# *7TH INTERNATIONAL ORCHID CONSERVATION CONGRESS*

Jodrell Laboratory
Royal Botanic Gardens, Kew
28 May – 1 June 2019

# *Welcome to Kew!*

The International Orchid Conservation Congress (IOCC) series started in Western Australia in 2001, and subsequent meetings were held in Florida, Costa Rica, the Czech Republic, La Réunion and Hong Kong. At the meeting in Hong Kong, it was decided that IOCCVII should be held at the Royal Botanic Gardens, Kew. The title for IOCCVII is "Orchid Conservation: the Next Generation", and we hope that the focus will be on the importance of involving the next generation of orchid conservation biologists and on the use of next-generation techniques.

We are delighted to welcome more than 150 delegates from around the world to share their experiences of orchid conservation with each other. Orchids are among the most highly threatened groups of plants, with threats to orchids including habitat destruction, legal and illegal trade and climate change. As a result, our mission to conserve orchids is of increasing importance – if we don't act, then we may be the last generation to see some groups of orchids, notably slipper orchids, in the wild.

We are grateful to the Royal Botanic Gardens, Kew, for the use of the venue and for logistical support. The Lennox Boyd Trust, the Linnean Society of London and Orchid Conservation International and friends and family of the late Amy Morris provided financial support which has allowed us to provide bursaries for orchid specialists from Developing Countries so that they can attend the congress.

We have an exciting and busy programme – with many talks and posters and other events including a demonstration of orchid propagation, a "World Café" session on conservation planning (arranged with the Conservation Planning Specialist Group) and a meeting of the Orchid Specialist Group.

We will keep you busy, but we hope that you will also take the opportunity for a walk in the gardens between the talks. On Friday, we have three field trips that will give delegates the opportunity to see Wakehurst Place and the Millennium Seed Bank, native chalk grassland flora or the home of Charles Darwin.

The Organising Committee would like to take this opportunity to extend a warm welcome to Kew, and we hope you all enjoy the congress!

Royal Botanic Gardens
Kew

# General information

## Meeting location

Jodrell Laboratory
Royal Botanic Gardens, Kew,
Kew Road
Richmond, Surrey TW9 3DS

The closest National Railway station is Kew Bridge (a 15-min walk) and the closest London Underground Station is Kew Gardens station, located in Kew village (a 10-min walk) (see maps below).

All talks and panel discussions will take place in the Jodrell Laboratory. Refreshment breaks, lunches and the evening poster session and drinks reception will also be held in the Jodrell Laboratory.

To access the building, please enter the Gardens via the Jodrell Gate on Kew Road. A member of staff will be present at the gate on Monday afternoon (14:00 – 17:00) and on Tuesday morning (08:00 – 09:00). If you arrive/depart outside these hours or the gate is unattended, please call RBG Kew Constabulary using the telephone in the yellow box on the wall by the gate. You will be connected automatically when you pick up the receiver. Please give Constabulary your name and let them know that you are attending the Orchid Congress. They will then remotely release the pedestrian gate.

## Poster presentations

Posters will be open for viewing on Wednesday afternoon. Please ensure that your poster is mounted by Wednesday lunchtime. A designated poster session will take place from 16:00–18:00 on Wednesday 29th May.

## Internet access

Free Wi-Fi access is available in all our buildings including the Jodrell Laboratory. To connect to our Wi-Fi, please select Kew-Visitors from the available networks and accept the terms and conditions. If you have access to Eduroam, then this is also available in the venue.

## Twitter

If you are tweeting about the congress, please use the hashtag #IOCCVII.

## First aid

Should you need assistance, please ask at the Jodrell reception desk or speak with a member of staff.

**Emergencies**

For emergencies, please dial 020 8332 3333 (or extension 333 from a Kew telephone). Please DO NOT dial 999, as emergency calls need to be co-ordinated by the on-site Constabulary team.

## Fire

The signal to evacuate is a loud, two-tone alarm. On hearing the alarm, please proceed directly to the nearest emergency exit. DO NOT use the lifts. The fire assembly point is the GRASS GARDEN at the rear of the Jodrell Laboratory, beyond the waterlily pond.

