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Seed lipid thermal fingerprints of Mediterranean terrestrial orchids can be used to optimize ex-situ conservation

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The family Orchidaceae

- **One of the largest families of flowering plants** in the world, numbering about 28,000-30,000 species.
- It is considered one of the most charismatic group, often referred to as the ‘**pandas of the plant world**’.
- Unfortunately, it is also **one of the most endangered groups** due to a wide range of intrinsic and extrinsic factors, directly or indirectly caused by human activities, include habitat change, loss and destruction, climate change, weed invasion and illegal collection.
- **Terrestrial orchids** are particularly susceptible to habitat and environmental changes, so they are often the first plant species lost from disturbed habitat.
- **This research aims to improve the prospects for the successful ex situ conservation of European orchids.**

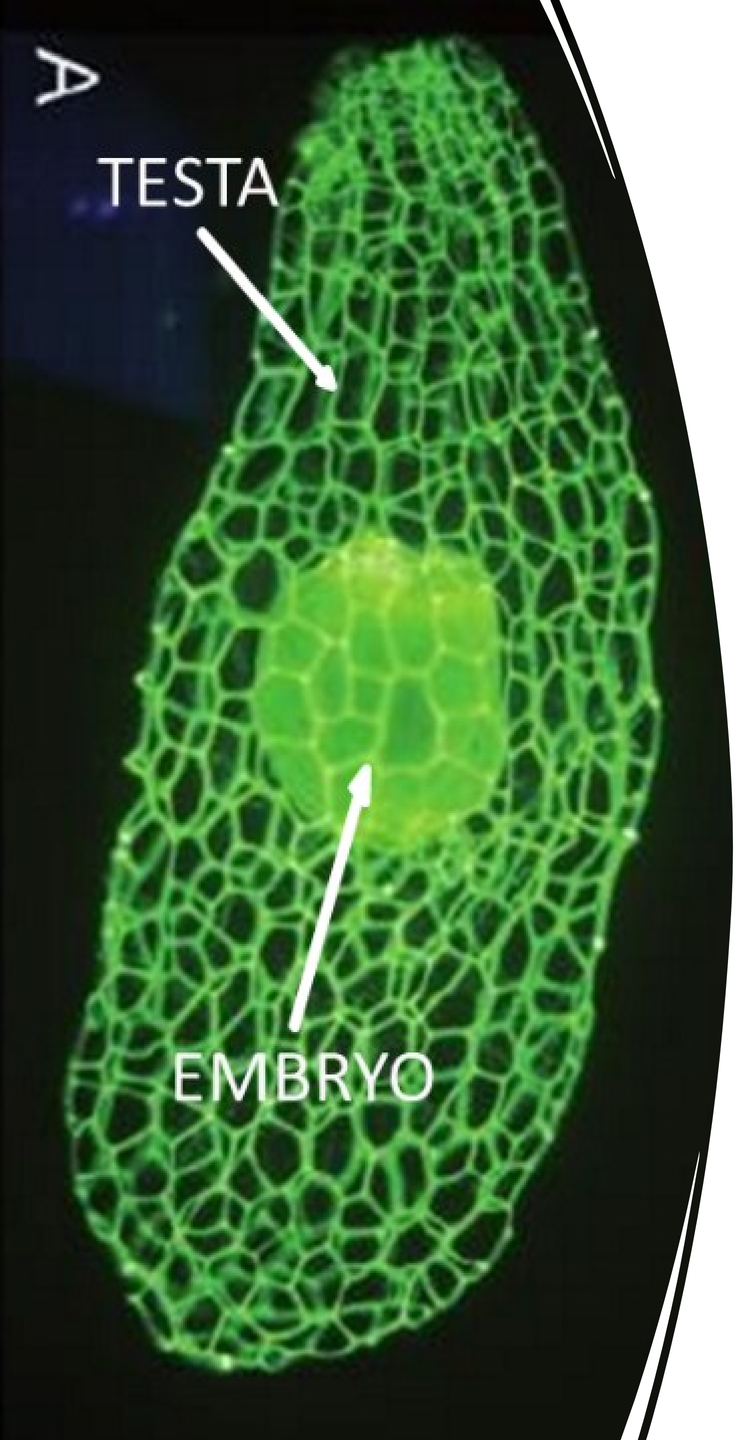
Ex-situ seed storage

- **Seed banking is the most widely adopted, practical key component for effective ex-situ conservation of plant diversity**, providing a long-term security backup (extinction-proofing) for species and their genetic diversity.

- **Seed banking is increasingly being adopted for the ex-situ conservation of orchids.**

