CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

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# DEVELOPMENT OF A MOLECULAR METHOD TO RAPIDLY IDENTIFY SPECIES OF THE GENUS *GALANTHUS* (AMARYLLIDACEAE, CITES APPENDIX II)

1. This document has been prepared by the German CITES Scientific Authority.

Summary

2. To identify those species of the genus *Galanthus* (Amaryllidaceae, CITES App. II), which are in legal and illegal commercial trade, a rapid species identification method was developed on the base of the species-unique DNA-PCR-Marker. The project was carried out by the University of Osnabrück (Germany) on behalf of the German CITES Scientific Authority.

3. The identification method is quick and cost-effective and has already provided evidence in detecting mislabelled *Galanthus* species, when imported into Switzerland. It also recently revealed a tendency to import *Galanthus* bulbs from the Caucasus region which were not traded yet.

4. Beside its relevance and importance for the species identification in trade, the study indicated taxonomic relationships within the genus *Galanthus* which do not correspond with classifications based on morphological data.

## Project background

5. The genus *Galanthus* L. (Amaryllidaceae) comprises 19 species and which occur naturally in Europe, Turkey and in the Caucasus region.

6. Snowdrops are popular winter-flowering garden plants and millions of bulbs are sold annually, mainly *Galanthus nivalis*, *G. elwesii* and *G. woronowii*. Most of the bulbs of *G. elwesii* and *G. woronowii* are collected in the wild in Turkey and Georgia, for which the collecting and trade quota is fixed, whereas *G. nivalis* bulbs are mostly coming from cultivation. *Galanthus* species are presently listed on CITES Appendix II.

7. The monitoring of trade in *Galanthus* is very difficult, since plants are usually imported as bulbs and only very limited identification can be undertaken at this stage of the life cycle. Before identification of a *Galanthus* specimen can be undertaken, any bulb has to be grown at a nursery facility for identification at a later date (2–8 months): a costly and time-consuming exercise. Therefore, CITES enforcement officers are interested in quick and cost effective species identification methods for the genus *Galanthus.*

8. There have been report that some *Galanthus* species are collected for which trade has been nationally banned, or which are not allowed to be imported into countries of the European Union (e.g. *G. rizehensis, G. krasnowii, G. alpinus*). In some cases *G. elwesii* and *G. woronowii* are declared

as *G. nivalis* from cultivation, for which large-scale commercial trade is allowed. In a survey in Germany, bulbs of up to five *Galanthus* species in one package were found.

Project description

9. The research project aimed to develop a rapid species identification method on the base of the species unique DNA-PCR-Marker to identify most *Galanthus* species, which are in legal and illegal commercial.

10. 320 accessions from different *Galanthus* species have been investigated as reference plant material. Most of the studied accessions were collected from naturally occurring populations in Georgia, Ukraine, Russia, Greece, and Turkey. Some accessions represent herbarium specimens from Moscow, St-Petersburg, Tbilissi, Batumi, Vena, and Frankfurt a. M, some other accessions came from living plants at the Botanic Gardens Bonn, Botanic Garden Osnabrück, and the National *Galanthus* Collection of Alresford, Hampshire, UK. Fresh material was collected in Georgia in March 2004 and in Turkey in April 2005.

11. By using methods of molecular biology like DNA sequencing and PCR sequence amplification, species unique DNA-PCR marker were developed and primers of 18-20 base pairs were designed to amplify some short, unique fragment of DNA for most *Galanthus* species.

Results

12. a) Species of the genus *Galanthus* are divided into the five alliances *krasnovii*-, *nivalis*-, *elwesii*-, *woronowii*-, and *alpinus*-group.

    b) The species Galanthus nivalis, G. elwesii and G. woronowii, which are important for commercial trade, are genetically clearly different and build monophyletic clades on the cpDNA and nrDNA analysis.

    c) Relationships based on the DNA data (*trn*L-*trn*Fspacer and ITS) do not correspond with classification based on morphological data.

    d) The identification method is quick, cost effective and reqires very little plant material. It has provided evidence in detecting *Galanthus* species, falsely declared, when imported into Switzerland.

    e) For identification of closely related taxa, DNA fingerprints from reference species have to be used.

Conclusions

13. The method is easy to apply with a basic molecular lab equipment. A test takes about 6 hours and costs approximately 40 Euro per 10 bulbs. The German CITES SA is ready to supply the primer code to all interested CITES Management and Scientific Authorities. For additional DNA based identification, the database for all DNA sequenced *Galanthus* species is available in the Botanic Garden of the University of Osnabrück.

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