## Taxis

Kew Cars: 020 8568 6666

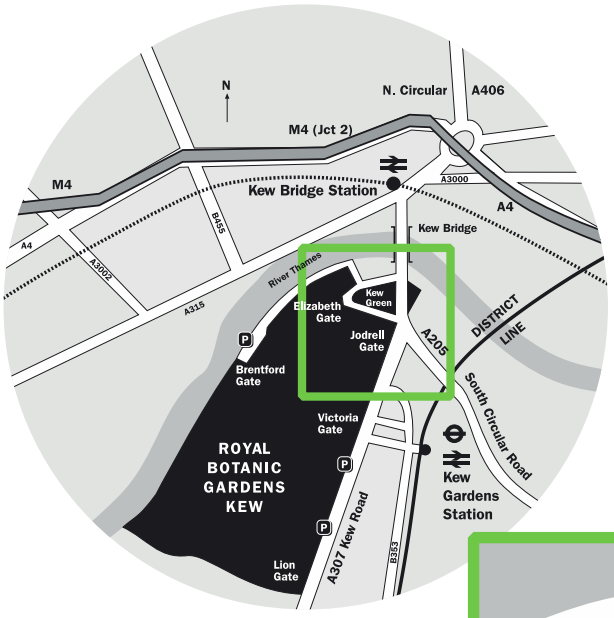
## Nearby facilities

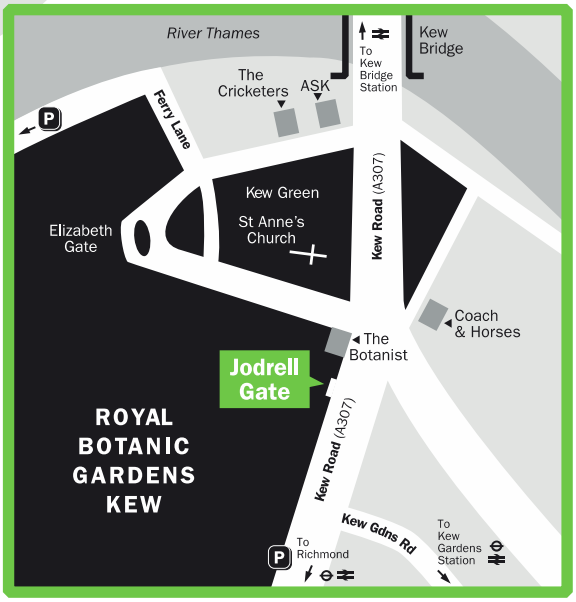
The retail store at Victoria Plaza (situated by Victoria Gate) sells a variety of books, gifts and gardening supplies. Delegates are offered a 10% on all full price products (including books, gifts etc.) in the Victoria Gate shop at Kew during the Congress. To obtain this discount, you will need to show your delegate badge. You can also find a range of shops and restaurants near to Kew Bridge railway station, Kew Gardens station and Kew Green.

## Car parking

There is no parking available within the Royal Botanic Gardens, but there is a public car park which can be reached from Kew Green via Ferry Lane. Alternatively, there is on-street parking around Kew Green (but note that the side towards the buildings is for residents only).

# How to get to **Jodrell Gate**



# 7TH INTERNATIONAL ORCHID CONSERVATION CONGRESS

## Royal Botanic Gardens, Kew

## 28 May – 1 June 2019

## PROGRAMME AT A GLANCE

## #IOCCVII

**Monday 27th May**

14.00 – 17.00: Registration desk open, Jodrell Laboratory (entry through Jodrell Gate)

**Tuesday 28th May**

| | |
|---|---|
| 08:00 – 09:00 | Registration desk open |
| 09:00 – 09:30 | **Opening Ceremony & Welcome**<br>Opening remarks: *Prof. Michael F. Fay, Chair, Organising Committee*<br>Official Welcome: *Prof. Alexandre Antonelli, Director of Science, RBG Kew* |
| **09:30 – 10:15** | **Plenary Lecture: Leaping into the decade of orchid restoration**<br>***Kingsley Dixon, Curtin University*** |
| 10:15 – 10:45 | Tea Break |
| **10:45 – 16:00** | **Session 1: Conservation** |
| 12:45 – 14:00 | Group Photo and Lunch |
| **4:00 – 16:00** | **Session 1: Conservation (continued)** |
| 16:00 - 16:30 | Tea Break |
| **16:30 – 17:45** | **Session 2: Restoration** |

**Wednesday 29th May**

| | | | |
|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: Creating sustainable conservation of orchids through sound business development - saving China's slipper orchids**<br>***Wenqing Perner, Hengduan Mountains Biotechnology*** | | |
| **09:45 – 11:00** | **Session 3: *Ex Situ* Conservation** | | |
| 11:00 – 11:15 | Tea Break | | |
| **11.15 – 12:00** | **Session 3: *Ex Situ* Conservation** | | |
| **12:00 – 13:00** | **Orchid Specialist Group meeting and discussion (all welcome)**<br>***Chair: Michael F. Fay*** | | |
| 13:00 – 14:00 | Lunch | | |
| **14:00 – 14:45** | **Session 4: Education** | **14:00 – 15:00** | **Session 5: Population Biology** |
| 15:00 – 18:00 | **Writhlington School – demonstration of orchid propagation** | | |
| **16:00 - 18:00** | **Poster Presentations and Reception** | | |