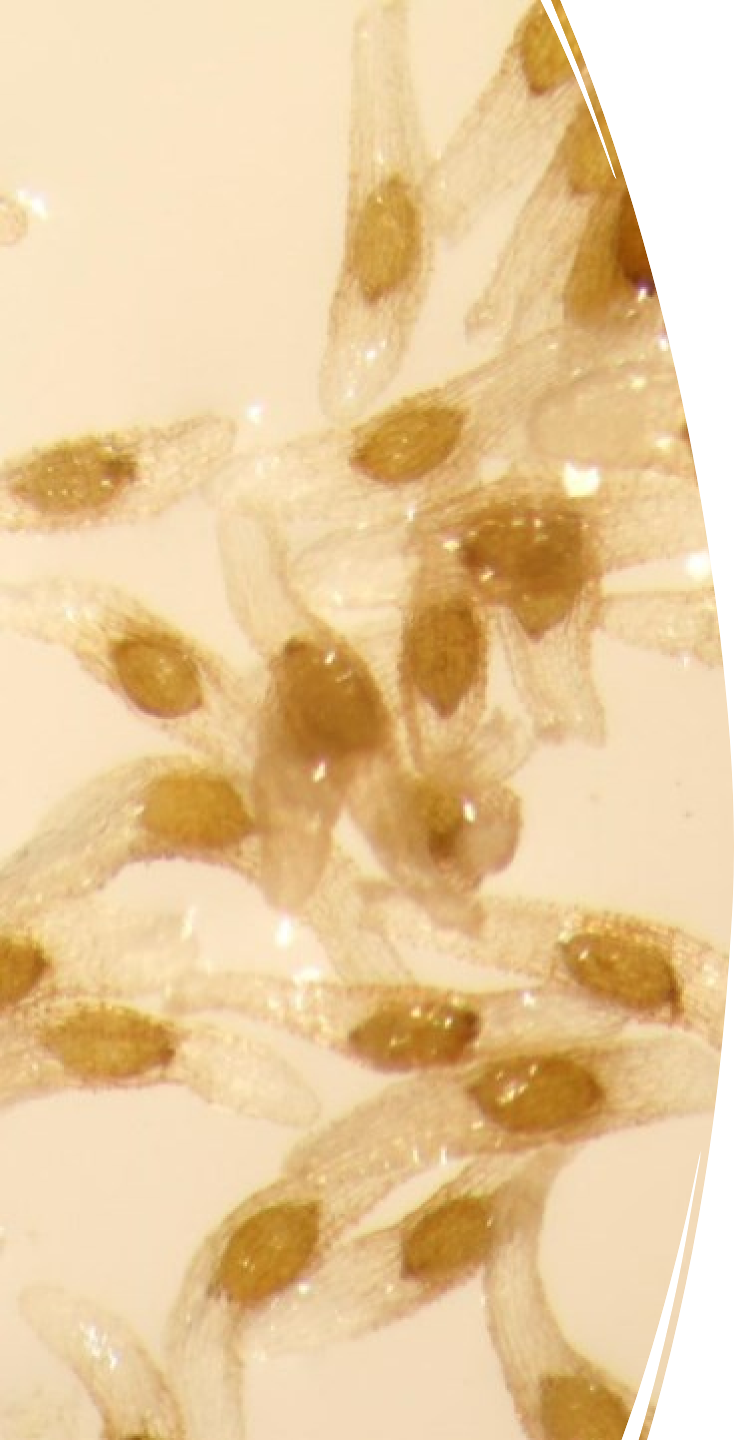
Ex-situ seed storage

- Terrestrial orchid seeds are **orthodox in storage behavior**, so their ex-situ conservation in seed banks can be a cost-effective tool to provide a long-term backup of their genetic diversity.
- Due to their minute size, large numbers of dust-like **seeds can easily be stored in small volumes**, making orchids ideal for seed-banking without the need for large facilities (Colville et al. 2016; Swartz and Dixon 2017).



Orchid seeds

- The mature embryo of an orchid seed is just an ovoid mass of cells without any differentiation of the tissues.
- A thin external integument (or testa) and an inner one involving the embryo (carapace).
- Cell walls of testa contain a wide range of lipids, suberins, and polyphenolic deposits, including lignins and tannins, all contributing to the hydrophobic nature of mature seeds.
- **No endosperm, only a few small starch grains and lipids as the main high-energy storage compound**.



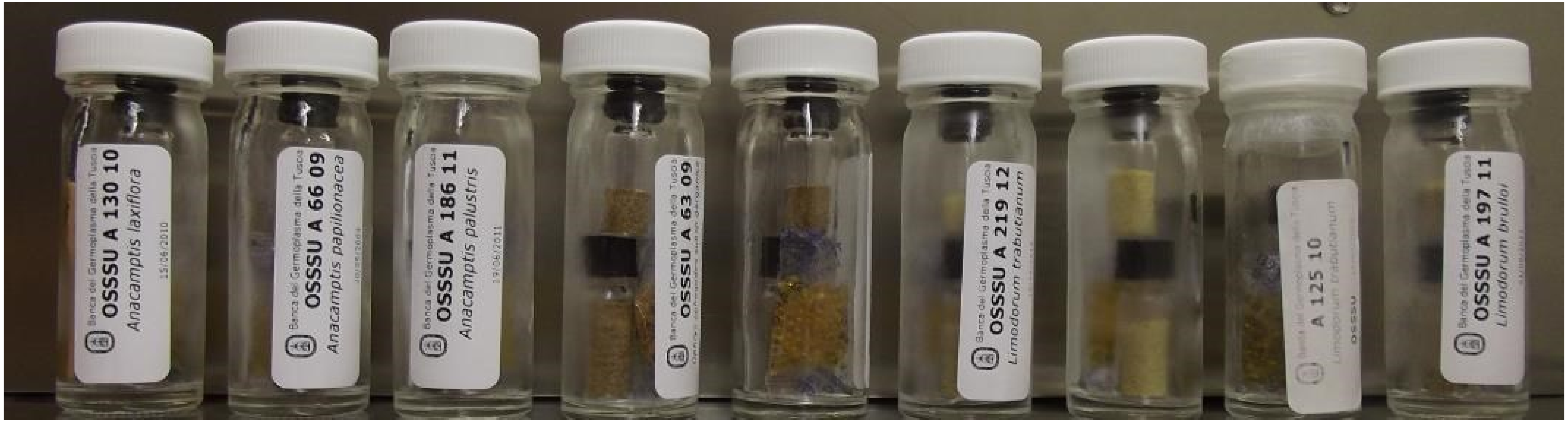
But orchid seeds are reported to have a short lifespan... Why?

