downloadable from the website of the International Plant Genetic Resources Institute IPGRI under Descriptors for *Allium* spp. (IPGRI et al., 2001). These descriptors are also the backbone for the characterisation of the accessions in this catalogue. Some deviations may, however, occur.

As an example, how the phylogeny of garlic corresponds to the infraspecific grouping, the bolting behaviour of the plants is described here. It is thought that garlic, during the history of its cultivation, lost gradually its generative reproduction strategy by permanent positive selection of the vegetatively most productive plants which are clearly those with smaller or no inflorescence stalk.

The inflorescence itself changed from structures with flowers and many small bulbils to a lower number of larger bulbils accompanied by total loss of flowers. Then, the stalk shortened more and more, ending finally in an inclusion of the inflorescence into the bulb. The bulbs appeared more and more clove-like. The resulting structure is then an irregular compound bulb with no separable inflorescences at all. One of the intermediate types of this evolutionary line is forming even bulbils in several, more than one, levels of the stalk.

And these are real examples of the gradual reduction of the inflorescence:



The infraspecific groups of the Isozym/RAPD classification according to Maaß and Klaas (1995) are explained here with respect to their correspondence to the morphological characterization:

The *Longicuspis* Group (I and IV)
Bolting and coiling scapes, small bulbils, numerous flowers with exserted anthers, inner filaments with two or four lateral teeth. Many accessions produce more or less fertile flowers in dependence on the wheather conditions. Ia and IVe correspond to Pooler & Simon's 'fertile, pollen-shedding *ophioscorodon* types'. Ic correspond to Pooler & Simon's 'yellow-anthered pollen sterile *ophioscorodon* types'. The description is in agreement with that of *A. longiscuspis* sensu Regel 1875 in Vvedensky (1935), Wendelbo (1971) and Kollmann (1984).

The *Pekinense* Subgroup
Not distinguishable with the markers so far used by Maaß & Klaas from the *Longicuspis* Group and localized in group IV b. Height 40-75 cm, relatively broad leaves, non-coiling inflorescence stalk, few relatively large bulbils, a very long and often non-opening spathe.

The *Sativum* Group (II)
IIa/b: Bolting and coiling (IIa corresponds to group V, IIb corresponds to group I according to Messiaen et al. [1993], bolting later than other groups, flowers do not open under our conditions). IIc/d: Incompletely or non-bolting (correspond to group III after Messiaen et al., [1993] and 'non-bolting *sativum'* type of Pooler & Simon [1993]).

The *Ophioscorodon* Group (III)
Corresponds to group IV after Messiaen et al., (1993) and 'early senescing, non fertile flowering type' of Pooler & Simon (1993). Bolting and coiling, colour of spathes whitish-green, flowers often deformed, being four-merous instead of three-merous, inner filament usually with two lateral teeth, the outer with four, yellow anthers, sterile pollen, spathe remains closed in some types.

V - Subtropical group
No bolting observed in our conditions (presumably because of too weak growth).

Key to the infraspecific formal groups of garlic

1. Complete plants, bolting and coiling of the upper part of the unripe scape, inflorescence complete, spathe slender, numerous small spindle-shaped bulbils, well-formed flowers tending to be fertile (***Longicupsis* type**; isozyme/RAPD types I and IV [IVb p.p.])
2. Plants tending more or less to reduction of the generative part (in cases of stronger reduction also changed bulb structure)
   2.1. Spathe very long, inflated; few large bulbils, shorter scapes without coiling or with simple curvature, some plants with incomplete bolting (***Pekinense* type**; isozyme/RAPD type IVb p.p.)
   2.2. Spathe not inflated, if long, then slender, or inflorescence not visible
      2.2.1. Well-expressed scapes
         2.2.1.1. Scapes coiling in unripe stages, few large bulbils; few, weakly developed or deformed flowers (***Ophioscorodon* type**; isozyme/RAPD type III)
         2.2.1.2. Scapes coiling or with simple curvature, numerous small, then mostly spheroidal, or few larger bulbils, flowers do not open o rare malformed (***Sativum* type, bolting;** isozyme/RAPD types IIa/b)
      2.2.2. Incomplete or no bolting, sometimes single plants with inflorescences, irregular bulb structure (structure type VI) (***Sativum type, non-bolting***; isozyme/RAPD types IIc/d)

Morphological classification of shallot is much less documented. The main fact is that shallot forms a part of the genepool of *Allium cepa* and those fertile shallots may be freely crossed with onion. The mainly differing characters which are, however, not as suitable for sharp distinction are the branching tendency, the concomitant tendency of vegetative multiplication and tendency to lose ability to flower, which are connected with the clonal breeding strategy of shallot. At present, this border is more and more blurred by breeding of true seed shallots. At present, based on the existing slight differences, shallot is divided as *Aggregatum* Group from the onion proper (termed as Common Onion Group). There is some grouping within shallot into shallot *s. str*. and potato onion (also called multiplier onion). Some authors describe potato onion as being larger than shallot and possessing round or flat-round bulbs growing together in smaller groups. Shallots s. str., however, have elongated bulbs in dense clumps of higher bulb numbers (Fritsch & Friesen, 2002). Furthermore, there is a tunica formed from remainders of old scales, which remains longer in shallots than in potato onion and even persists during harvest (Gladis, 2000).

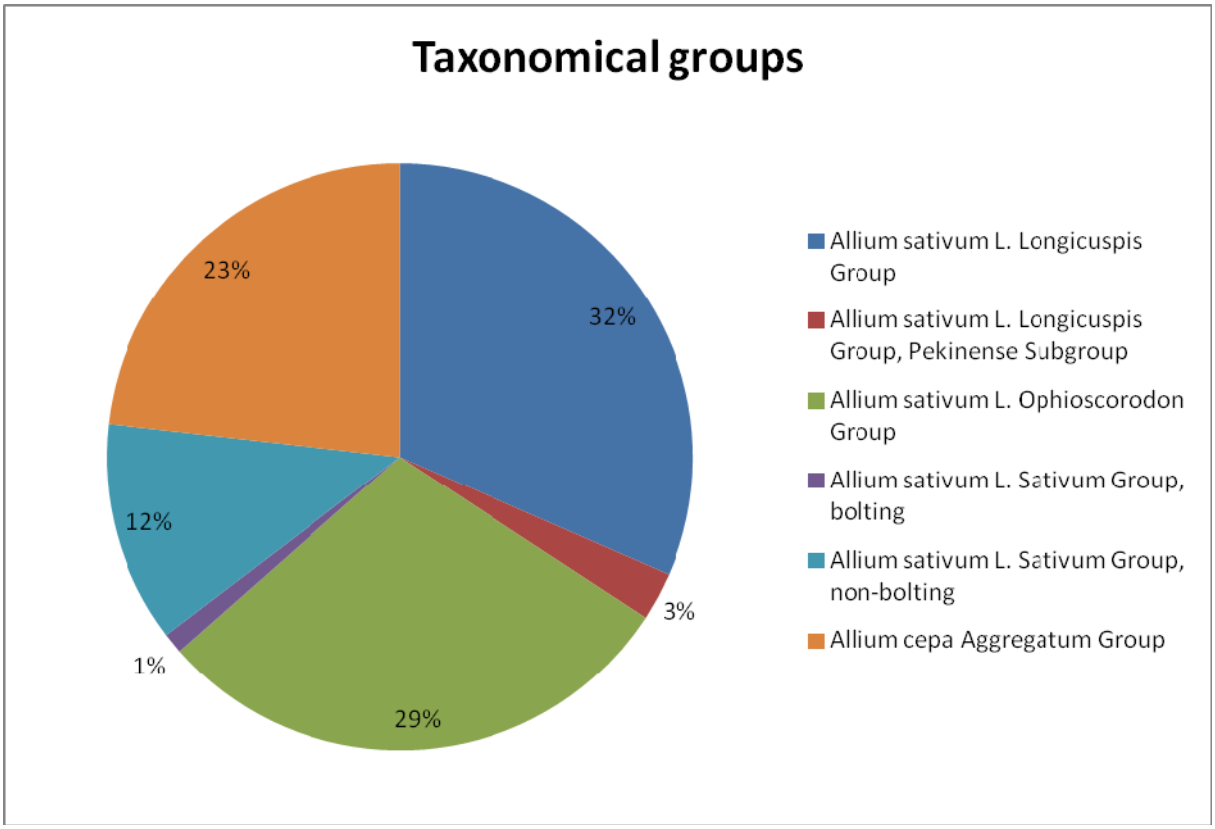
At present, examples for this grouping can be given under reserve only, because the characterization is not completed yet:

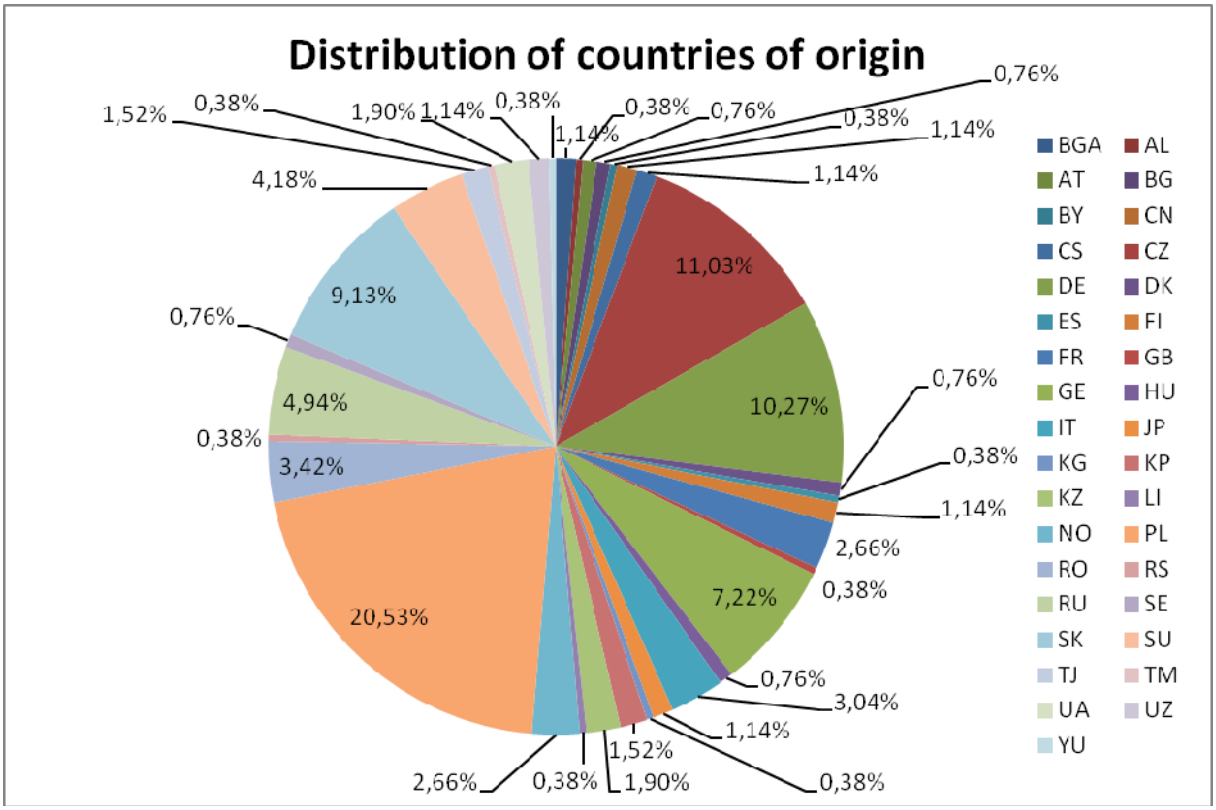
Potato onion All 591 (IPK collection)

Shallot All 609 (IPK collection)

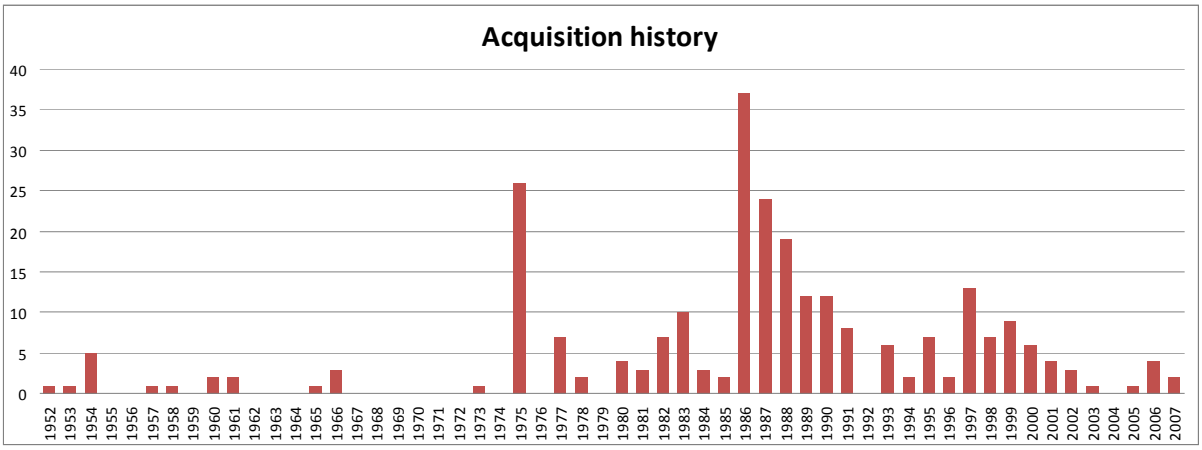
In large genebanks, it is not possible to treat all the material with the same intensity. Therefore, a part of the accessions is selected and arranged as a Core Collection (Van Hintum, 1996, 1999). The strategy to form core collections in order to facilitate the use of genebanks is one of the main actions in the FAO Global Plan of Actions agreed at the Leipzig Conference on June 17-23, 1996. There it is Activity 9: Expanding the characterization, evaluation and number of core collections to facilitate use. This is not only useful for the internal management but also for a broader external use insofar, as various users are recommended to rely on the same material, which will increase comparability of results in science. There are various aspects and parameters to select a Core Collection. It may be that breeding questions determine the scope of the ensemble or a special character like interesting chemical constituents or disease resistances. It may also be the greatest diversity within a given taxon. This was also one substantial aspect for the selection of EURALLIVEG's Core Collection presented in this catalogue. Another, more practical aspect, at least for garlic, was the performance of a given accession in cryopreservation. Only material was included whose regeneration rate was better than 30 % (related to the number of introduced explants) in a control of 25 explants, representing 125 explants introduced into liquid nitrogen, or better than 10 % in a sample of double this size. Finally, the EURALLIVEG collection is delimitated on material from the own country of the collection holder, countries with respective agreements with the collection holders or collected in an Eurasian country until end of the year 1993 in case of no agreements in order to meet the restrictions set by the Biodiversity Convention (CBD).