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**Thursday 30th May**

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|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: Illegal trade in wild orchids: an overlooked conservation challenge**<br>***Luciano Ramos Zandoná, Botanical Institute of São Paulo, Brazil*** | | |
| **09:45 – 11:30** | **Session 6: Trade** | | |
| 11:30 – 11:45 | Tea Break | | |
| **11:45 – 12:10** | **From Orchids to Oryx: species conservation planning and the CPSG**<br>***Jamie Copsey, Conservation Planning Specialist Group*** | | |
| **12:15 – 17:30** | **Session 7: Conservation Planning, Red Listing and Conservation Assessment** | **12:15 – 15:15** | **Section 8: Molecular Tools and Morphology** |
| 13:00 – 14:00 | Lunch | | |
| 14:00 – 15:30 | **Conservation Planning "World Café" (Sign-up session)**<br>***Led by Jamie Copsey*** | | **Session 8 (continued)** |
| 15:15 – 15:45 | Tea Break | | |
| 15.45 – 16.30 | **Conservation Planning "World Café" (continued) (Sign-up session)** | **15:45 – 17:30** | **Session 9: Demography** |
| 16:30 – 16:45 | **Section 7 (continued)** | | **Section 9 (continued)** |
| 17:30 – 18:45 | Film on orchid trade/Free time for a walk in the Gardens | | |
| **19:00-21:30** | **Congress dinner: Orangery Restaurant (inside Kew Gardens)** | | |

**Friday 31st May:**

**Congress Field Trips – these leave from the Elizabeth Gate at 09:00 and return c. 18:00.**

**Saturday 1st June**

| | | | |
|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: How future proof are our orchids? Orchid conservation in the genomics era**<br>***Barbara Gravendeel, Naturalis Biodiversity Center Netherlands*** | | |
| **09:45 – 11:15** | **Session 10: Genetics** | **09:45 – 14:30** | **Session 11: Mycorrhizal Associations and Seed Germination** |
| 11:15 – 11:45 | Tea Break | | |
| **11:45 – 13:00** | **Session 12: Interactions with Other Organisms (Pollination)** | | **Section 11 (continued)** |
| 13:00 – 14:00 | Lunch | | |
| **14:00 – 15:15** | **Session 13: Ecology** | | **Section 11 (continued)** |
| 15:00 – 15:30 | Tea Break | | |
| **15:30 – 16:30** | **Closing ceremony** | | |

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# *FULL PROGRAMME*

**Monday 27th May**

14.00-17.00: Registration desk open

**Tuesday 28th May**

| | |
|---|---|
| 08:00 – 09:00 | Registration desk open |
| 09:00 – 09:30 | **Opening Ceremony & Welcome**<br>Opening remarks: *Prof. Michael F. Fay, Chair, Organising Committee*<br>Official Welcome: *Prof. Alexandre Antonelli, Director of Science, RBG Kew* |
| **09:30 – 10:15** | **Plenary Lecture: Leaping into the decade of orchid restoration**<br>***Kingsley Dixon, Curtin University.*** |
| 10:15 – 10:45 | Tea Break |
| **10:45 – 16:00** | **Session 1: Conservation**<br>***Chair: Ken Cameron*** |
| 10:45 – 11:00 | Orchid conservation via habitat preservation<br>*Steve Beckendorf* |
| 11:00 – 11:15 | Orchid conservation achievements from China National Orchid Conservation Center<br>*Meina Wang* |
| 11:15 – 11:30 | The importance of private reserves in orchid conservation – case studies from Colombia<br>*Marta Kolanowska (and Sebastián Vieira)* |
| 11:30 – 11:45 | Vanilla crop wild relatives and their microbiome: perspectives for conservation and sustainable use<br>*Nicola S. Flanagan* |
| 11:45 – 12:00 | When age old tools are still required for modern orchid conservation practices<br>*Hildegard Crous* |
| 12:00 – 12:15 | The Palau Orchid Conservation Initiative: an integrated approach to orchid research and management<br>*Benjamin J. Crain* |
| 12:15 – 12:30 | Medicinal orchids of Nepal: efforts for conservation, challenges, and future directions<br>*Bijaya Pant* |
| 12:30 – 12:45 | Integrating systematics and reproductive biology in African orchid conservation: a case study with the genus *Cyrtorchis* Schltr. (Angraecinae)<br>*Laura N. Azandi* |
| 12:45 – 14:00 | Group Photo and Lunch |