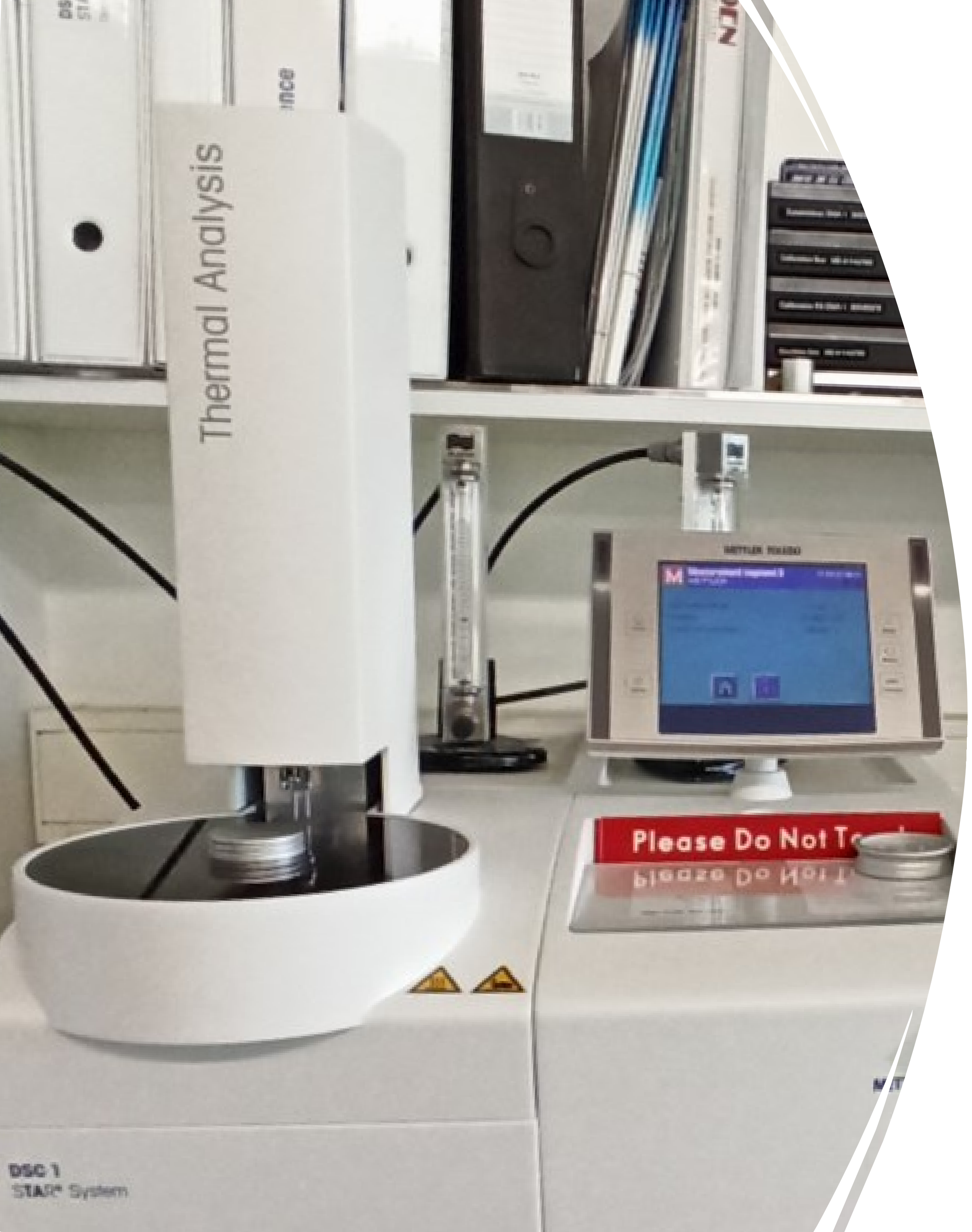
- Seed lipids have long been thought to be a determinant of seed ageing, with lipid composition impacting differing susceptibility to oxidation and to variation in thermal behavior.
- In particular, the thermal characteristics of lipid melting and crystallization are hypothesized to influence the storage stability of oily seeds.

Aim of the research

In this study, we have used **Differential Scanning Calorimetry to explore potential links between poor storage performance and lipid thermal fingerprints of seeds** of terrestrial orchid species.
We interpret our data in relation to the risk of lipid crystallisation during cold storage and attempt to answer the question:

What is the optimal storage temperature for the long-term preservation of orchid seeds?



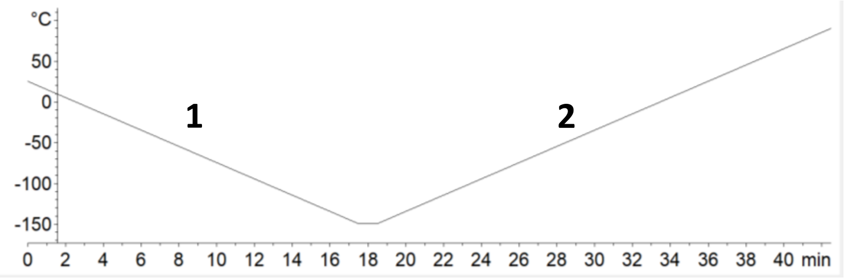


Materials & Methods

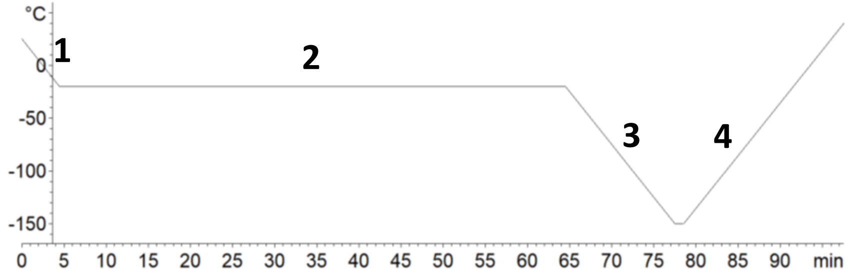
- Lipid thermal behavior of dry seeds was carried out on **15 terrestrial orchid species from the Tuscia Germplasm Bank**, collected in Italy.
- Analyses of the melting behaviour were performed using a **Differential Scanning Calorimetry at the Millennium Seed Bank.**

DSC methods

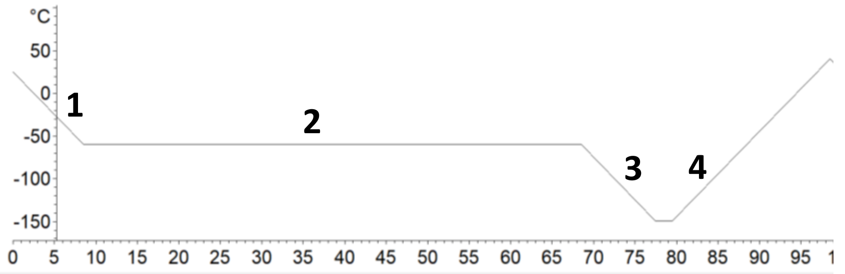
- Standard method:
  - 1: cooling: from 25°C to -150°C @ 10°C/min
  - 2: warming: from -150°C to 90°C @ 10°C/min