Some surveys of the EURALLIVEG Collection as it has been established until end of the project in summer of year 2011 (263 accessions) is given in the following diagrams: pie chart of sub-taxa, pie chart of countries of origin and history of acquisition of accessions:





Rem.: Country abbreviations according to the international standard ISO 3166 (ISO, 2011); BGA – initial origin unknown - material from botanic gardens



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1.2. Short history of EURALLIVEG (Vegetative Allium, Europe's Core Collection, Safe and Sound)

EURALLIVEG (abbreviation for EURopes ALLIums VEGetatively propagated) was a project granted by the European Commission, Directorate-General for Agriculture and Rural Development, under the Council Regulation No 870/2004 under the project number AGRI GEN RES 050. Its duration was from April 1, 2007, until March 31, 2011.

AGRI GEN RES is the abbreviation of the programme 'Genetic resources in agriculture'. This programme promotes genetic diversity and the exchange of information including close co-ordination between Member States and between the Member States and the European Commission for the conservation and sustainable use of genetic resources in agriculture. It facilitates also co-ordination in the field of international undertakings on genetic resources. The total budget allocated to this programme amounts to 10 million Euro. Please read further details on the website of the GENRES programme. In the frame of this programme, it is a targeted action whose total costs amount to 1.089.000 Euro including co-funding by EU of 544.500 Euro.

Before EURALLIVEG started, several other activities had been performed in this field. Various members of the ECP/GR Group were full partners in a first project GEN RES #20, regulation 1467/94 (1996-2000), which involved all aspects of genetic resources work on onion/shallot, garlic, leek and chives, including documentation, characterization, regeneration, evaluation, core collection development and the collection of material to fill gaps in the collections. This project was the fundament for all *Allium* crops, which was especially useful for the rationalization of onion and leek resources and for the development of a worldwide usable guide for morphological descriptors, documented by IPGRI. The routine application of in vitro propagation and virus elimination was established which resulted in a first European Garlic Core Collection, shared between Germany and Spain. In the Agriculture and Fisheries Programme (FAIR) implemented (4th Framework), a project about onion breeding (1996-2001) was performed, and another project was funded by the Programme Quality of Life and Management of Living Resources (5th Framework), "Garlic & Health" (1998-2003). The latter contributed considerably to the knowledge about the genetic resources of garlic. Later, *Allium* was one of the four model cases of the project AEGIS (2004-2006). Herein, the focus was again on vegetatively propagated alliums with the justification by the distinctly higher labour and cost requirements of this germplasm. Because AEGIS was initially a project establishing proposals for model cases in genetic resources but not covering their implementation, this project provided the background for the project EURALLIVEG. Thus, the idea to create a Tripartite Cryopreservation Collection comprising the AEGIS designated Most Appropriate Samples and their safety duplicates is a product of the AEGIS discussion. It was adopted by the last *Ad hoc* Meeting of the *Allium* Working Group 2007 in Prague. The EURALLIVEG proposal was a consequence of a Europe-wide discussion of genetic resources rationalization strategy concerning vegetatively propagated alliums. Whereas research has been done in the past in part supported by EU grants, the present project was the first opportunity to apply previously elaborated biotechnological methods in a large-scale routine action on the European level.

Plant germplasm which has to be propagated and maintained vegetatively is the most expensive part of all the material held in genebanks. Therefore, projects to rationalise this part of the genetic resources will have high impact on costs and labour requirement for the management of living plant collections. Garlic and shallot are such crops held in Europe in several genebanks. They are the target species of this project.

To use efficient alternatives to the laborious field cultivation which is at risk by many biotic (pests and diseases) and abiotic (flood, drought, cold winters) factors, new ways of modern biotechnology were used in this project. Cryopreservation formed the core activity of the project. It was organized in a Cryobanks Network, initially formed by three partners, the Czech, German and Polish genebanks, and being open for joining of other institutions. To get right material in this base collection, the accessions are unique and meet requested criteria of a Most Appropriate Accession (MAA) also called European Accession.

The objectives of the EURALLIVEG project were:

Main objective:
Establishment of European Core Collection of vegetative alliums, covering garlic including molecular characterization, cryopreservation and virus elimination, and molecular characterization of shallot.

Specific Objectives:

- Use European Allium Database to screen garlic and shallot germplasm.
- Screen 1600 garlic and 550 shallot accessions for redundant duplication by general molecular marker system.
- Confirm interrelationships by morphological character lists.
- Develop a structured Core Collection under elimination of redundant duplicates.
- Cryopreserve the 200 most important garlic accessions using vitrification.
- Exchange safety duplicates of cryopreserved garlic to establish the Tripartite Cryopreservation Genebank
- Disseminate CGP documents to facilitate joining other European partners.
- Virus elimination to free 125 most important garlic accessions from viruses, prove of virus-free state.
- Conclusions for future expanding preservation from garlic to shallot and other vegetative alliums.

In course of this project four major, four smaller meetings and three training courses were held.

Major meetings
1. Start-up meeting 12.-13.4.2007, Gatersleben, Germany
2. Meeting 8.-9.7. 2008, Potenza, Italy
3. Meeting, 9.-10.3. 2010, Skierniewice, Poland
4. Final Meeting 1.-2.3. 2011, Prague, Czech Republic

Smaller meetings:
1. Special Work Packages coordinators' meeting 6.3.2008, Wageningen, Netherlands
2. Technical Meeting 4.12. 2008, Prague, Czech Republic. Objective: Improving *in vitro* culture, cryopreservation and virus elimination
3. Technical Meeting 27.1. 2008, Gatersleben, Germany. Objective: Drawing conclusions from the molecular marker analyses

4. Technical Meeting 23.10. 2009, Skierniewice, Poland. Objectives: Improving *in vitro* culture, cryopreservation and virus elimination

Training courses in IPK Gatersleben, Germany:
1. Training course 16.-27.4. 2007. Techniques of cryopreservation for RIVC, Poland
2. Training course 21.5.–1.6. 2007. Techniques of virus elimination for UNIBAS, Italy
3. Training course 31.3.–4.4. 2008. Techniques of micropropagation and cryopreservation of *in vitro* plants for CRI, Czech Republic

Due to some technical and biological reasons, not all parameters were completely fulfilled. However, the most important objective, namely the establishment of a collection whose accessions are ready for labelling as European accession within the AEGIS strategy was attained. The product of this work is summarized in the special part of this catalogue.