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| **14:00 – 16:00** | **Session 1: Conservation (continued)**<br>***Chair: Steve Beckendorf*** |
| 14:00 – 14:15 | Safeguarding imperiled orchid species in the southeastern United States and Caribbean<br>*Emily Coffey* |
| 14:15 – 14:30 | Strategies for orchid conservation of the Atlantic Forest in Legado das Águas, São Paulo, Brazil<br>*Luciano Ramos Zandoná* |
| 14:30 – 14:45 | The eastern underground orchid *Rhizanthella slateri* (Diurideae) – biology, phylogeny and conservation<br>*Mark Clements* |
| 14:45 – 15:00 | Conservation of North America's native *Vanilla* species<br>*Ken Cameron and W. Grant Morton* |
| 15:00 – 15:15 | Recent advances and dispelled myths: propagation, flowering and conservation of the epiphytic ghost orchid (*Dendrophylax lindenii*)<br>*Michael E. Kane* |
| 15:15 - 15:30 | Orchid diversity: environmental drivers and consequences for orchid conservation in Mediterranean archipelagos<br>*Michele Lussu* |
| 15:30 - 15:45 | NGS-based multidisciplinary approach for the conservation of the Mediterranean *Orchis patens*<br>*Jacopo Calevo* |
| 15:45 – 16:00 | New insight into orchid conservation in the Indo-Burma Biodiversity Hotspot: a case study from *Bulbophyllum bicolor* Lindl.<br>*Hu Ai-Qun* |
| 16:00 - 16:30 | Tea Break |
| **16:30 – 17:45** | **Session 2: Restoration**<br>***Chair: Kingsley Dixon*** |
| 16:30 – 16:45 | Restoration of orchid populations in cultural landscape: fungus availability matters<br>*Tamara Těšitelová* |
| 16:45 – 17:00 | The Million Orchid Project: non-traditional approach to orchid conservation and restoration<br>*Jason L. Downing* |
| 17:00 – 17:15 | Survival and prosperity of common alvar orchids after habitat restoration<br>*Kadri Tali* |
| 17:15 – 17:30 | Evolution of the integrated conservation strategy for fen orchid in England<br>*Tim Pankhurst* |
| 17:30 – 17:45 | Efforts to establish new populations of Spicer's paphiopedilum: a critically endangered orchid in South China<br>*Jiang-Yun Gao* |

**Wednesday 29th May**

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|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: Creating sustainable conservation of orchids through sound business development - saving China's slipper orchids**<br>***Wenqing Perner, Hengduan Mountains Biotechnology*** | | |
| **09:45 – 11:30** | **Session 3: *Ex Situ* Conservation**<br>***Chair: Noushka Reiter*** | | |
| 09:45 – 10:00 | Horticulture and biotechnology as strategies for *in-situ* and *ex-situ* orchid conservation in Colombia<br>*Tatiana Arias* | | |
| 10:00 – 10:15 | Conserving the UK's Orchids at the Millennium Seed Bank (Royal Botanic Gardens, Kew)<br>*Jennifer L. Peach* | | |
| 10:15 – 10:30 | Are the dust-like seeds of orchids always dormant?<br>*Hugh W. Pritchard* | | |
| 10:30 – 10:45 | Seed viability assessment of *Cymbidium* species and their conservation using cryopreservation<br>*Namrata Pradhan* | | |
| 10:45 – 11:00 | Rescuing orchids from logging concessionaries for rapid documentation and conservation strategy in Peninsular Malaysia<br>*Rusea Go* | | |
| 11:00 – 11:15 | Tea Break | | |
| 11:15 – 11:30 | Chikanda: Zambia's wild edible orchids<br>*Geophat Mpatwa* | | |
| 11.30 – 11.45 | Propagation for conservation: edible orchids of Zambia<br>*Jonathan P. Kendon* | | |
| 11:45 – 12:00 | Rediscovery and conservation of the rare *Octomeria estrellensis*. Production of seedlings traced with guarantee of origin, a great step in favour of conservation<br>*Luciano Ramos Zandoná* | | |
| **12:00 – 13:00** | **Orchid Specialist Group meeting and discussion (all welcome)**<br>***Chair: Michael F. Fay*** | | |
| 13:00 – 14:00 | Lunch | | |
| **14:00 – 14:45** | **Session 4: Education**<br>***Chair: Phil Seaton*** | **14:00 – 15:15** | **Session 5: Population Biology**<br>***Chair: Tiiu Kull*** |
| 14:00 – 14:15 | A teacher's view - thirty years of the Writhlington School Orchid Project<br>*Simon Pugh-Jones* | 14:00 – 14:15 | Small populations on small islands: what chance does an orchid have?<br>*James D. Ackerman* |
| 14:15 – 14:30 | The students' experience of the Writhlington School Orchid Project<br>*Jess Buckle* | 14:15 – 14:30 | Irresistibly fragrant: a key innovation for the long-distance dispersal of wild *Vanilla* seeds<br>*Adam Karremans* |
| 14:30 – 14:45 | Conservation education<br>*Philip Seaton* | 14:30 – 14:45 | Features of population biology of some Neottieae species in Russia<br>*Ekaterina Zheleznaia* |
| | [set up for propagation demo] | 14:45 – 15:00 | Demographic and genomic consequences of landscape transformation for a tropical Andean epiphytic orchid<br>*Nhora Helena Ospina-Calderón* |
| | | 15:00 – 15:15 | Revisiting the life cycle of Helen's bee orchid (*Ophrys helenae* Renz) using a dynamic population model<br>*Martha Charitonidou* |
| 15:00 – 18:00 | **Writhlington School – demonstration of orchid propagation** | | |
| **16:00 - 18:00** | **Poster Presentations and Reception** | | |