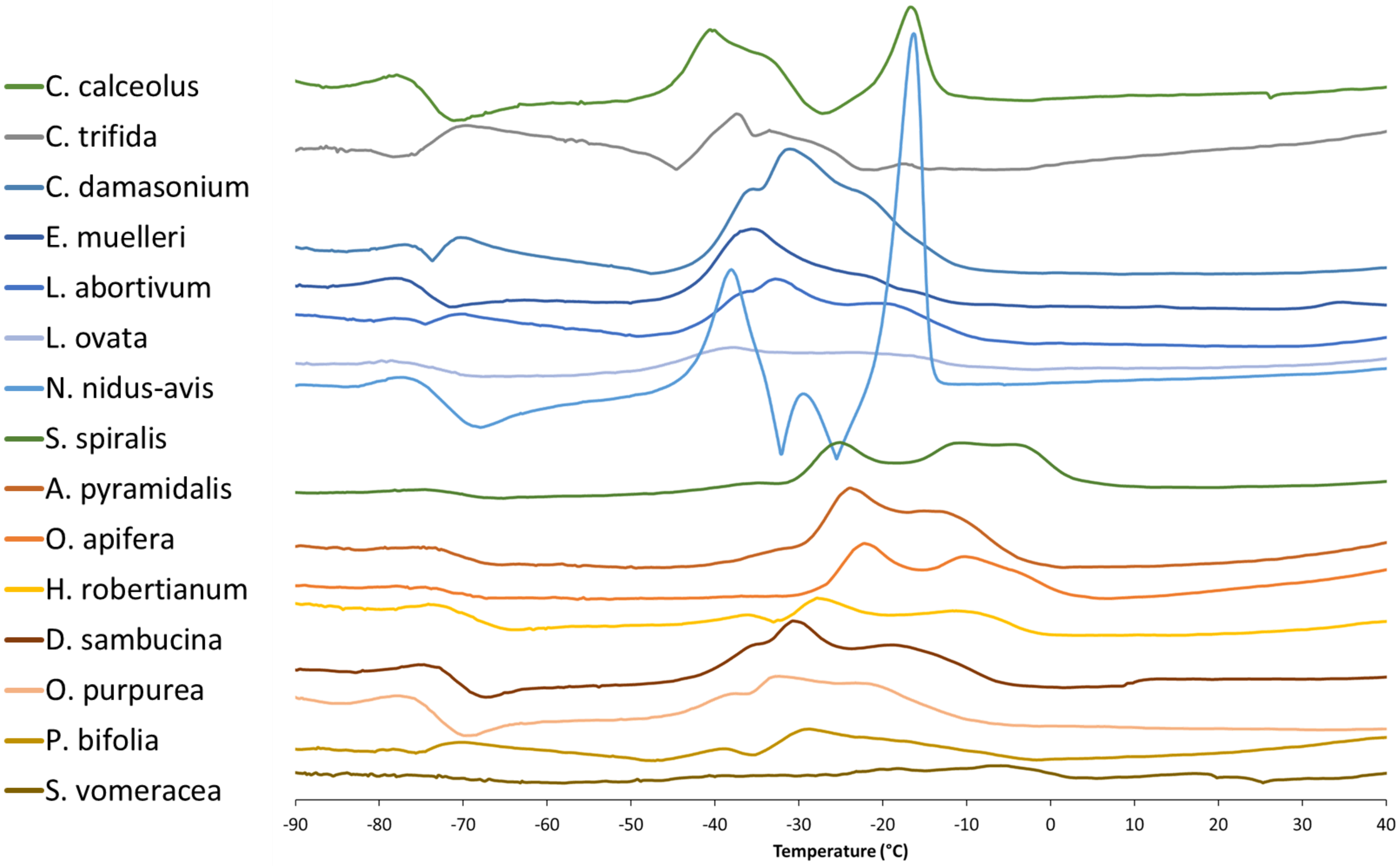
- Annealing (short storage) at -20°C :
  - 1: cooling : from 25°C to -20°C @ 10°C/min
  - 2: annealing at -20°C (60 min)
  - 3: cooling : from -20°C to -150°C @ 10°C/min
  - 4: warming: from -150°C to 40°C @ 10°C/min



- Annealing (short storage) at -70°C
  - 1: cooling : from 25°C to -70°C @ 10°C/min
  - 2: annealing at -70°C (60 min)
  - 3: cooling : from -70°C to -150°C @ 10°C/min
  - 4: warming: from -150°C to 40°C @ 10°C/min