1.3. Introduction into the Collections Participating in EURALLIVEG

The *Allium* collection of P0, IPK Gatersleben, Germany

The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben is a large and internationally renowned plant research centre working on problems in modern biology by focusing on cultivated plants. It is a non-university research centre associated with the German Leibniz Gemeinschaft (WGL). Since its foundation on 1 January 1992, it has carried forward the scientific work in the fields of basic and applied research pursued by the former Central Institute for Genetics and Crop Plant Research. The foundation this institution dates back to 1943. From this beginning, its basic, application oriented and interdisciplinary research seeks to collect new knowledge and to find new technologies aiming at the extensive use of plant genetic resources for optimised nutrient production and environmentally friendly agriculture. The central *ex situ* gene bank contains more than 2,700 botanic species of about 800 different genera. The living collection currently totals approximately 147,000 specimens. The genebank accessions are reproduced in the fields (18 ha) and in various greenhouses (6000 m^2). The main bulk of the germplasm is stored as seeds. Seed storage is managed in large cold chambers, maintained at 0 or -15 °C. Seeds are kept in glass jars, covered with bags containing silica gel.

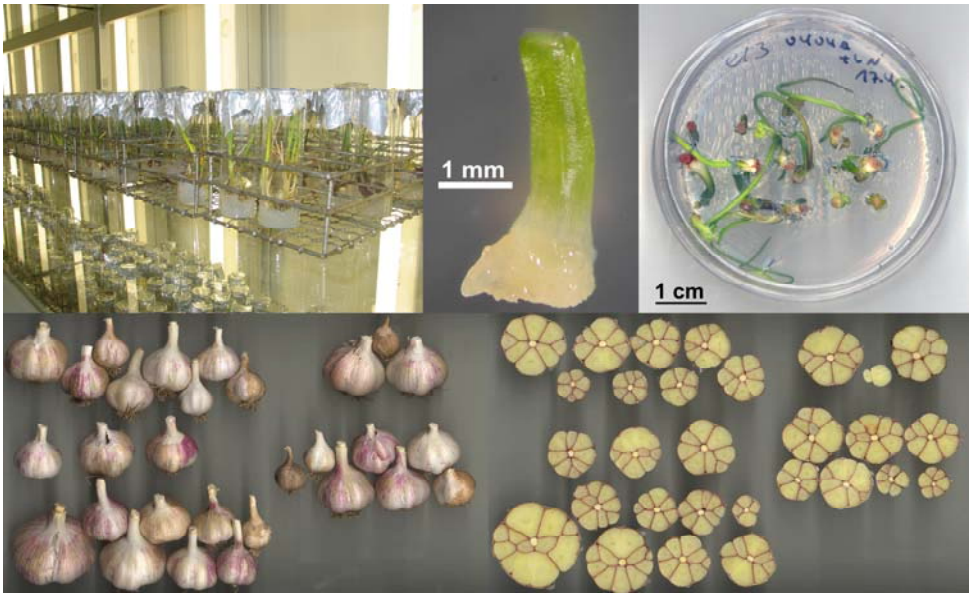
The *Allium* collections of IPK consist of two parts Taxonomic *Allium* Reference Collection and the Genebank Allium Crop Collection. The Taxonomic *Allium* Reference Collection amounts to about 1800 ones finally determined which belong to 340 *Allium* species and subspecies. The number of original samples is 1500. They were either collected by the IPK staff or obtained from botanic gardens. They are regarded as most original samples because of their origins. The collection is managed by a special contract gardening service in a special reference garden. A database was established which contains head data and taxonomic affiliation of more than 3600 definitely determined accessions which are or were ever present in IPK. It shows up to five pictures per accession.

The genebank *Allium* Crop Collection consists of 1400 accessions both seed-propagated (onion, leek, bunching onion, chives, some wild relatives) and vegetatively maintained (garlic, shallot, great-headed garlic, top onion, hybrids etc.). The main bulk of garlic and other *Allium* crop accessions are cultivated in field plots, which are replanted every four years. However, a special part, IPK's garlic core collection, is replanted annually. Shallots are planted in spring and harvested in late summer. The bulbs are stored over winter in cool places under the roofs of the buildings. The focus of the collection of vegetatively propagated allium is concentrated on German local landraces (mainly in the frame of a campaign in East Germany in 1975) and on collaborative activities with other countries (e.g. on the basis of an agreement with Georgia).

The IPK genebank maintains garlic also by means of modern biotechnological methods such as *in vitro* storage and cryopreservation. Micropropagation, isolation of small explants for cryopreservation and recovery of small plantlets from this storage (upper row) led to fully developed healthy material. In August 2011 the total number of garlic accessions in cryopreservation amounted to 94, amongst them 61 from the EURALLIVEG project. Fifty of them are in virus-free state, which was attained by meristem culture and proven by ELISA indexing.

Upper row: field plot in the main *Allium* collection. Lower row: plots of the Garlic Core Collection.

Shallot collection



Laboratory collections: Upper row: *in vitro* storage (left), explant for cryopreservation (middle), Petri dish with plantlets regenerating from cryopreservation (right); lower row, material from a cryopreservation experiment in the second year after regeneration - left bulks control, right bulks after liquid nitrogen storage

The *Allium* collection of P1, CRI Prague Ruzyne, Workplace Olomouc, Czech Republic

The Centre of Applied Research of Vegetables and Special Crops of the Crop Research Institute, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Olomouc is the holder the germplasm collections of vegetables, medicinal, aromatic and culinary plants (MAPs). The collections have had very long and rich history in Olomouc since 1951. The bases of collections are originated from the Research Institute of Vegetables Growing and Breeding in Olomouc (RIVGB). This institute was founded in 1951 and abolished in 1994. In 1994, the Department of Genetic Resources, RIVGB, in Olomouc was affiliated to the Crop Research Institute (CRI) as the Department of Vegetables and Special Crops. Since 2010 this department has been part of the Centre of the Region Haná for Biotechnological and Agricultural Research. The Department has the germplasm collections of vegetables crops, which are typical for Central Europe: *Allium, Beta, Brassica, Capsicum, Cucumis, Daucus, Lactuca, Lycopersicon, Phaseolus, Pisum* and medicinal, aromatic and culinary plants of the genera *Carum, Hypericum, Lavandula, Ocimum, Origanum, Plantago, Salvia etc*. Currently the department maintains 9268 accessions of vegetables and 884 of MAPs. The sum amounts to 10152 accessions. It presents 20 % of all accessions in the Czech Republic.

The Department maintains the germplasm collections of both vegetatively and generatively propagated *Allium*. The collection of vegetatively propagated alliums has two parts – garlic and shallot. The collection of garlic is divided to three parts according to their ability to produce scapes: group of bolting garlic: 327 accessions, group of non-bolting garlic: 212, and group of semi-bolting garlic: 108 accessions. The actual collection comprises material from 30 countries of origin. The important part of the collection represents the old garlic landraces collected in the White Carpathian Mountains and in Southern Moravia as well as the advanced Czech varieties. Wild *Allium* species and primitive forms of garlic from Central Asia and Siberia were collected during international collecting missions in the Central Asia (1988) and West Siberia (1990). The shallot collection has 124 accessions. The main part of collection contains material from Scandinavia (Norway and Finland) and from the Czech Republic. The collections are maintained as field collection. Cryopreservation is used as one method of safe duplication.

From 2002, all collections have been described according the Descriptors for *Allium* (IPGRI, 2001). Passport data of collections are on-line available on the web application EVIGEZ (Catalogue of Plant Genetic Resources in the Czech Republic) http://genbank.vurv.cz/genetic/resources/. The passport information is included also in the ECPGR European Allium Database. The collection cooperates with other genebanks within the framework of ECPGR and takes part in national and international projects.