**Thursday 30th May**

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|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: Illegal trade in wild orchids: an overlooked conservation challenge**<br>***Luciano Ramos Zandoná, Botanical Institute of São Paulo Brazil.*** | | |
| **09:45 – 11:30** | **Session 6: Trade**<br>***Chair: Amy Hinsley*** | | |
| 09:45 – 10:00 | A study on wild orchid trade in a Chinese e-commerce market and its application in conservation<br>*Shan Wong* | | |
| 10:00 – 10:15 | Is over-harvest a major threat to the Chinese orchids? An analysis of the Chinese Redlist<br>*Hong Liu* | | |
| 10:15 – 10:30 | An assessment of medicinal orchid trade in Nepal<br>*Reshu Bashyal* | | |
| 10:30 – 10:45 | Waiting for the salep storm: what future for the protected orchids of Greece?<br>*John M. Halley* | | |
| 10:45 – 11:00 | Commercial interest in endangered terrestrial orchids demonstrated by systematic analysis of patent applications using salep<br>*Susanne Masters* | | |
| 11:00 – 11:15 | Trade profiles of Malagasy orchids under the light of data from literature and citizen science<br>*Narindra Randriamialisoa* | | |
| 11:15 – 11:30 | Study on edible orchids activities in the Nyika National Park and the surrounding communities with focus on drivers and consequences to find entry points for action<br>*Heejoo Lee* | | |
| 11:30 – 11:45 | Tea Break | | |
| **11:45 – 12:10** | **From Orchids to Oryx: species conservation planning and the CPSG**<br>***Jamie Copsey, Conservation Planning Specialist Group*** | | |
| **12:15 – 17:30** | **Session 7: Conservation Planning, Red Listing and Conservation Assessment**<br>***Chair: Hassan Rankou*** | **12:15 – 15:15** | **Session 8: Molecular Tools and Morphology**<br>***Chair: Barbara Gravendeel*** |
| 12:15 – 12:30 | From IUCN conservation assessments to seed bank: conserving threatened orchids in Africa and Madagascar<br>*Simon Verlynde* | 12:15 – 12:30 | Target sequence capture in orchids: developing a kit to sequence hundreds of single copy loci across all orchids<br>*Lauren A. Eserman* |
| 12:30 – 12:45 | Global conservation prioritization of Orchidaceae<br>*Demetra Rakosy* | 12:30 – 12:45 | Next-generation sequencing of orchids and their mycorrhizal fungi<br>*Sarah Unruh* |
| 12:45 – 13:00 | A continental scale analysis of threats to orchids in Australia<br>*Jenna Wraith* | 12:45 – 13:00 | Investigating hybridization and gene flow between *Orchis militaris* and *O. purpurea*: evidence from nuclear microsatellites and geometric morphometrics<br>*Leif Bersweden* |
| 13:00 – 14:00 | Lunch | | |
| | **Section 7 (continued)**<br>***Chair: Tariq Stévart*** | | **Session 8 (continued):**<br>***Chair: Barbara Gravendeel*** |
| 14:00 | **Conservation Planning "World Café" (Sign-up session)**<br>***Led by Jamie Copsey, Conservation Planning Specialist Group*** | 14:00 – 14:15 | Genomic diversity in Australian orchid complexes and its implications for taxonomy and conservation<br>*Katharina Nargar* |
| | | 14:15 – 14:30 | Utility of DNA Barcodes and Stable Carbon Isotope Signatures on Philippine Orchid Taxonomy and Ecology<br>*Maribel Agoo* |