RESULTS: Diversity of the thermal profiles



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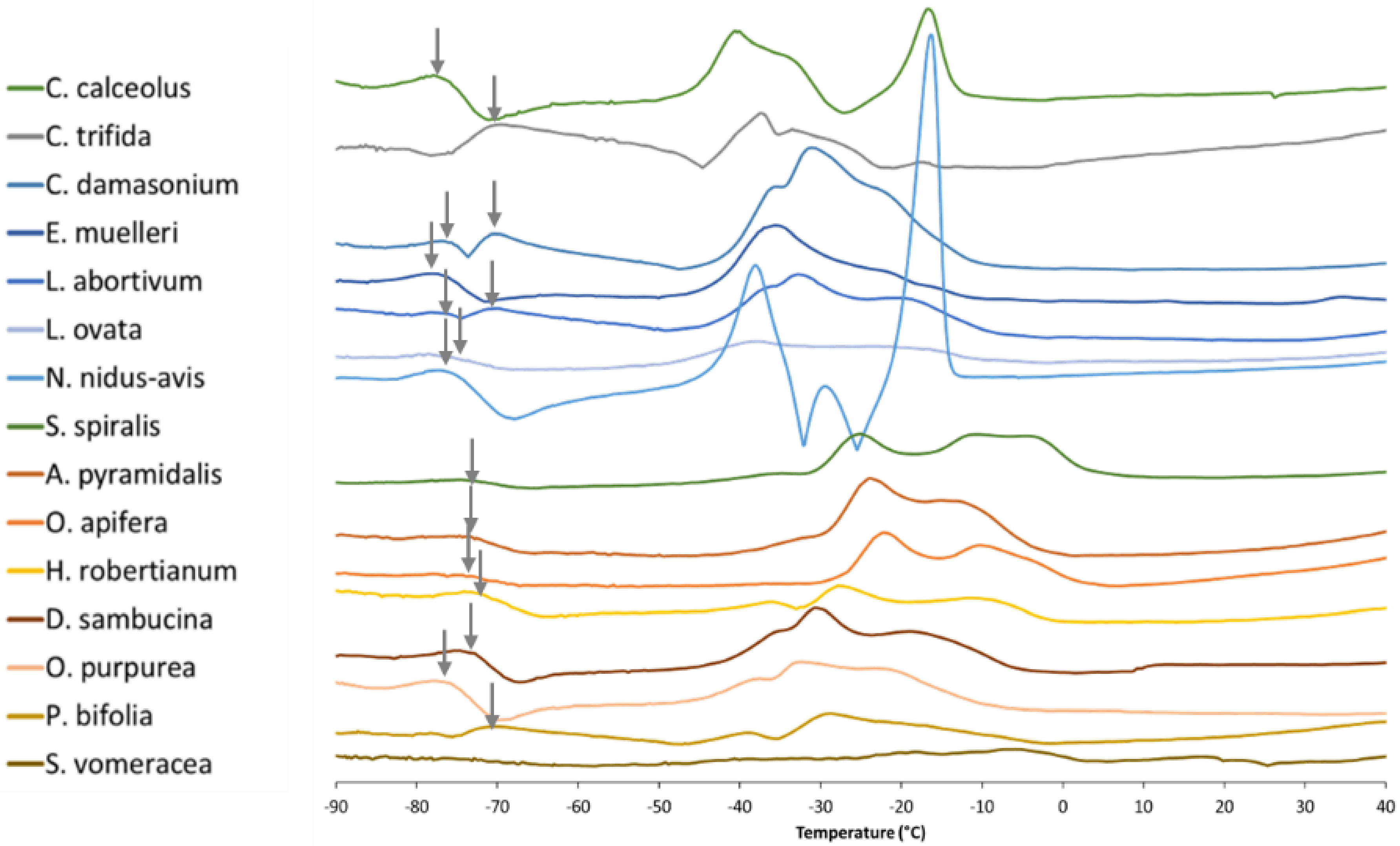
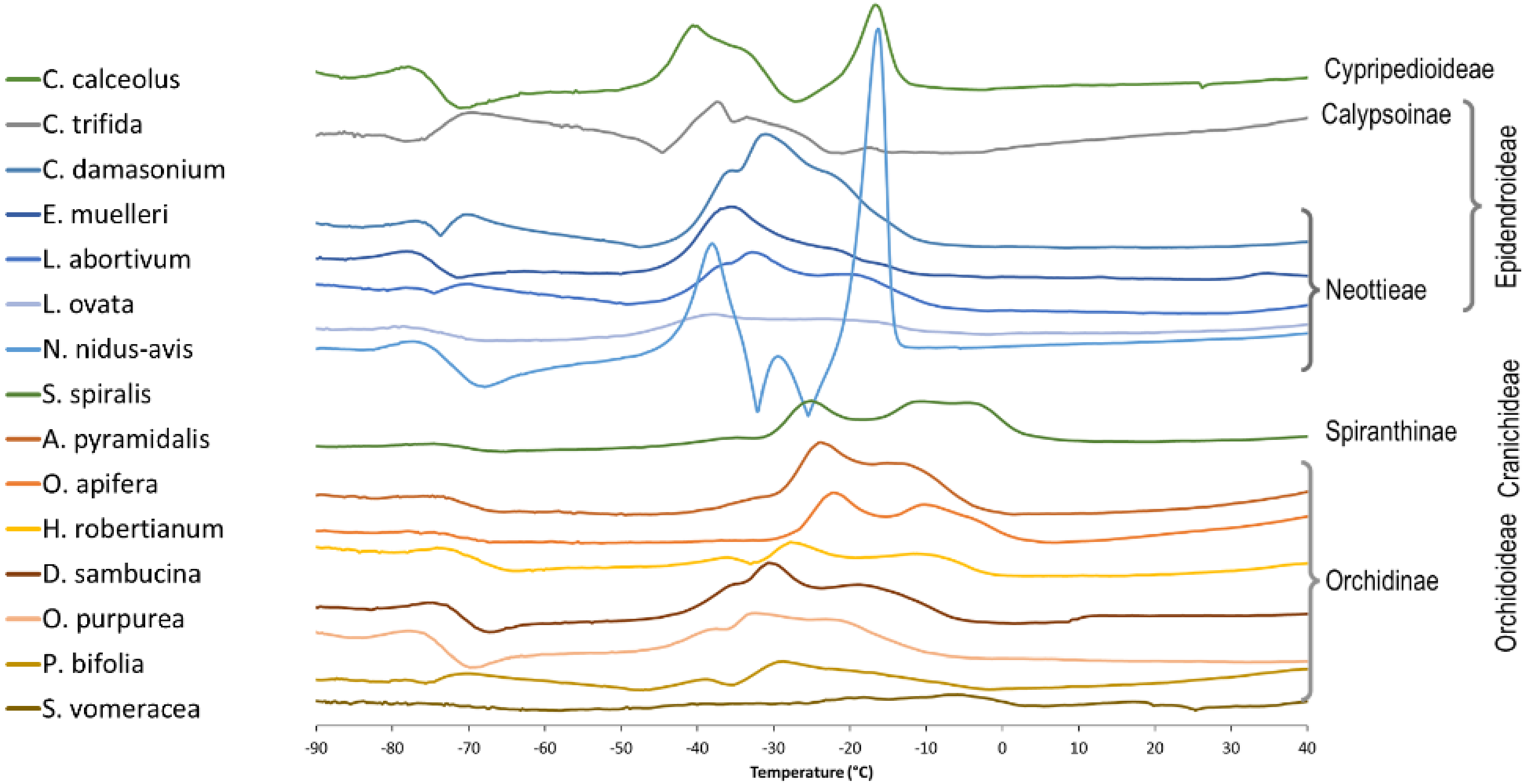
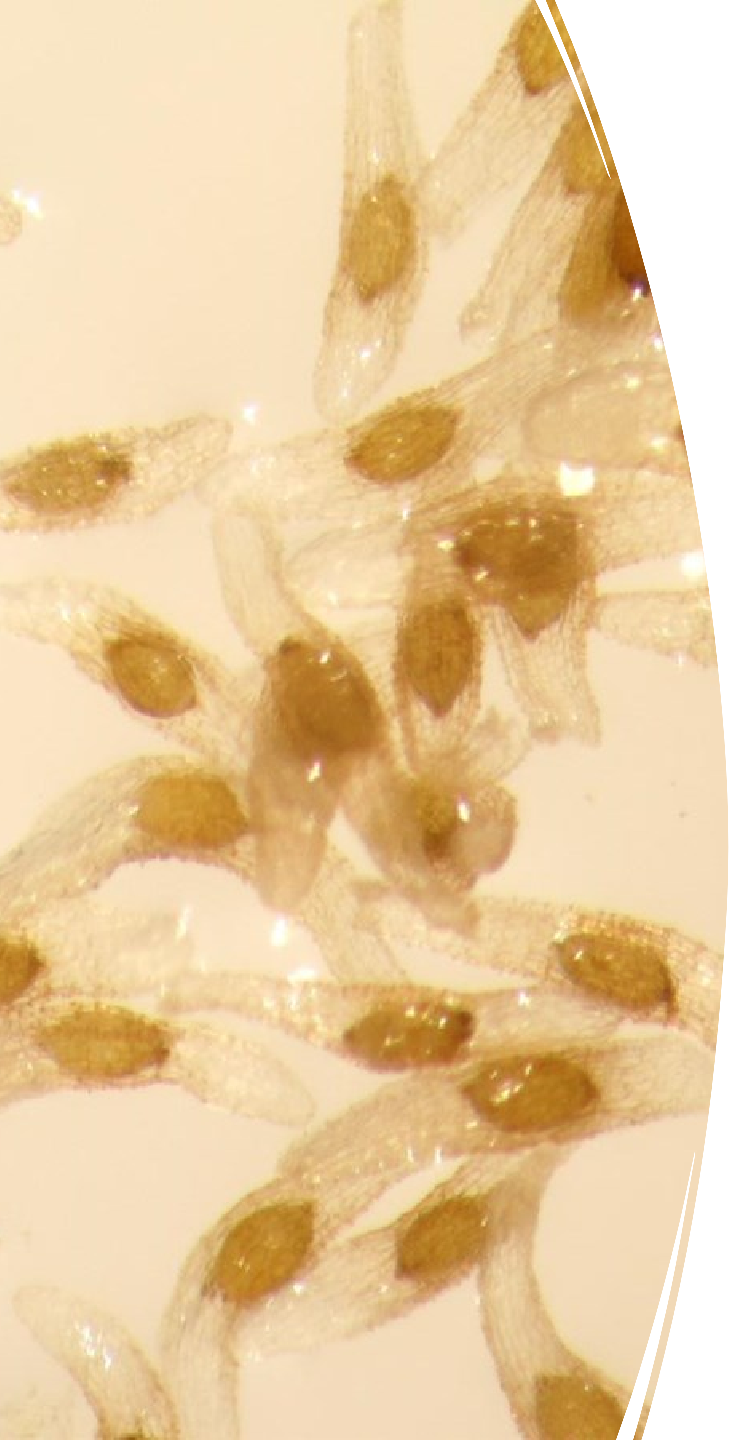


