Genmeda

NETWORK OF MEDITERRANEAN PLANT CONSERVATION CENTRES

ISSN: 1988-5881

Odissea Seminum

GENMEDA Newsletter

Issue 7: October 2022

ARTICLE

Orchid-related research and activities at the NKUA Seed Bank and Research Team

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The National and Kapodistrian University of Athens (NKUA) Seed Bank constitutes, arguably, the oldest seed bank for native wild plants in Greece. The Seed Bank was established in 1991 by the Assistant Professors (and currently Professors Emeriti) Kyriacos Georghiou and Costas A. Thanos. The Seed Bank currently hosts more than 700 seed lots from 403 taxa; 56 of these taxa belong to the family Orchidaceae and are represented by 120 seed lots. Both scientific responsibles of the Seed Bank are currently active as supervisors or associates of scientific projects and have accumulated significant experience in both seed biology (mainly germination ecophysiology) and plant conservation (both *in situ* and *ex situ*).

Figure 1. *Cephalanthera cucullata* in full flower, in the Plant Micro-Reserve of Koustogerako (Crete). The entire plant shoot (in the left) is 20-25 cm tall.

The earliest actions for conserving the orchids of Greece in the NKUA Seed Bank date about 20 years ago with the **LIFE project** CRETAPLANT (LIFE04 NAT/GR/000104, scientific coordinator Prof. Emer. Costas A. Thanos) implemented in collaboration with the plant conservation team of the Mediterranean Agronomic Institute of Chania (Crete). One of the target species of the CRETAPLANT project (2004-2007) was the Cretan endemic *Cephalanthera cucullata* (Figure 1), a Community priority (92/43/EEC), mycoheterotrophic orchid, characterised as EN by both the 1995 and 2009 Red Data Books of the rare and threatened plant species of Greece. The species shows declining populations, mainly affected by overgrazing. During the project, a Plant Micro-Reserve at Koustogerako was established through fencing of a 12 ha area for the control of grazing. During the 15 years of annual monitoring of the PMR, a 3-fold increase of the population has been observed (Oikonomidis et al. 2021), signalling a remarkable success of the particular **Plant Micro-Reserve of *Cephalanthera cucullata***. Trials for the *ex situ* propagation of the species have been repeatedly made as well but, as it is known for most of the *Cephalanthera* spp., their germination is particularly difficult in asymbiotic cultures and thus no positive results have been obtained so far. Even though no germination was achieved till now, it is quite interesting that the latest viability tests for all the seed lots collected and stored in the NKUA Seed Bank, showed that a considerable percentage of seeds collected during the project (~20-30%) still remain viable.

Figure 2. Post flowering *Ophrys kotschyi* in the Plant Micro-Reserve of Mitsero (Cyprus). The inset shows a flower of the species

During the same period of CRETAPLANT implementation, our team worked (2005-2008) on two low-budget, **NKUA funded projects** (under the call name Kapodistrias) for the *ex situ* conservation of plant diversity of Mt. Hymettus (coordinated by Prof. Emer. Costas A. Thanos). Through the implementation of these projects, the locations of several species of orchids in Mt. Hymettus were recorded while for two orchid species, seed collections for long-term storage were made.

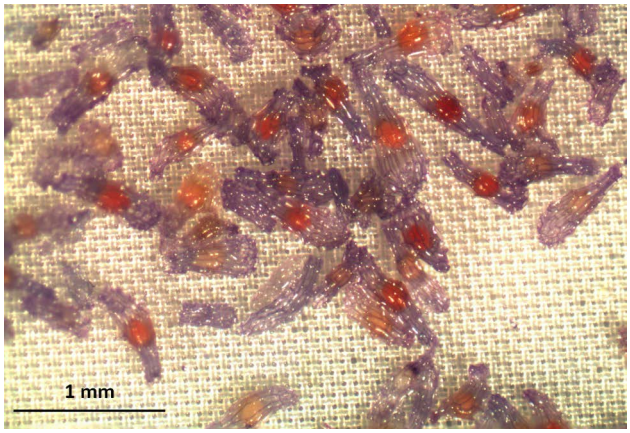

Figure 3. Viability staining in *Ophrys kotschyi* seeds with the use of the double-staining technique (tetrazolium and trypan blue, Magrini et al. 2019)

