Molecular methods in studying biosystematics in wild potatoes (*Solanum* section *Petota*)

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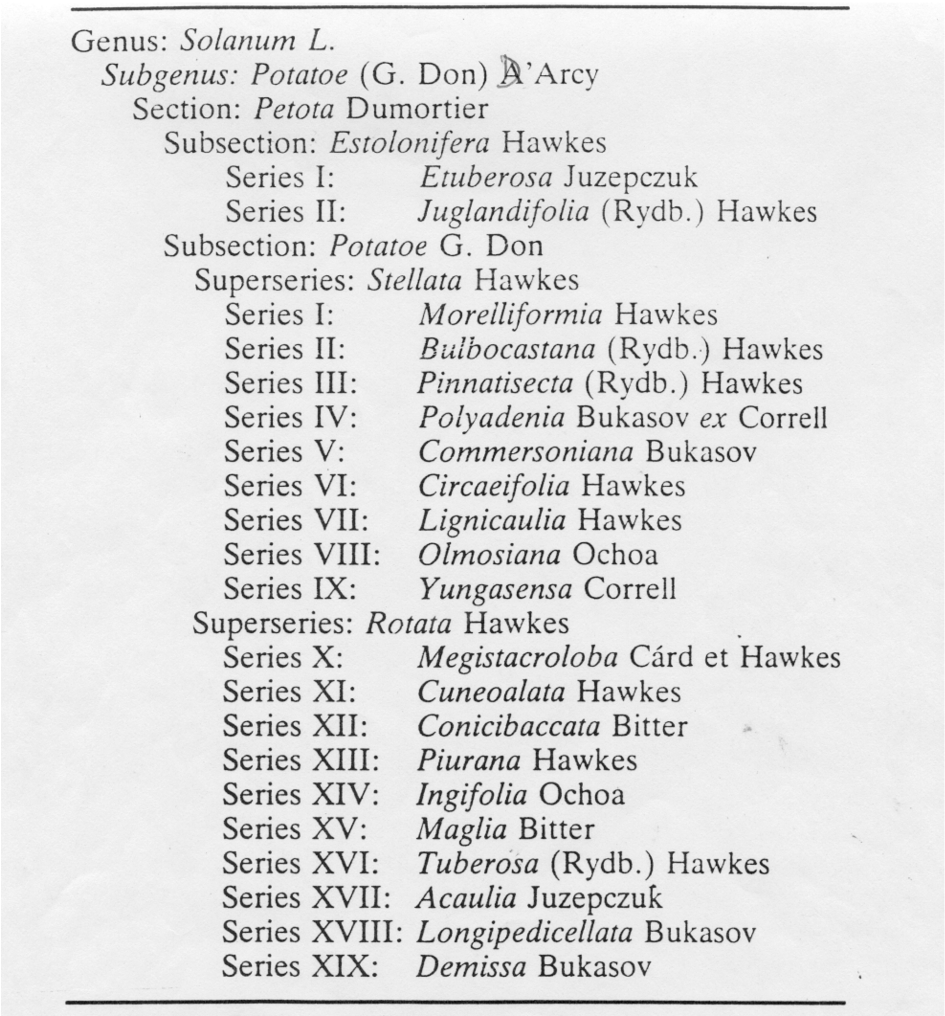
Value of wild potatoes

- Potato 4th food crop in the world
- Large secondary genepool (227 – 188 wild species)
- Important source for novel resistance and quality traits
  - Nematode resistance
  - Phytophthora resistance
  - Non-enzymatic tuber browning
  - Frost tolerance etc.