Lipid crystallization events during warming, after a small melting peak and prior to the main melting transitions are evident in 14 out of 15 species at onset temperature between -77°C (*Orchis purpurea*) and -69.5°C (*Himantoglossum robertianum*).
No crystallization events were noted in *Serapias vomeracea*.

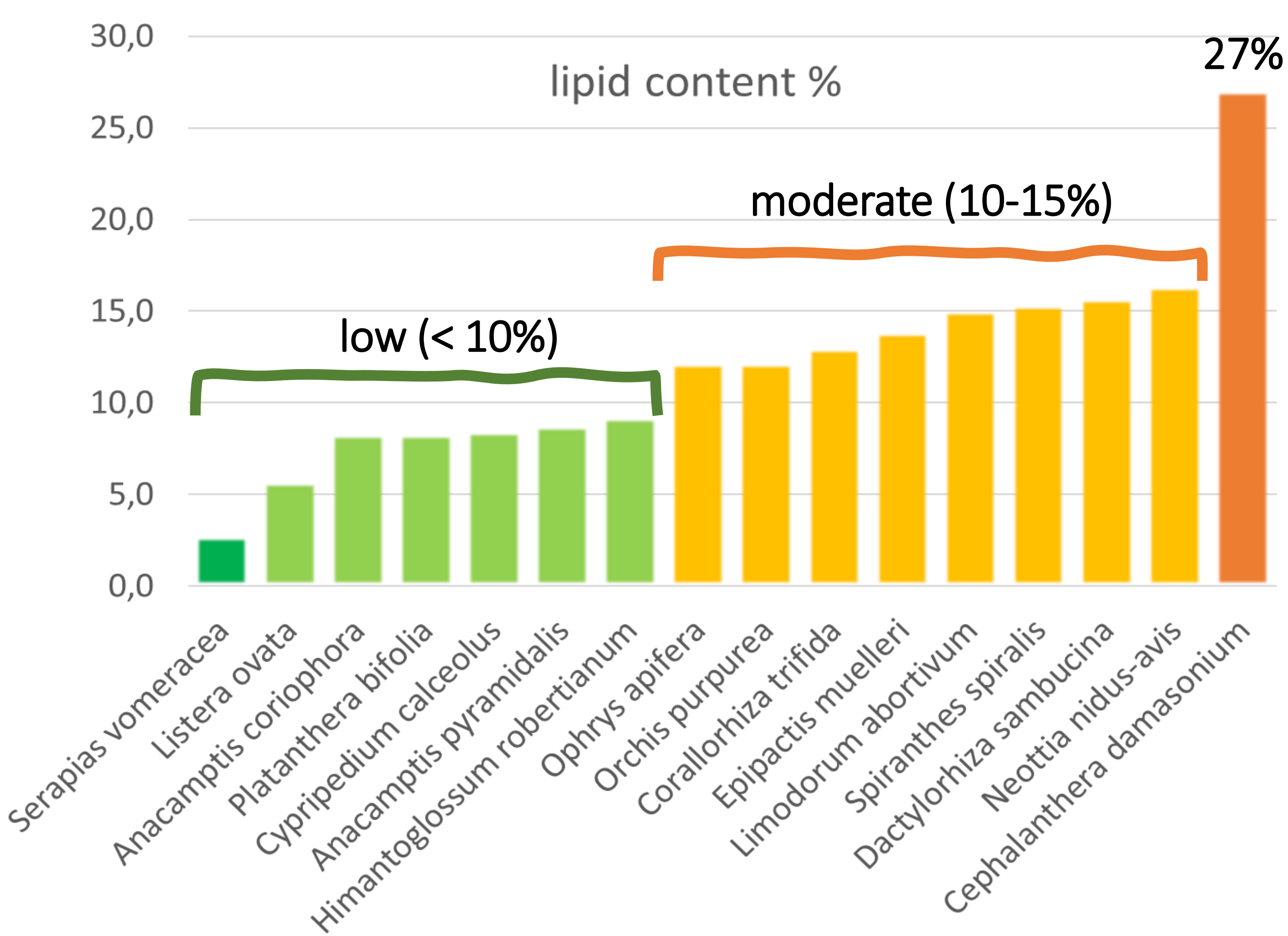
RESULTS: Diversity of the thermal profiles

Is the thermal profile correlated with phylogeny?