DNA preparation in the year 2008

Freeze-drying of samples Bulb storage Disinfection

Garlic field collection

Harvest of bulbils and bulbs

Garlic and shallot in isolation cages

The *Allium* collection of P2, Institute of Horticulture Skierniewice**, Division of Vegetable Crops, Poland**

The Institute of Horticulture, Division of Vegetable Crops, is the main research unit in Poland developing the scientific and practical bases for production of vegetable crops in the field and under cover as well as for mushrooms. Most of the research work carried out at RIVC is related to priority areas of agricultural research defined by the Ministry of Agriculture and Rural Developments including the following subjects: genetics, breeding and biotechnology; technology of vegetable production in open field, under cover and mushroom growing; plant protection against diseases, pests and weeds; technology of vegetable storage, processing and evaluation of quality and biological value. The Institute is responsible for the programme of plant genetic resources of vegetable crops in Poland, which is a branch of the national genebank. This programme includes the seed collection of around 10,000 accessions representing genetic resources of 70 vegetable species and maintaining over 1700 accessions of vegetatively propagated species in field collections.

The collection of genetic resources of garlic (*Allium sativum* L.), established in 1986, is maintained at the Research Institute of Horticulture in Skierniewice, Poland (former name until end of 2010: Research Institute of Vegetable Crops - RIVC).The base collection includes 448 accessions of garlic: 212 non-bolting garlic accessions suitable for spring growing season and 236 accessions of bolting garlic for autumn growing season. The garlic accessions originated from 23 countries. The collection of garlic germplasm consists of two parts:

- collection of garlic landraces located in southern part of Poland,
- collection of garlic clones derived from landraces located in Skierniewice

The accessions of garlic, after initial multiplication, are included in 3-year trials (3–4 replications) to characterise and evaluate their economic value. After a 3-year research cycle, the accessions are maintained in the field collection in one replication (50 -100 plants per accession). Characterisation is conducted according to the descriptors for *Allium* developed by IPGRI (IPGRI et al., 2001).

Bolting garlic accessions revealed great variability of the weight and number of bulbils and also flower number in the inflorescence. On the base of measurements of these parameters in bolting garlic accessions, five classes for weight and number were distinguished. Because these traits are stable over years, they can be used as criteria four grouping accessions in collections. Twenty-one types of cloves arrangement on transversal cut of the head were observed in collected garlic accessions of both forms.

The annual maintenance of the vegetatively propagated garlic collection in the field causes phytosanitary troubles and high costs. Therefore, it is necessary to search for alternative methods of long term storage of the most valuable garlic germplasm. With this objective, cryopreservation using the vitrification method is applied in the garlic research program.

The main building of the institute

The field collection of garlic

Clean bench and culture cabinet

In vitro cultures after regeneration from cryopreservation

The *Allium* collection of P3, Department of Sciences of the Systems Crops, Forest and of the Environment, University of Basilicata - Faculty of Agriculture (UNIBAS), Italy

The need to develop work on conservation of crop genetic diversity in the form of traditional varieties or 'landraces' is emphasized in many of international scientific organization (Convention of Biological Diversity, Agenda 21, International Treaty on Plant Genetic Resources for Food and Agriculture), all of which confirm the conservation as an essential component of sustainable agriculture and seek to improve the well-being of present and future generations of people.

As a part of the University of Basilicata (UNIBAS), a new Italian university place born in Southern Italy on 1980, operate the Crop System, Forestry and Environmental Sciences Department involved in teaching and researching in agricultural field. Among various activities, a small group of stakeholder scientists, since then, started to collect garlic (*Allium sativum* L.*)* accessions around the university place first and then in regions close to it, in which garlic clones are cultivated by growers mainly in home gardens and less in open fields, as an aromatic vegetables from long time ago in the past.

They are traditional crop varieties, generally known as 'landraces', 'farmer varieties', 'local varieties', 'primitive varieties' that have been continuously maintained by growers within their local biological, cultural and socio-economic context. The vegetative propagation habit of garlic has been contributed to enhance the biodiversity of the species especially in Southern Italian variegated lands. At this moment the Italian garlic collection account more than 110 accessions collected mainly in Basilicata, Apulia, Calabria, Sicily, Sardinia and Campania regions and some of them have been acquired in the other Italian regions or, in some cases, from abroad.

The collection held by UNIBAS as a curator is open with the aim to collect all the Italian garlic biodiversity yet available. The most important accessions from the collection are reported in this catalogue.


Local small garlic field in Southern Italy Garlic field collection in June 2007



Garlic in storage


Garlic diversity presented in the EURALLIVEG meeting


Virus-free plantlets for the EURALLIVEG project *in vitro* (left) and in pot culture (right)

The *Allium* collection of P5, INRA Station Ploudaniel, France

The Joint Research Unit for Plant Genetics and Biotechnologies (UMR-APBV) of INRA, Agrocampus Ouest and University of Rennes 1 is located at three sites in Le Rheu, Rennes and Ploudaniel. A total of 130 people are employed in the UMR-APBV among which 30 % are researchers and engineers.

The aim of the UMR-APBV is to obtain, communicate and exploit knowledge of the genetics, genomics and postgenomics of major crops (rapeseed, pea and potato). The activities of the UMR-APBV include the development and protection of biodiversity, the identification of genetic determinants for characters of agricultural interest, the transfer of results obtained from model species (*Arabidopsis*, *Medicago truncatula*, rice), and the assessment of the impacts resulting from the breeding innovations.

Research in the unit is divided into four main areas of scientific activity:

- Biodiversity and polyploidy
- Resistance to bio-aggressors
- Rapeseed oil yield under nitrogen and water constraints
- Innovative plant material

The research that is conducted in Ploudaniel focuses on genetics, breeding and genomics for disease resistance in potato. The steps in the process include exploitation of genetic resources, study of the genetic factors involved in disease resistance, study of the durability of these genetic factors, development of molecular markers useful in marker-assisted selection.

The research team is also responsible for genetic resources conservation of different vegetatively propagated species: potato and its related species, shallot and garlic, *Tulipa* and *Gladiolus* and of *Brassica* species.

The *Allium* collection maintained in Ploudaniel consists of French garlic and shallot landraces and varieties. It is vegetatively propagated in the field. Passport data of these genotypes are available in the ECPGR *Allium* database. The accessions have also been characterised for a few plant descriptors.