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| | | 14:30 – 14:45 | New evidence of hybridization in *Platanthera* species<br>*Marilin Mõtlep* |
| | | 14:45 – 15:00 | Chemical identification promotes the conservation of cryptic sexually-deceptive orchid taxa<br>*Alyssa M. Weinstein* |
| | | 15:00 - 15:15 | True staminodes of Cypripedioideae<br>*Marpha Telepova-Texier* |
| 15:15 – 15:45 | Tea Break | | |
| 15.45 – 16.30 | **Conservation Planning "World Café" (continued) (Sign-up session)** | **15:45 – 17:30** | **Session 9: Demography**<br>***Chair: James Ackerman*** |
| | | 15:45 – 16:00 | The demography of orchids: life history, population dynamics, and global rarity<br>*Tiiu Kull* |
| | | 16:00 – 16:15 | Inherently at risk? Does morphological variation predict conservation status?<br>*Raymond L. Tremblay* |
| | | 16:15 – 16:30 | Impact of root system type and elevation on the richness and distribution of orchid flora in the Czech Republic<br>*Zuzana Štípková* |
| 16:30 – 16:45 | Red Listing of New Guinea orchids<br>*André Schuiteman and Helen Chadburn* | 16:30 – 16:45 | Habitat and phorophyte comparisons of the mule-ear orchid, *Trichocentrum undulatum* between the core and edge distribution: a Cuba and southern Florida story<br>*Haydee Borrero* |
| 16:45 – 17:00 | Opportunities and pitfalls in rapid conservation assessment methods for orchids<br>*Barnaby E. Walker* | 16:45 – 17:00 | *Tridactyle* (Orchidaceae): a story of speciation and colonisation in São Tomé and Príncipe<br>*Tania D'haijère* |
| 17:00 – 17:15 | *Habenaria occlusa*, *Eulophia schweinfurthii*; are they Rare, Extinct (EX) or Critically Endangered (CR)?<br>*Lourance Mapunda* | 17:00 – 17:15 | Preliminary floristic checklist of orchids: Flora of Gashaka Gumti National Park, Nigeria<br>*George Nodza* |
| 17:15 – 17:30 | A proposal for the integration of seven orchid species endemics of Sardinia in the IUCN list: a tool for conservation of endangered species<br>*Pierluigi Cortis* | 17:15 – 17:30 | Orchids from Galápagos Islands<br>*Galo Jarrín* |
| 17:30 – 18:45 | Film on orchid trade/Free time for a walk in the Gardens | | |
| **19:00-21:30** | **Congress dinner: Orangery Restaurant (inside Kew Gardens)** | | |

**Friday 31st May**

**Congress Field Trips:**

Field Trip 1: Box Hill (chalk grassland and woodland flora).
Field Trip 2: Down House (Darwin's home and garden).
Field Trip 3: Wakehurst Place and Millennium Seed Bank.

**Saturday 1st June**

| | | | |
|---|---|---|---|
| **09:00 – 09:45** | **Plenary Lecture: How future proof are our orchids? Orchid conservation in the genomics era**<br>***Barbara Gravendeel, Naturalis Biodiversity Center Netherlands.*** | | |
| **09:45 – 11:15** | **Session 10: Genetics**<br>***Chair: Juan Viruel*** | **09:45 – 14:30** | **Session 11: Mycorrhizal Associations and Seed Germination**<br>***Chair: Larry Zettler*** |
| 09:45 – 10:15 | Origins of allotetraploids in *Dactylorhiza* (Orchidaceae): insights from coalescent and maximum likelihood analyses of RADseq data<br>*Mark Chase* | 09:45 – 10:15 | The use of stable isotope natural abundance (d13C, d15N, d2H) to elucidate orchid mycorrhizal nutrition: an update with perspectives for orchid conservation concepts<br>*Gerhard Gebauer* |
| 10:15 – 10:30 | Evolutionary diversification of *Lepanthes* (Pleurothallidinae): a hyper-diverse Neotropical orchid lineage<br>*Diego Bogarín* | 10:15 – 10:30 | Ecology informing orchid conservation translocations: the ménage à trois of orchid, fungi and pollinator<br>*Noushka Reiter* |
| 10:30 – 10:45 | Population genetics of *Cypripedium calceolus* L. and implications for conservation<br>*Roberta Gargiulo* | 10:30 – 10:45 | Exploring orchid-mycorrhizal interactions in New Zealand to understand hot-spots and guide conservation actions<br>*Carlos A. Lehnebach* |
| 10:45 – 11:00 | Applying the RAD-seq to test the hybrid speciation of *Paphiopedilum wenshanense*<br>*Yung-I Lee* | 10:45 – 11:00 | Diversity of orchid mycorrhizal fungi from endemic and widespread orchids native to the Republic of Palau in Micronesia (Western Pacific)<br>*Melissa K. McCormick* |
| 11:00 – 11:15 | The impact of chromosome number changes on the diversification of angraecoids in tropical Africa (Epidendroideae: Vandeae: Angraecinae)<br>*João N. M. Farminhão* | 11:00 – 11:15 | Isolation and identification of fungal endophytes from orchids native to the Republic of Palau in Micronesia (Western Pacific)<br>*Luigi Erba* |
| 11:15 – 11:45 | Tea Break | | |
| **11:45 – 13:00** | **Session 12: Interactions with Other Organisms (Pollination)**<br>***Chair: Thierry Pailler*** | | **Section 11 (continued)**<br>***Chair: Gerhard Gebauer*** |
| 11:45 – 12:00 | Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid<br>*Daniela Scaccabarozzi* | 11:45 – 12:00 | The symbiotic germination mechanism of Chinese traditional medicinal plant *Dendrobium officinale* (Orchidaceae) inoculated with *Tulasnella* sp.<br>*Juan Chen* |