RESULTS: Lipid content



RESULTS: Lipid content & composition

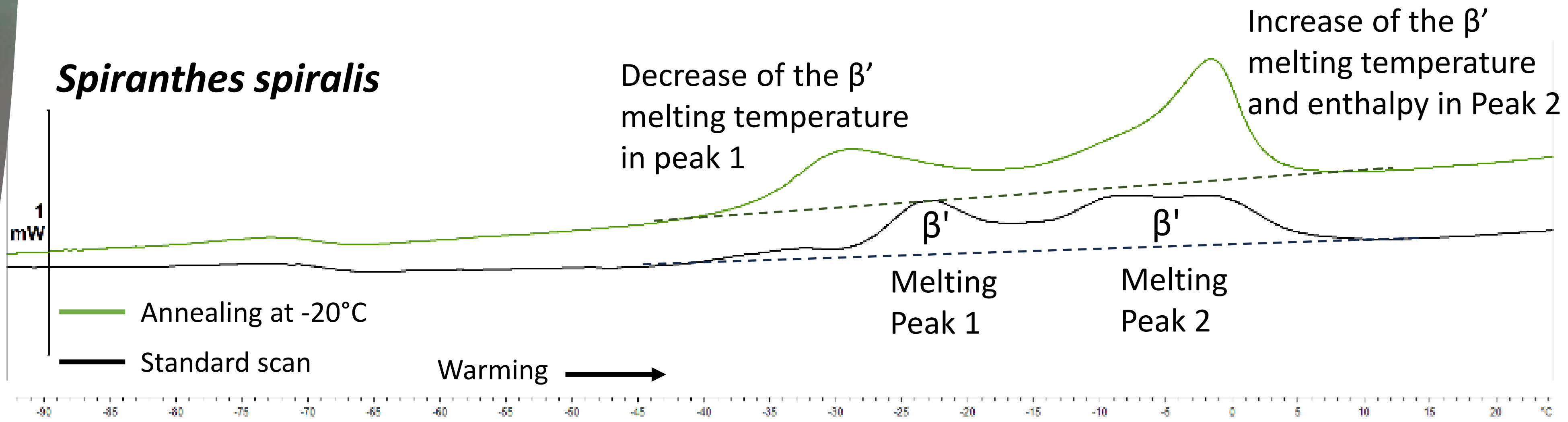
Species	Onset main β' melt	Onset other β' melt	Enthalpy main β' melt (mJ mg^{-1} DW)	Predominant FA[a]	Other FA	Lipid content % (estimated)[b]
Listera ovata	-46.6	-29.7	5.0 (0.1)	Linolenic	Linoleic	5.7
Corallorhiza trifida	-45.6	-35.1	11.4 (0.7)	Linolenic		13
Cypripedium calceolus	-45.4	-21.1	7.4 (0.7)	Linolenic	Linoleic	8.4
Limodorum abortivum	-43.1	-24.9	13.2 (0.7)	Linolenic	Linoleic	15
Epipactis muelleri	-42.8	-25.5	12.2 (0.6)	Linolenic	Linoleic	13.9
Orchis purpurea	-39.1	-26.8	10.7 (0.9)	Linolenic	Linoleic	12.2
Dactylorhiza sambucina	-38.4	-	13.8 (1.9)	Linolenic		15.7
Cephalanthera damasonium	-38.2	-	23.8 (1.0)	Linolenic		27
Platanthera bifolia	-35.4	-	7.3 (0.6)	linolenic		8.3
Himantoglossum robertianum	-33.2	-17.2	8.1 (1.1)	Linolenic	Linoleic	9.2
Anacamptis coriophora	-32.6	-	7.3 (1.2)	Linolenic		8.3
Spiranthes spiralis	-31.0	-5.5	13.5 (1.4)	Linolenic	Linoleic. oleic	15.3
Anacamptis pyramidalis	-30.1	-17.9	7.7 (1.2)	Linolenic	Linoleic	8.8
Ophrys apifera	-27.8	-14.6	10.7 (0.3)	Linolenic	Linoleic	12.2
Neottia nidus-avis	-20.4	-42.7	14.4 (0.1)	Linoleic	Linolenic	16.4
Serapias vomeracea	-19.2	-	2.4 (0.3)	Linoleic		2.7

Low temperature melting lipids: unsaturated FAs

[a] based on β' temperatures for simple TAG obtained from Small (1988) and Hageman (1972)
[b] considering that enthalpy of melt for linolenin and linolein = 88 J/g lipid*

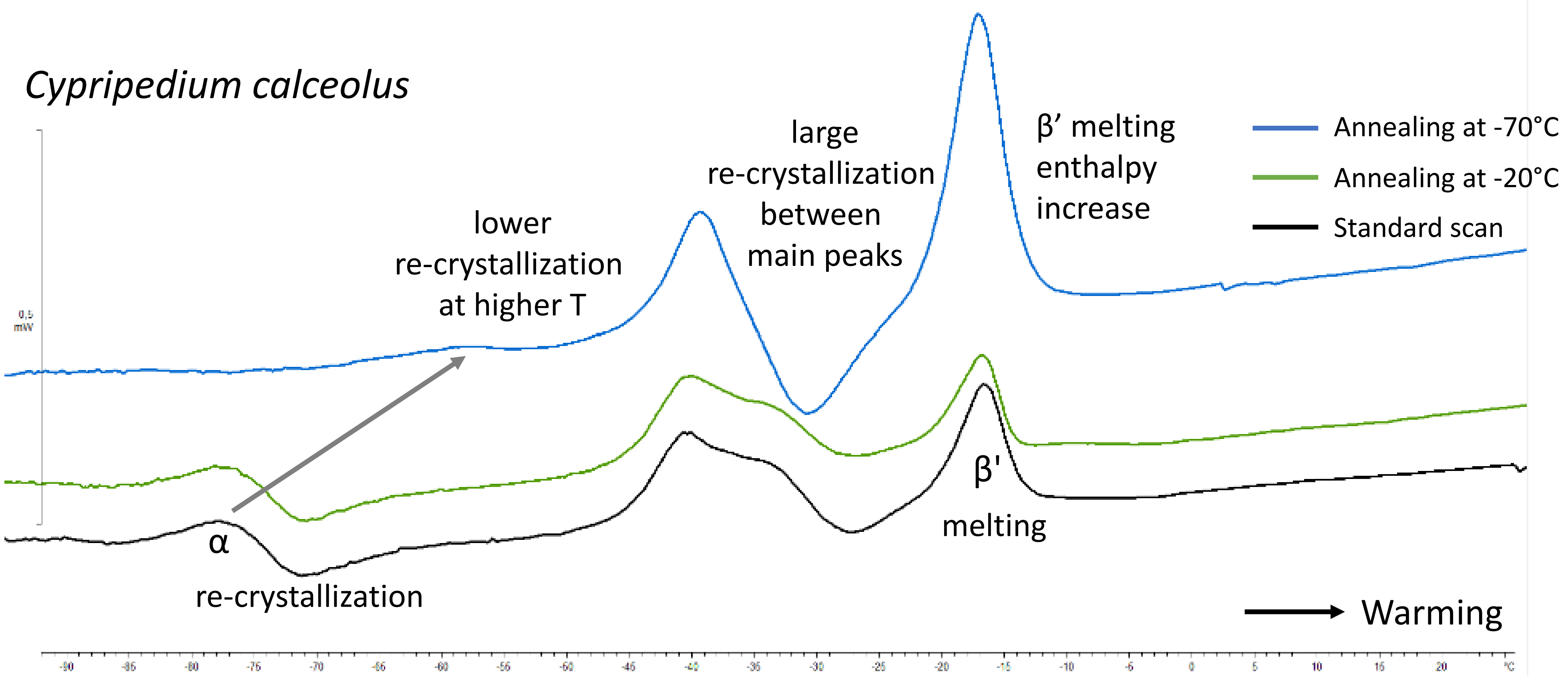
Potential damages under seed bank storage conditions