Geography and Ecology of wild potatoes

- From the southern states of the USA and Mexico to the South of Argentina
- Wide diversity of habitats
  - Mountains (800 – 4000 m)
  - In deserts
  - In cloud forests
  - Epiphytic

Classification of Hawkes (1990)



- Genus: *Solanum* L.
  - *Subgenus: Potatoe* (G. Don) D’Arcy
    - Section: *Petota* Dumortier
      - Subsection: *Estolonifera* Hawkes
        - Series I: *Etuberosa* Juzepczuk
        - Series II: *Juglandifolia* (Rydb.) Hawkes
      - Subsection: *Potatoe* G. Don
        - Superseries: *Stellata* Hawkes
          - Series I: *Morelliformia* Hawkes
          - Series II: *Bulbocastana* (Rydb.) Hawkes
          - Series III: *Pinnatisecta* (Rydb.) Hawkes
          - Series IV: *Polyadenia* Bukasov *ex* Correll
          - Series V: *Commersoniana* Bukasov
          - Series VI: *Circaeifolia* Hawkes
          - Series VII: *Lignicaulia* Hawkes
          - Series VIII: *Olmosiana* Ochoa
          - Series IX: *Yungasensa* Correll
        - Superseries: *Rotata* Hawkes
          - Series X: *Megistacroloba* Cárd et Hawkes
          - Series XI: *Cuneoalata* Hawkes
          - Series XII: *Conicibaccata* Bitter
          - Series XIII: *Piurana* Hawkes
          - Series XIV: *Ingifolia* Ochoa
          - Series XV: *Maglia* Bitter
          - Series XVI: *Tuberosa* (Rydb.) Hawkes
          - Series XVII: *Acaulia* Juzepczuk
          - Series XVIII: *Longipedicellata* Bukasov
          - Series XIX: *Demissa* Bukasov

- ► 19 series of tuber-bearing Solanum species
- ► ordered from primitive to advanced
- ► Based on morphology , cytology, and crossability

Previous molecular studies

- Molecular studies: RAPD, RFLP, AFLP and DNA sequences
- Most studies only cover a small number of the series described by Hawkes, difficult to link all species together
- cpDNA RFLP studies by Spooner and coworkers: 4 clades
  - 1) Mexican diploids,
  - 2) *S. bulbocastanum* and *S. cardiophyllum*,
  - 3) *Piurana* plus a few other species,
  - 4) remaining South American species and polyploid Mexican/North American species

Goal of the project

- Elucidate the systematic relationships of wild tuber-bearing *Solanum* species
    - include as many species as possible
    - focus on the wild tuber-bearing *Solanum* accessions in the CGN collection, supplemented with material from other genebanks
    - Use the information on the systematic relationships to select material in the second part of the project (search for new sources of R genes against *P. infestans*)
    - Make the information available to others scientists and users (breeders) in CBSG project
- Search for new sources of R genes
    - Generate markers for *P. infestance* resistance