Numbers of accessions in the collection:

Shallot	*A. cepa Aggregatum Group*	Long type : 24 accessions Half-long type : 10 accessions Round type : 24 accessions
Garlic	*A. sativum Sativum Group*	French traditional cultivars : 21 accessions French varieties: 3 accessions European collection: 14 accessions Far-East collection: 3 accessions
	A. sativum Longicuspis Group	24 accessions


Institute’s building


Greenhouses

Shallot field collection

The *Allium* collection of P6, Nordic Genetic Resource Center (NordGen), Alnarp, Sweden

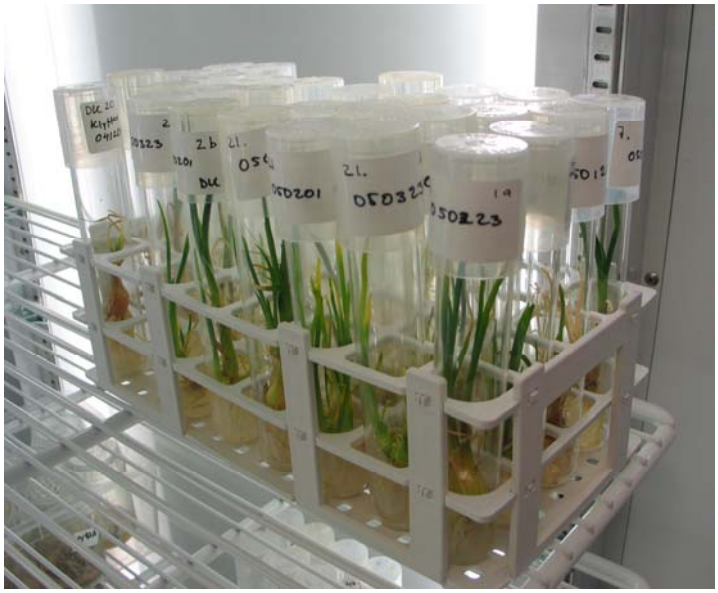
The Nordic Genetic Resource Center (NordGen) is a Nordic organization dedicated to the safeguarding and sustainable use of plants, farm animals and forests. The Nordic countries have been co-operating for more than 30 years on conservation of genetic resources. NordGen was established in January 2008 as a result of a merger between the Nordic Gene Bank, the Nordic Gene Bank Farm Animals and the Nordic Council for Forest Reproductive Material. NordGen is mainly financed by the Nordic Council of Ministers.

NordGen's primary task is to contribute to securing the broad diversity of genetic resources linked to food and agriculture. This is done through conservation and sustainable use, solid documentation and information work and international agreements. Our goals are to secure genetic diversity for agriculture and forestry in the Nordic countries and to promote in a visible, pro-active and effective way, the Nordic co-operation on sustainable conservation and use of genetic resources for agriculture, forestry and food production.

NordGen runs the genebank for seeds and seed potato for the five Nordic countries (Sweden, Denmark, Norway, Finland, and Iceland). The Nordic collaboration is carried out by crop-specific working groups with specialists from each nation. In total there are more than 30,000 unique seed accessions maintained in NordGen's seed collection. The collection is split into two components, the ordinary collection and the special collections. The ordinary collection consists of breeding lines, old landraces and cultivars and their wild relatives, while the special collections consist of material derived from different research projects. The collections of the Nordic vegetative alliums are in responsibility of the national programs. These *Allium* accessions are kept in national clonal archives run by each nation. NordGen facilitates a back-up of *in vitro* material of shallots and some other crops.


NordGen's buildings

Seed store of NordGen

In vitro storage of shallot

1.4. Phylogenetic trees developed in the project

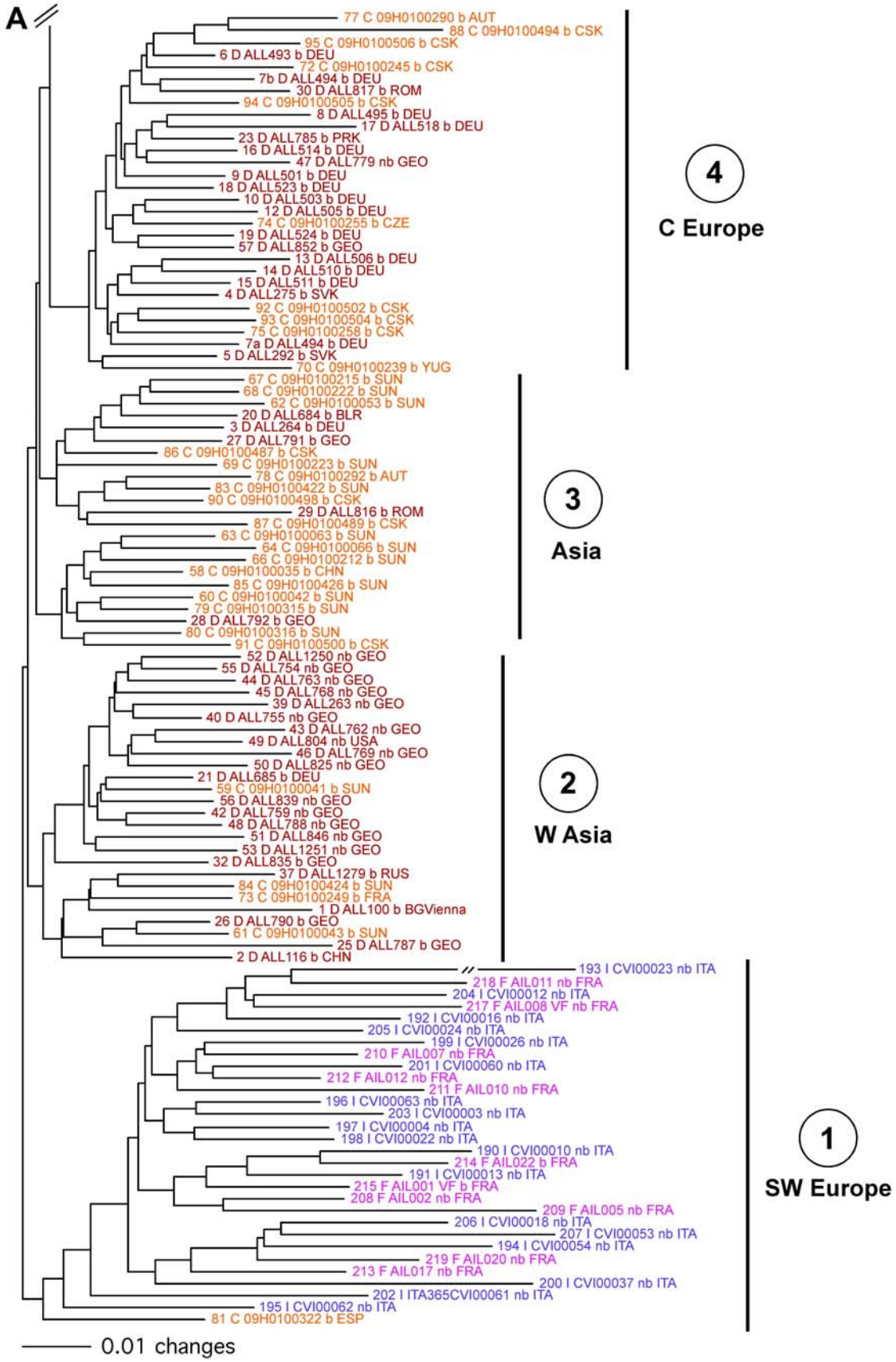
Since unfortunately, the SNP analysis failed, which was intended to be performed within the project, a limited analysis of the structure was performed in a subset, which is almost completely congruent to the collection presented in this EURALLIVEG catalogue. In the field “Further remarks” of the special part, it is indicated for every description of the respective accessions whether it was included in the analyses or not.