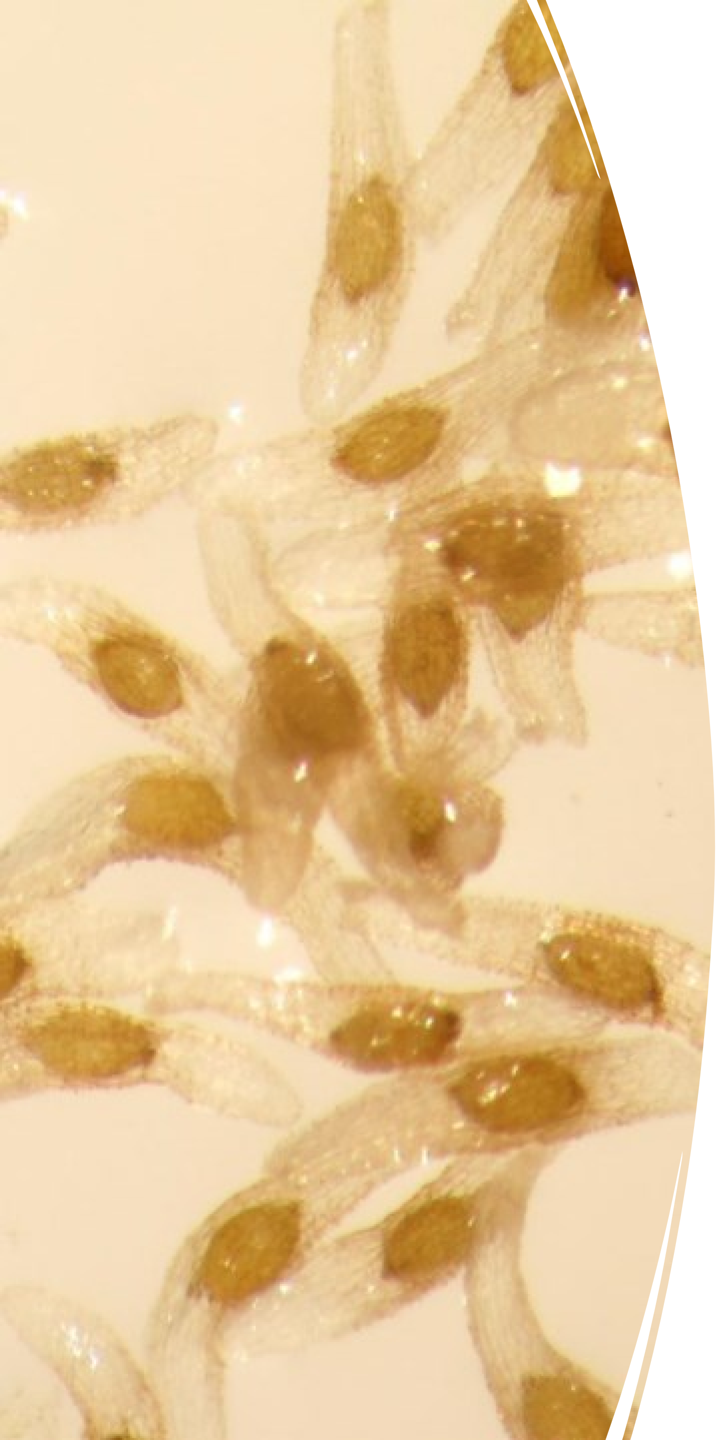
- Conventional seed bank conditions: storing seeds at sub-zero temperatures, between -24 and -18°C.
- **DSC can detect if there are changes in crystallisation kinetics that may affect storage stability**.
- During -20°C annealing, changes in the onset temperature and enthalpy of the melting peaks can be used to predict if terrestrial orchid seeds may present storage issues.



What happens at colder temperatures?

The seed lipid thermal fingerprint was also studied after 60 min annealing at -70°C to assess the potential effect of ultra-low storage as an alternative conservation method.





Conclusions

- High diversity of lipid thermal profiles, with some species (e.g. *Neottia nidus-avis* and *Cypripedium calceolus*) showing complex profiles.
- **Thermal profiles tend to be conserved phylogenetically**, except for *N. nidus-avis*.
- **All species show the main lipid melting peaks <-20°C**, indicating a high degree of unsaturated fatty acids (linoleic, linolenic) as is typical for seeds of **temperate environments**. Only *Spiranthes spiralis* and *Ophrys apifera* show secondary melting peaks with a higher temperature end that may correspond to TAG composed of oleic acid.
- Orchid seeds studied are estimated to have **moderated (10-15%) to low (5-10%) or very low (3% in *Serapias vomeracea*) lipid contents**, except for *Cephalanthera damasonium* (27%).

Conclusions
Long-term storage

- **Short storage times at -20°C induced large changes in the thermal profile of *Spiranthes spiralis* and *Ophrys apifera***, which may be indicative of **potential damage during storage** at the standard temperatures of a seed bank due to the fast crystallisation kinetics of the lipids in the dry seeds.
- **Short storage times at -70°C (re-crystallisation temperature of α crystals) induced large changes in the thermal profile of *Epipactis muelleri* and *Cypripedium calceolus***, which may also be indicative of potential damage during ultra-cold storage.

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Thank you for your kind attention!!!