In a subsequent **LIFE project** (PLANT-NET CY / 2010-2013, scientific coordinator Dr. Costas Kadis), a **Plant Micro-Reserve for *Ophrys kotschyi***, a threatened orchid of Cyprus, was established in Mitsero (Figure 2). The NKUA team contributed to the *in situ* monitoring of this endemic orchid of Cyprus (Eliades et al., 2020) and to the study of its seed biology as well, both by elaborating a successful germination protocol (Koutsovoulou et al., 2013) and by storing several lots of the species in the NKUA Seed Bank. Recently, after 10 years of storage, the viability tests of these seed lots indicate that they still remain viable (Oikonomidis et al. 2021) without significant changes, especially for the seeds produced by cross fertilization (Figure 3).

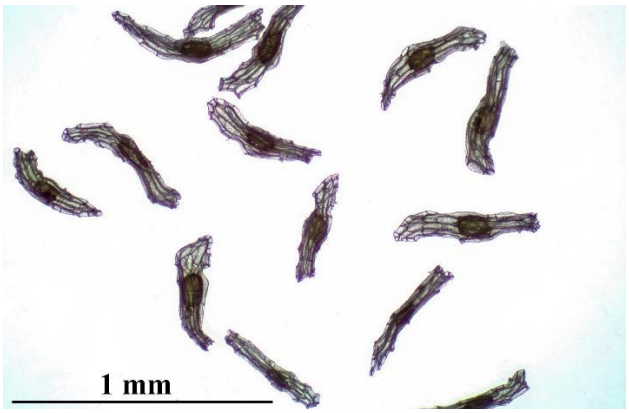

Figure 4. Seeds of *Neotinea maculata* under a stereomicroscope

In addition to the two LIFE projects and the two nationally funded ones for the conservation of Mt. Hymettus plant diversity, **four Diploma Dissertations** concerning orchid germination and *ex situ* conservation have been implemented under the supervision by Prof. Costas A. Thanos (Velianiti 2011, Peppa 2011, Ambelakiotou 2015, Oikonomidis 2019). These student studies resulted in the collection of 34 taxa, represented by 60 seed lots (Figure 4), while protocols of successful germination for several of these species have been proposed at national or international conferences (Velianiti et al. 2009, Peppa et al. 2011, Oikonomidis et al. 2018).