The AFLP tree of the 214 analysed garlic accessions shows 6 distinct geographical clusters. Within the Southwest European group (group 1) the accessions from partner P3 and P5 were found, which were, with two exceptions from France, all non-bolting (“nb” in accession name in the figure). They are mixed amongst each other within this group and show relatively high genetic diversity. In the groups, coming mainly from West Asia (group 2) and Asia (group 3), the accessions clustered closer. The West Asia group is divided into two subgroups which cover a smaller part of the bolting accessions (“b” in accession name) and a bigger part of non-bolting accessions mainly from Georgia, given by P0 into the analysis. Within the Asian group, bolting accessions clustered in three subgroups, which were mainly contributed by P1. They originated mainly from Russia. Only five accessions given by P0 were found within this group. In group 4, bolting accessions from Central Europe clustered in two main subgroups. They were introduced by P0 and P1. Within group 5, bolting accessions from East Asia clustered together with Eurasian accessions. Again these materials were introduced by P0 and P1. The genetic diversity is higher in this group 5 than in the other three groups (2, 3 and 4), in which also accessions from P0 and P1 clustered. Within the last, very big group 6 the genetic diversity is comparable to that of group 1. But nevertheless, five subclusters can be seen. In the first and second subgroups mainly bolting accessions from Poland, Uzbekistan and some of the Czech Republic grouped together. The third subgroup comprises non-bolting accessions originating mainly from Poland and Romania, which were closer related to each other than the bolting accessions. These accessions were also sent by P1 and P2. In the next subgroup mainly non-bolting accessions, sent by P1, clustered together, which had originated from the Czech Republic and Russia. Also two non-bolting accessions from Italy were found in this group. Within the last subgroup the distribution of the bolting accessions, sent by P1 and P2, was much less dense than that of the other four subgroups.

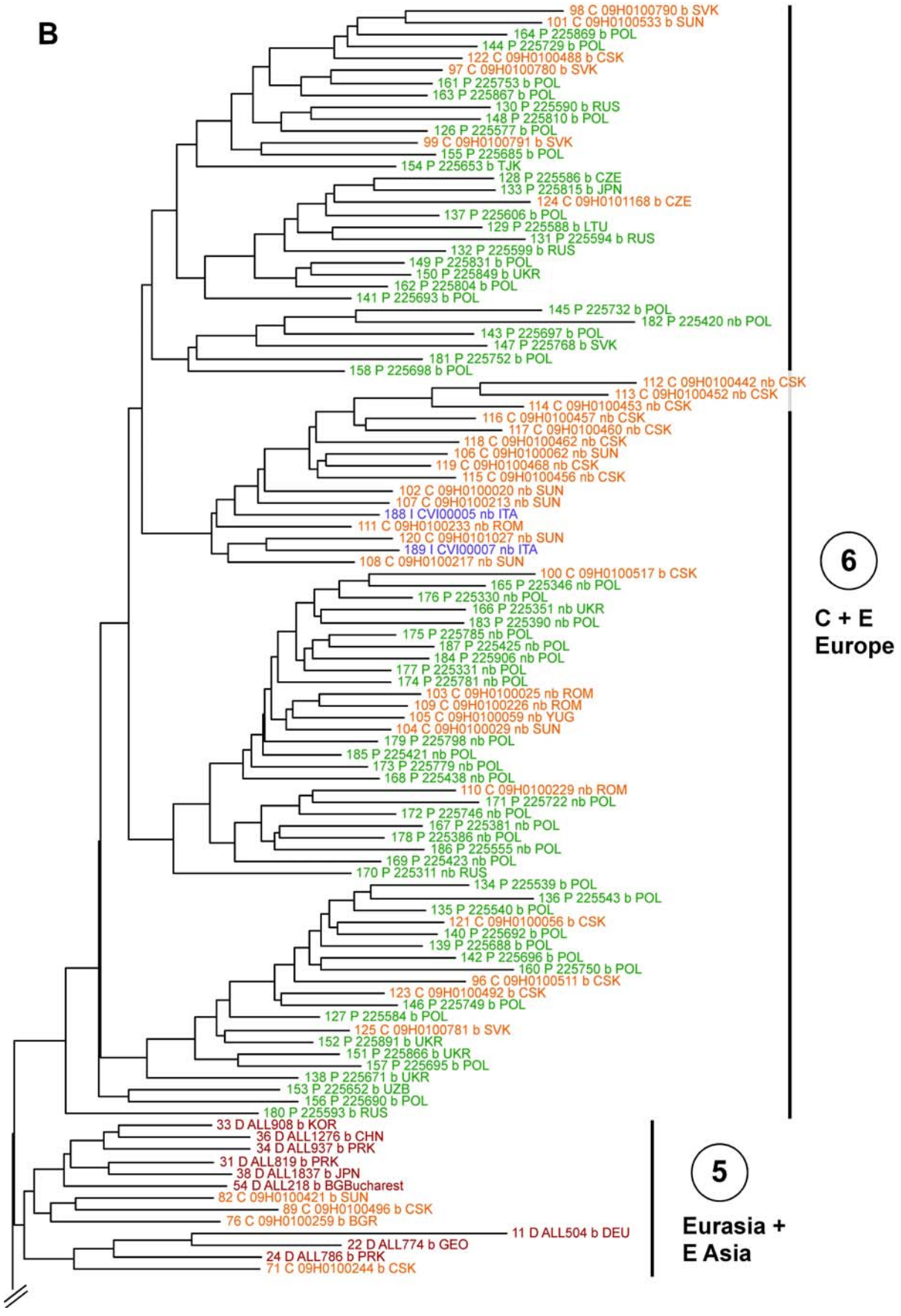
In general, the neighbour-joining tree of the garlic showed clear clusters, which had been introduced by different partners. These well-defined groups correlate mainly with geographic regions from which materials had been collected by the respective genebanks. Within the geographical groups, the accessions were more or less closely related to each other.

In contrast to garlic, the neighbour-joining tree of the 62 analysed shallot accessions did not show strong clustering. The genetic diversity is higher and relatively uniformly distributed. The material, which from the different genebanks is mixed and does not represent such narrow country-specific genotype distributions as it was found for garlic. This might indicate a much higher exchange of shallot accessions between countries (trade) during historic times than in garlic, where the materials seem to consist of locally adapted and selected lineages.