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| 12:00 – 12:15 | Pollination by sexual deception in *Pterostylis* (Orchidaceae)<br>*Tobias N Hayashi* | 12:00 – 12:15 | *In vitro* seed germination of native Ecuadorian orchids<br>*Paulina H. Quijia* |
| 12:15 – 12:30 | Evolution and development of pollination related floral traits in *Phalaenopsis*<br>*Dewi Pramanik,* | 12:15 – 12:30 | Orchid mycorrhizae research in southern Ecuador<br>*Alžběta Novotná* |
| 12:30 – 12:45 | First report of *Papilio* pollination in angraecoid orchids<br>*Thierry Pailler* | 12:30 – 12:45 | The mycorrhizal communities of four co-occurring *Habenaria* species affected by the distribution of mycorrhizal fungi and available nutrients in the soil<br>*Zhang Wenliu* |
| | | 12:45 – 13:00 | Two new approaches to acquiring the elusive mycorrhizal associate of the endangered Hawaiian bog orchid, *Peristylus holochila*<br>*Lawrence W. Zettler* |
| 13:00 – 14:00 | Lunch | | |
| **14:00 – 15:15** | **Session 13: Ecology**<br>***Chair: Maarten Christenhusz*** | | **Section 11 (continued)**<br>***Chair: Melissa McCormick*** |
| 14:00 – 14:15 | Relationships between environmental factors, life history strategies, and the richness and distribution of orchid species<br>*Pavel Kindlmann* | 14:00 – 14:15 | Effects of mycorrhizal fungi on seed germination and growth of an endangered medicinal orchid, *Dendrobium officinale*<br>*Xin-Ju Wang* |
| 14:15 – 14:30 | Phorophytes in epiphytic orchid ecology: a case of mutual exclusion?<br>*Hanne N. Rasmussen* | 14:15 – 14:30 | Effect of mycorrhizal fungi on seed development of *Rhynchostylis gigantea* (Lindl.) Ridl.<br>*Saijai Posoongnoen* |
| 14:30 – 14:45 | Biotic and abiotic factors affecting phorophyte preference in epiphytic orchids: a pilot study from southern China<br>*Lorenzo Pecoraro* | 14:30 – 14:45 | *Prasophyllum* conservation – investigating the causes of poor germination in a genus of threatened Australian orchids<br>*Marc Freestone* |
| 14:45 – 15:00 | | 14:45 – 15:00 | Route to recovery: use of symbiotic system for the yellow early marsh orchid using mycorrhizal fungus from a closely related species<br>*Faye McDiarmid & Tim Pankhurst* |
| 15:00 – 15:30 | Tea Break | | |
| **15:30 – 16:30** | **Closing ceremony** | | |

# 7TH INTERNATIONAL ORCHID CONSERVATION CONGRESS

## Talks

# Tuesday 28 May, 09:30 - 10:15

## *Plenary Lecture 1.*

### Leaping into the Decade of Orchid Restoration

**Kingsley Dixon**

Curtin University, Perth, WA 6102, Australia

With the March 1 announcement by the UN General Assembly that 2021-2030 is the Decade on Restoration the question is, where are the orchids? Though orchid science has delivered a potent research pool of information, the question remains on just how well that pool can build the conservation toolkit so that orchid conservation occurs in concert with the roll-out of the world's largest programs of ecological restoration. Here I will overview the chronology of orchid discoveries and link those to delivering restoration capacity. Importantly developing the 'next generation' is paramount for ensuring that what we do today has carriage into the decades ahead. And to tempt you, I will be revealing a new method of orchid production that promises to transform terrestrial orchid production for some difficult groups.