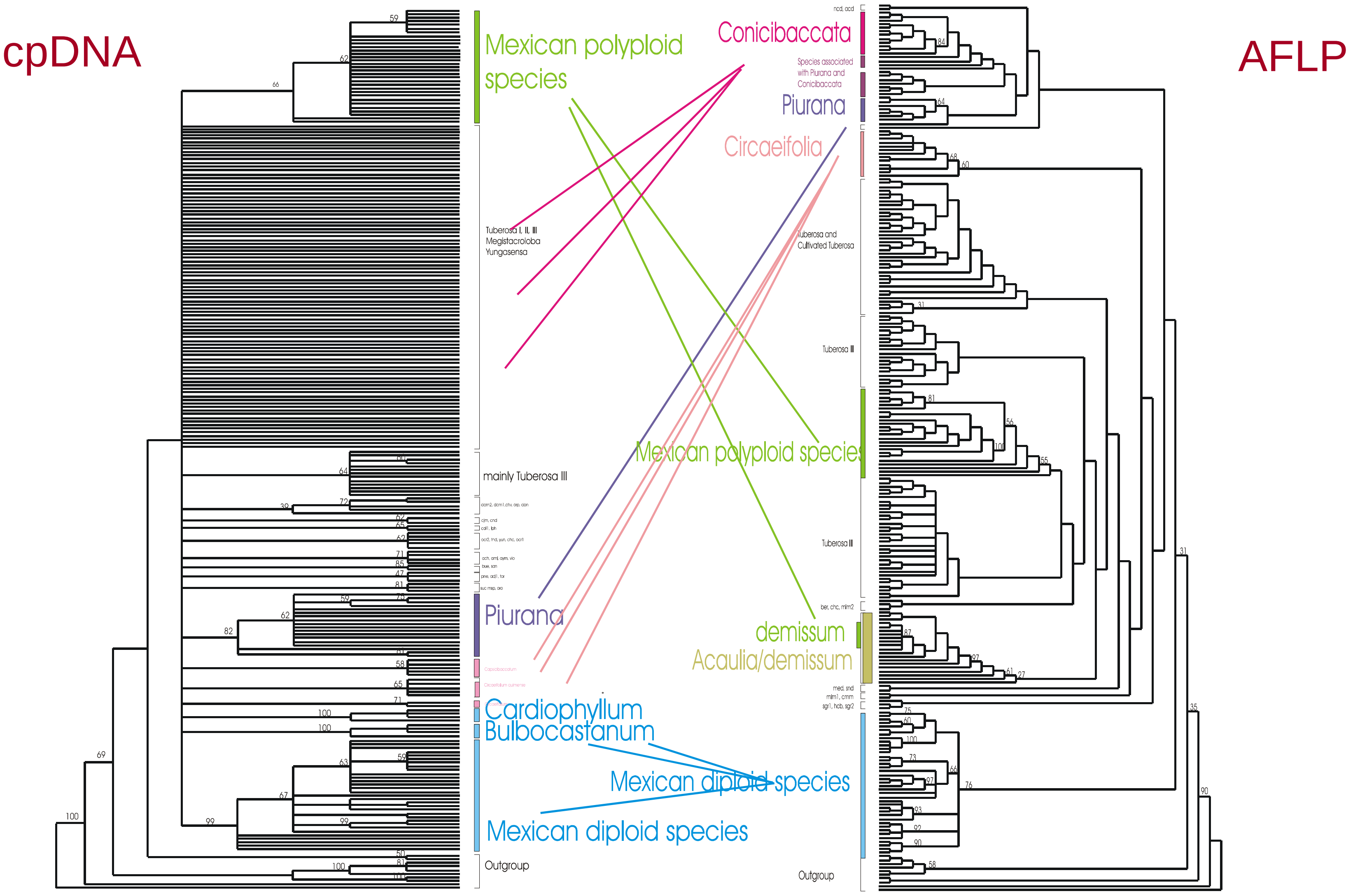
Original plan:

- Construct ‘backbone’ phylogeny using cpDNA sequencing
- Detailed phylogeny using AFLP
  - Scoring in groups based on cpDNA data

But:

- How much variation can be detected in cpDNA ?
- Do AFLP and cpDNA give similar results ?
  - Tested on a subset of the samples.

Comparing cpDNA and AFLP: results



Comparing cpDNA and AFLP: conclusions

- The cpDNA strict consensus tree is less resolved than the AFLP strict consensus tree
- Some groups appear in both trees
- incongruencies between the AFLP and cpDNA trees
  - cpDNA cannot be used for backbone phylogeny in section *Petota*
  - differences probably caused by differences in mode of inheritance: maternal versus biparental, hybridisation