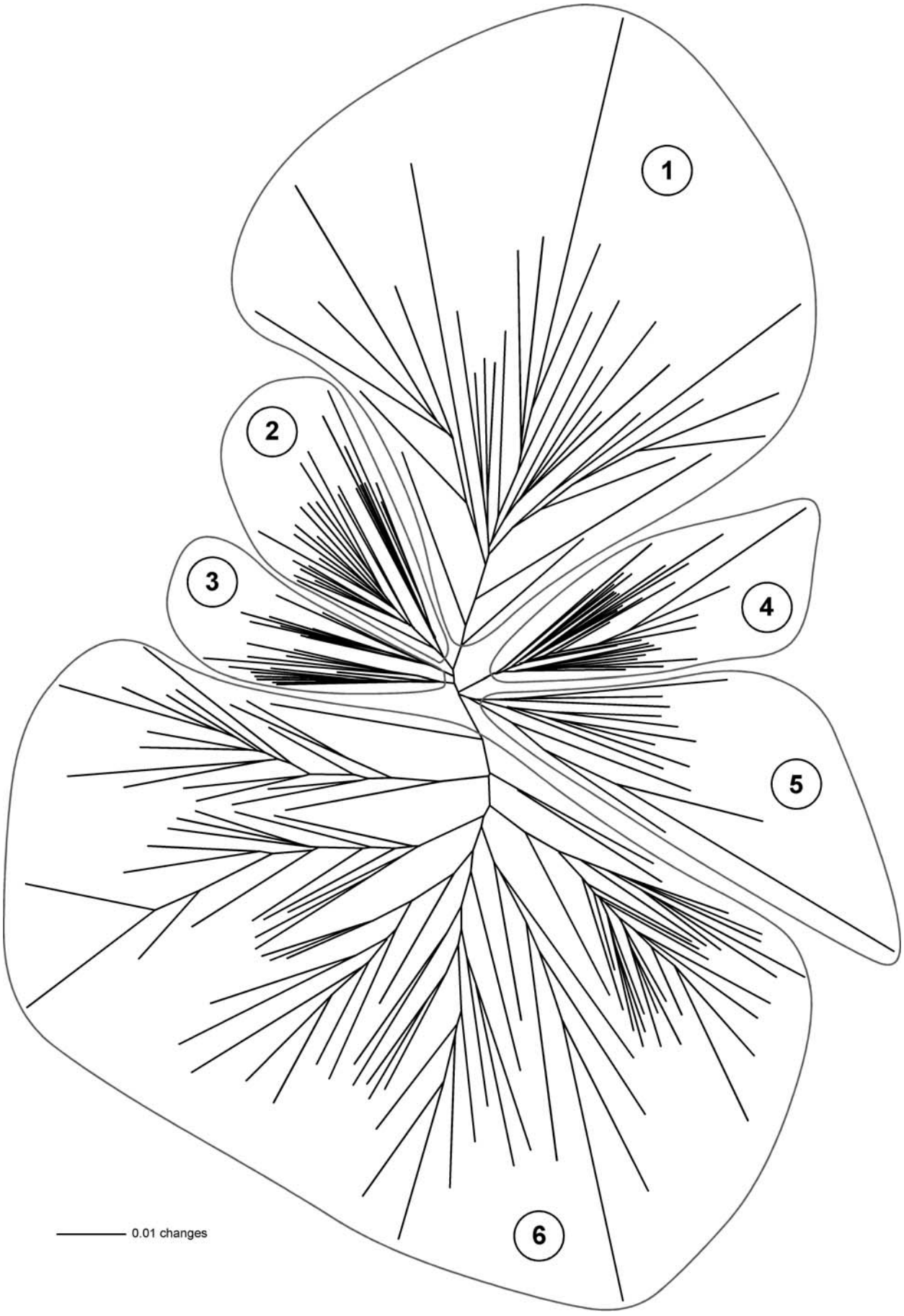
This tree analysis covers 72.5 % of the EURALLIVEG Core Collection. The substructuring of the species garlic is well reflected by the AFLP tree. There are different parts of clusters showing closer relationships and others with wider distances. However, the method is not able to show real duplicates, as has been expected from using SNP markers. If duplicate screen will be endeavoured, then real duplicates should be expected rather in the denser parts of the tree.



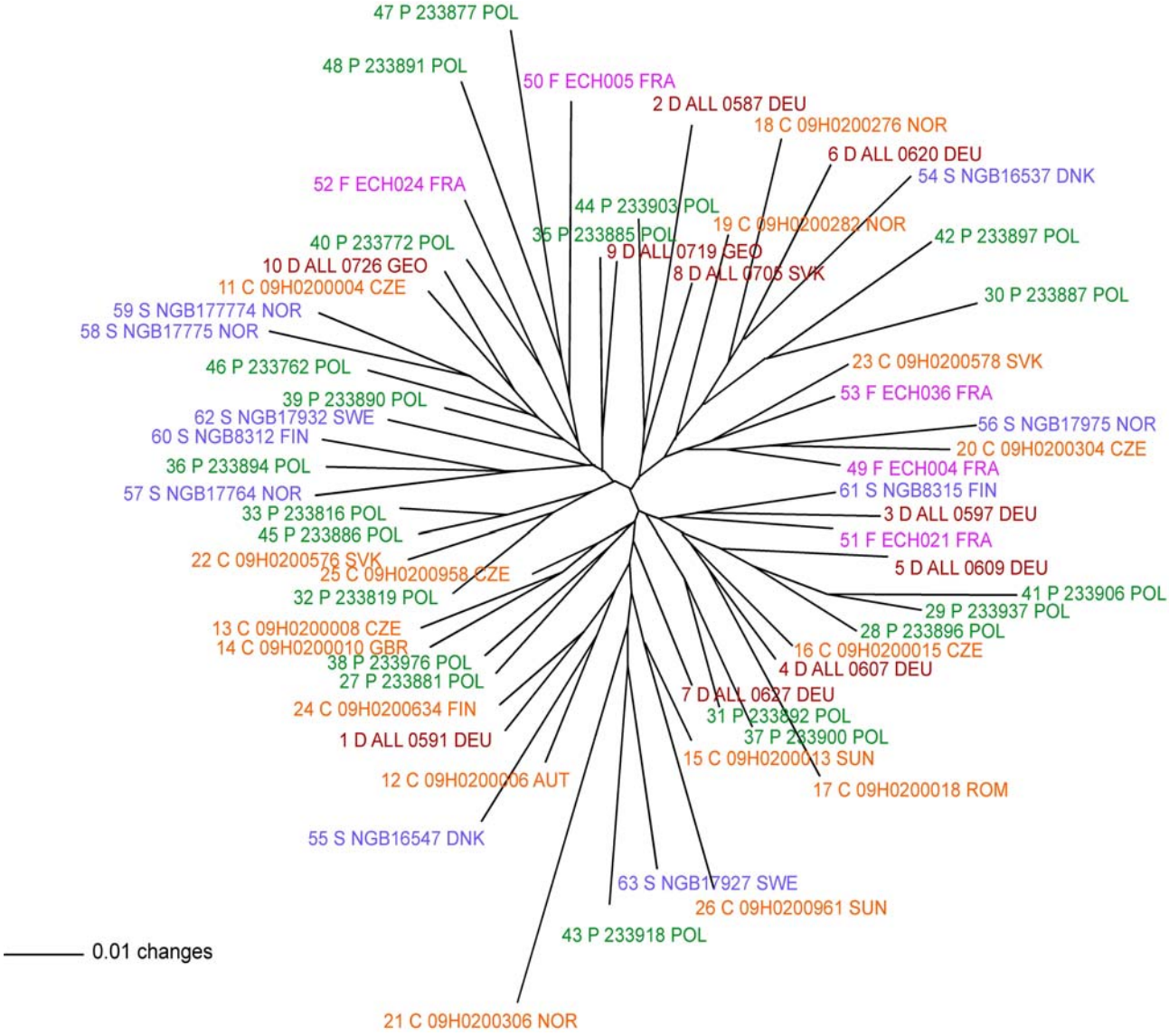
A. sativum AFLP analysis, Nei-Li distances, UPGMA tree, part 1; country abbreviations at the beginning of the accession numbers behind the sample figure: C Czech Republic, D Germany, F France, I Italy, P Poland



A. sativum AFLP analysis, Nei-Li distances, UPGMA tree, part 2



A. sativum AFLP analysis, unrooted UPGMA tree; numbers explained in the text



A. cepa Aggregatum Group AFLP analysis, unrooted tree;
all trees produced by Frank Blattner, IPK, in 2010