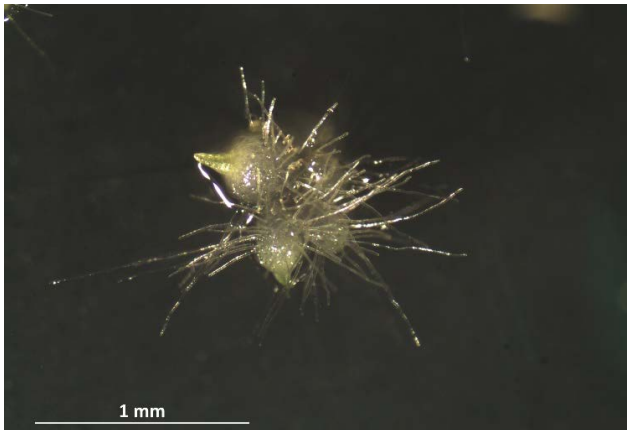

Figure 5. Protocorms and rhizoids of *Anacamptis sancta*

Starting early in 2020, a **PhD Thesis** (**S. Oikonomidis**) aiming to investigate the seed biology of the orchids of Greece was launched under the supervision of Prof. Emer. Costas A. Thanos. Within the context of this work, 22 new taxa and 60 new collections of orchids have already been added in the NKUA Seed Bank. Currently, a total of 56 taxa, i.e. more than one third of the orchid flora of Greece are conserved in the NKUA Seed Bank. During the ongoing PhD research, both the germinability (Oikonomidis et al. 2020, Oikonomidis & Thanos, 2021) and the longevity of the collected species are investigated (Figure 5). In addition, a database for the orchids of Greece and their functional traits is, currently, under development. Upon completion, the database will include all the data of our lab as well of the available national and international literature about the orchid species occurring in Greece and it will be hosted online along with an app, also under development, for the identification of the orchids of Greece, based on image recognition AI.

Starting in March 2022, a **new project in collaboration** with the **Royal Botanic Gardens of Kew** was launched. The new project entitled "Conserving the Flora of the Balkans: Native Plants of Greece (RBG KEW)" aims to the *ex situ* conservation of 500 native species of Greece and among those several orchid species will be collected and stored in the NKUA Seed Bank and in duplicates in the Millennium Seed Bank of RGB Kew. In the first year of the project, the priority species list

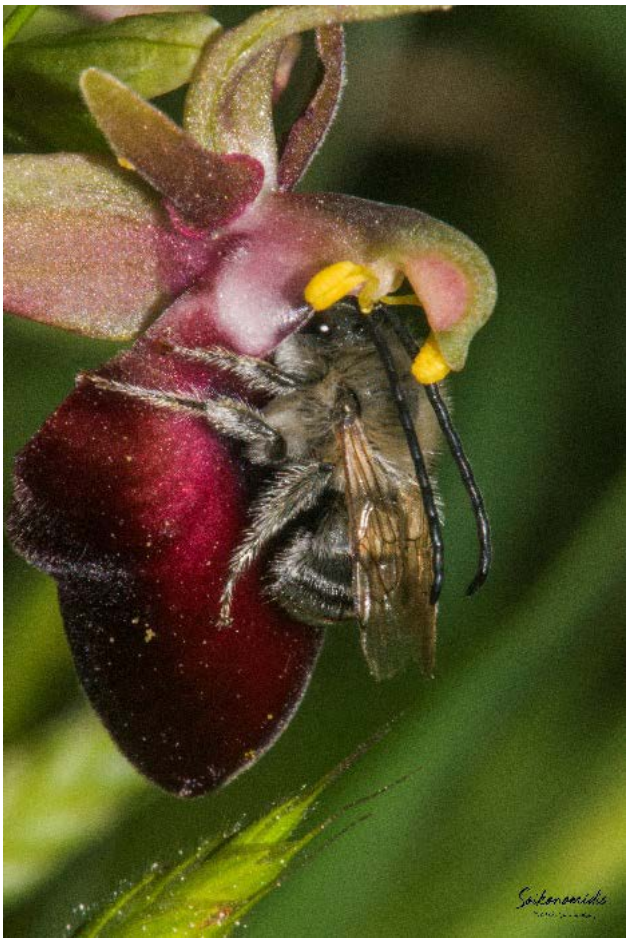

Figure 6. *Ophrys helenae* with a typical pollinator of the species

for collections contains 34 orchid taxa, i.e. 1/5 of the orchids occurring in Greece.

Lastly, even though the main focus of the work with orchids at the NKUA Seed Bank lies on their conservation through long-term seed storage, considerable effort is also being invested in the study of germination behaviour and seed longevity for the entire orchid flora of Greece. Finally, it is worth adding that certain important observations have been made in regard to the pollination of orchids. Especially in the case of *Ophrys helenae* and in collaboration with the University of Ioannina, two species of Coleoptera of the genera *Trichodes* and *Pygopleurus* (Oikonomidis et al. 2017 & 2021) have been recognised as true pollinators of the species (Figure 6), shedding further light on the pollination of the genus *Ophrys*.

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