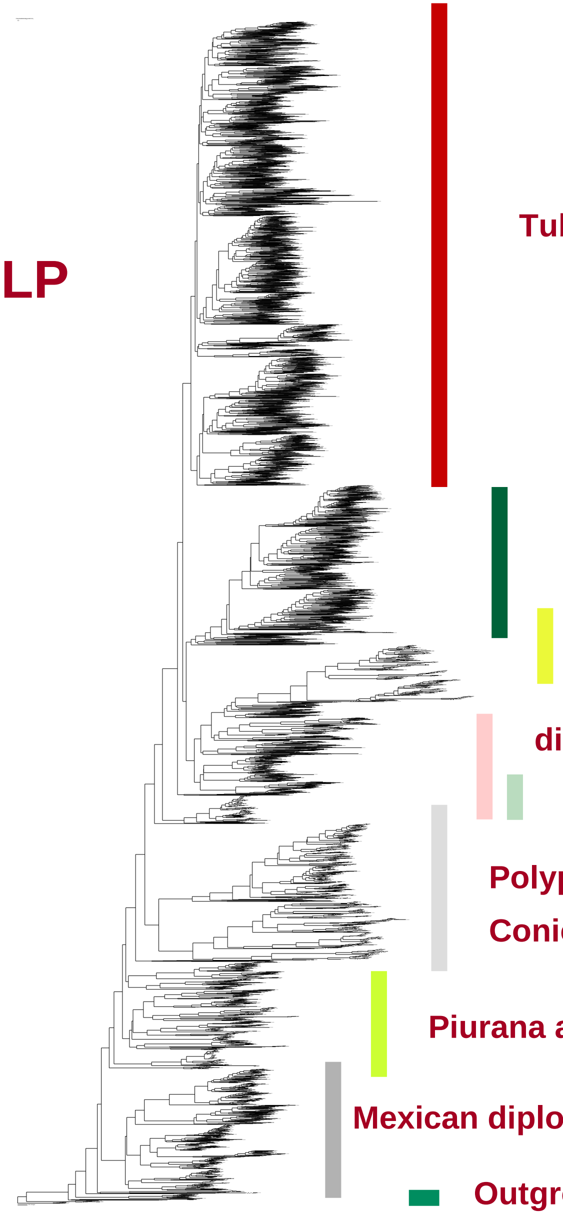
Analysis of large AFLP dataset: outline

- 1000 accessions, if possible 5 accessions per species, 5 genotypes per accession
  - 4929 OTU’s
- Capacity problems
  - only UPGMA and NJ could be succesfully applied so far
- New approach: light dataset
  - 1 genotype per accession, 916 OTU’s
  - mp and NJ jackknife trees (statistical support for found structure)

NJ tree

Complete dataset

global markers AFLP



Tuberosa from Argentina and Bolivia

Tuberosa Peru and cultivated

Acaulia and Demissa

diverse, most 2n Peru

verrucosum

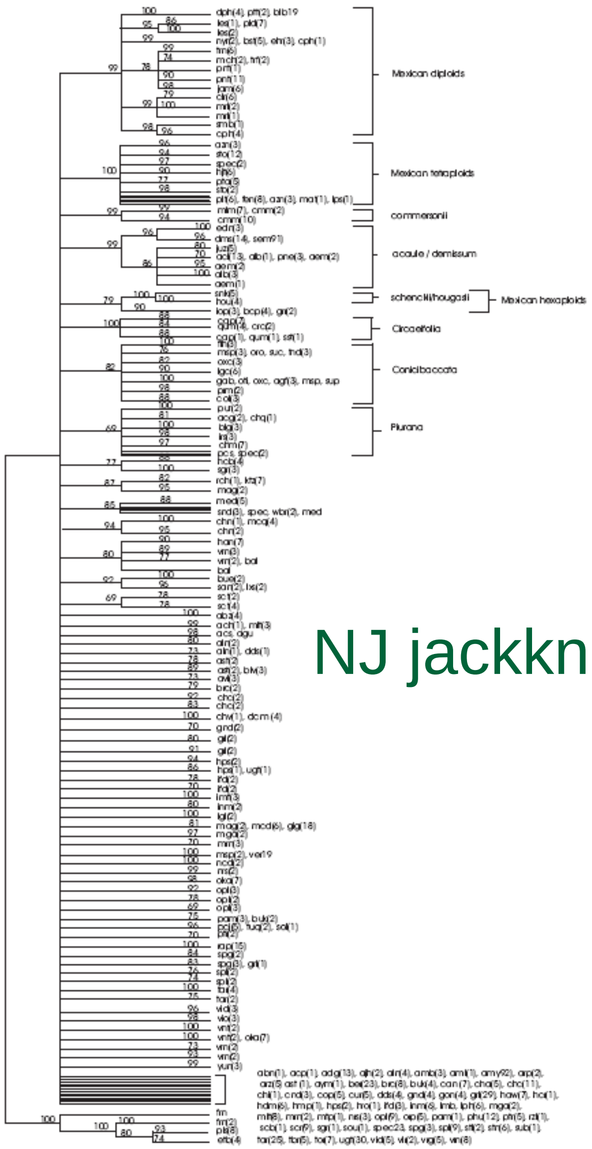
Polyploid Mexican/North American species and Conicibaccata

Piurana and Circaeifolia

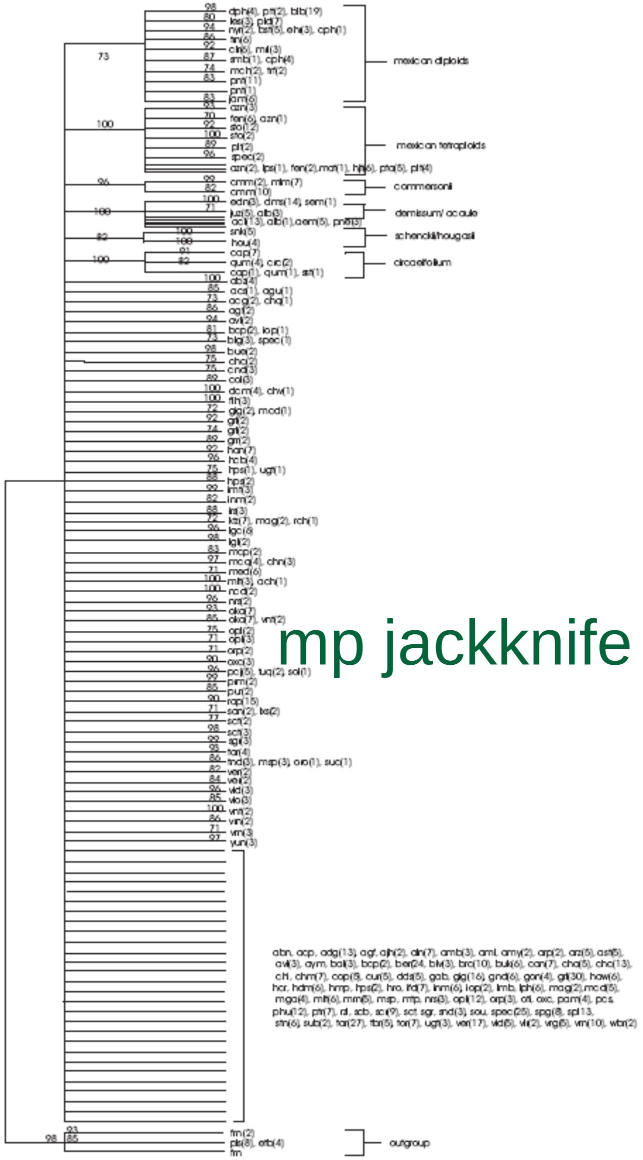
Mexican diploid species

Outgroup

AFLP light 916 dataset

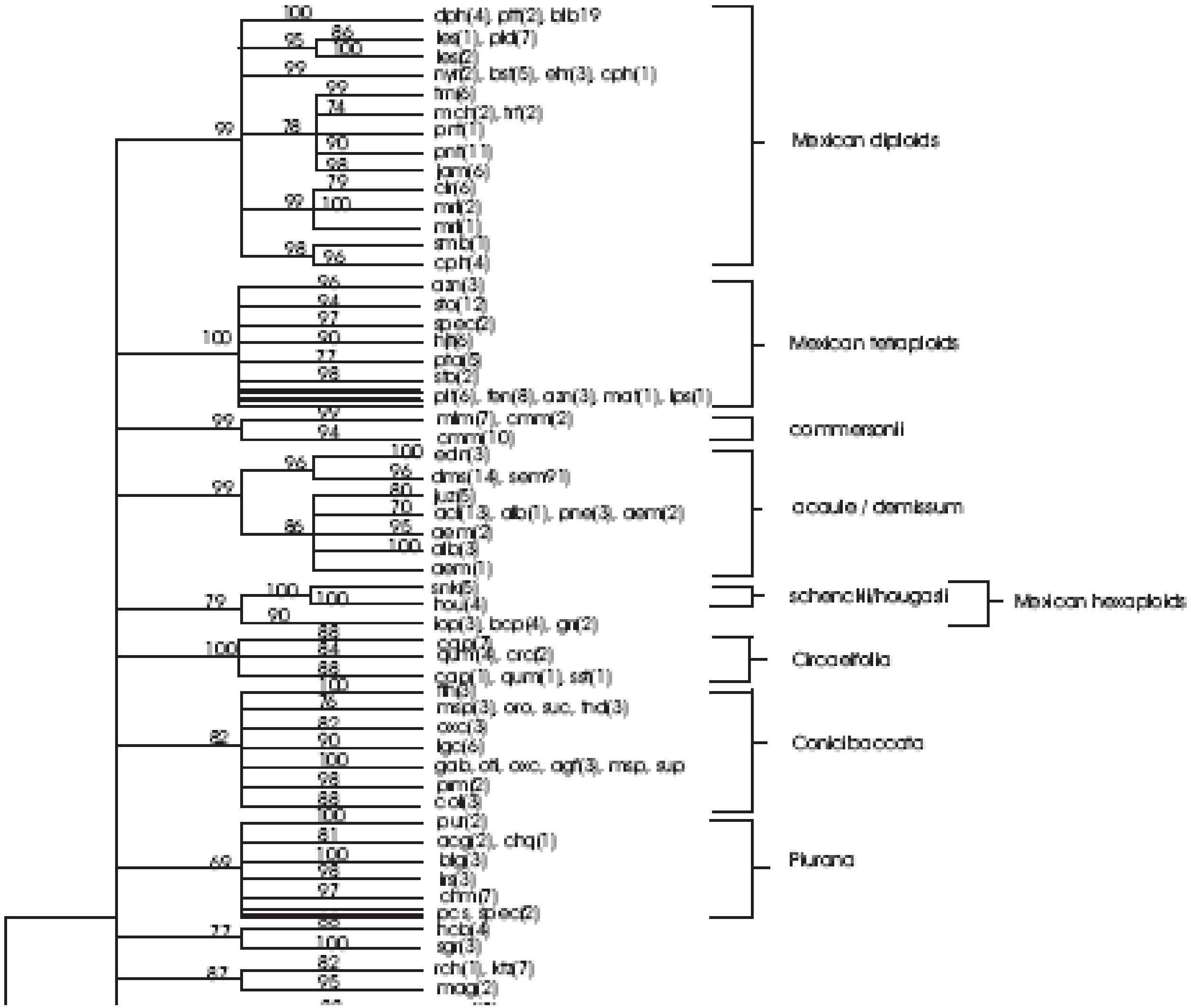


NJ jackknife tree



mp jackknife tree

AFLP light dataset: details of NJ jackknife tree



AFLP results

- NJ jackknife tree shows more structure than mp jackknife tree
- 3 levels of structured groups
  - groups well supported in both analyses:
  - groups only well supported in the NJ analysis
  - groups recognised in NJ and mp trees but without support

- Polytomies in both trees: difficult to split up the largest group of South American species
- Causes?
  - This may point at a relatively recent **radiation**, with a certain level of morphological divergence but less molecular divergence.
  - Plus: effect is probably worsened by **hybridisation.**

Detailed study on South American part

- Focus on the unresolved part of the AFLP tree
- Use a population genetic approach using STRUCTURE software and Fst
- Results: not more than 16 groups instead of 90 species labels.
- Causes: misclassification, overclassification and hybridization

Use of information in the project

- Strange (AFLP) results were discussed with CGN and Keygene
  - Wild potato accessions were planted in the field and evaluated for expected morphology
- Information was made accessible for users
  - Intranet (only accessed by CBSG members)
  - Neighbour Joining tree with search options for species labels and resistance data
  - Interactive module: select your own dataset: species, accession, genotypes. The built-in software will compute a dendrogram, similarity matrix or PCO or show the data on a map (based on Google Earth)

- The systematic information was used in the other part of the project and for other projects within CBSG
  - Useful in choosing plant material more efficiently

Important issues

- Need for revision of taxonomy of wild potatoes
  - Less species exist than species labels currently accepted
  - It will take some time before a change of view will be commonly supported
- Discrepancies in taxonomic labels
  - Change of species label is needed in some cases
  - Important to check the morphology of the material studied
- Contrast in morphology / molecular results
  - Morphological variation in wild potatoes is huge, but it cannot be captured with molecular markers AFLP nor cpDNA sequences
  - Other molecular markers are needed, but do they exist?
  - Managing/collecting genetic material: geographic distribution of accessions as a important alternative guideline

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