# Tuesday 28 May, 10:45 - 11:00

## Orchid conservation via habitat preservation

**Steve Beckendorf, Mary Gerritsen, Ron Kaufmann & Peter Tobias**

All authors are directors of the Orchid Conservation Alliance, 564 Arden Drive, Encinitas, CA 92024, USA

There is no doubt that multiple orchid species face extinction. *Ex situ* and *in situ* conservation as well as seed banking have been proposed to address this problem. In our view, the most efficient approach is *in situ* conservation, which preserves multiple species inhabiting the same plot of land (including orchid species as yet unidentified), preserves other flora and fauna and utilizes natural phenomena to nurture the orchids. There are drawbacks to *in situ* conservation: conserved spaces may be considered candy stores by unscrupulous collectors, conserved spaces may be subject to legal takeover if land rights are not vigorously protected, and natural phenomena such as landslides and forest fires can be very damaging. In our view, the advantages of *in situ* conservation prevail. Over the last decade and a half we have worked to raise money that enables the purchase of land as orchid reserves in the orchid-rich Neotropics. We have partnered in two ways to further this end. To fundraise, we have often partnered with the Rainforest Trust so that the funds we raise are matched, doubling the area we are able to protect. To support sustainable reserves, we have partnered with several organizations to purchase and manage land. These organizations include Fundación Ecominga in Ecuador, Instituto Uiracu in Brazil, and Fundaciónes Yumartán and Salvamontes in Colombia. These foundations understand how to own and manage land in their respective countries, and they provide at least protective services and sometimes research services as well. Our efforts have resulted in the expenditure of approximately $525,000 to enable purchase of approximately 3250 acres (1317 ha). Our fundraising methods include selling memberships in the Orchid Conservation Alliance, soliciting donations from individuals and orchid societies, and conducting ecotours to our reserves and other orchid rich areas of the world.

## Tuesday 28 May, 11:00 - 11:15

### Orchid conservation: major achievements at Chinese National Orchid Conservation Center

**Meina Wang**

Orchidaceae occupy a very important position in global biodiversity and are one of the key group of plants that need to be conserved. Although, all species in the family are listed in CITES, considerable numbers of orchids species are still facing the threat of extinction due to the unsustainable utilization of their ornamental and medicinal values. Hence, it is imperative to protect and restore wild orchids populations while meeting public economic benefits. The Chinese National Orchid Conservation Center (CNOCC) has made a lot of efforts and taken various measures to protect orchids, and some of these efforts have been a great success. Based on the investigation and conservation of orchid germplasm resources, the CNOCC has carried out research on various aspects including *ex-situ* conservation, artificial pollination, micropropagation, field reintroduction, genetic studies, understanding extinction mechanisms, drafting conservation strategies and attempts at commercialization. CNOCC has not only published high-standard research outcomes from the above activities but have also managed to conserve species in the wild. At present, nearly 1,500 species of orchids are being conserved at the Center, and a large number of plants have been successfully reintroduced in the wild, including *Paphiopedilum sinensis* (more than 3,000 plants). Additionally, multi-omic approaches have been used to understand the high biodiversity forming and ecological adaptation mechanisms of orchids, which reveal the causes of orchids endangerment; accordingly a conservation-utilization model have been designed for some species, such as *Dendrobium catenatum*. This conservation-utilization model may provide ideas and methods for the conservation and utilization of other endangered orchids.

## Tuesday 28 May, 11:15 - 11:30

# The importance of private reserves in orchid conservation – case studies from Colombia

**Sebastian Vieira[1,2] & Marta Kolanowska[3,4]**

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2. Corporación Salvamontes Colombia, Medellín, Colombia; 3-Department of Geobotany and Plant Ecology. University of Lodz, Poland; 4-Global Change Research Centre AS CR. Brno, Czech Republic

In Colombia, nature conservation has been traditionally the concern of public authorities, as the implementation of protected areas required public funding and was not regarded as a business. However, the privatization trend reached conservation activities in the late 1980s, mostly, from the personal interest of some local and foreign nature lovers concerned about the ravaging habitat destruction observed in the places they visited. Currently in Colombia, more than 860 protected areas are owned or otherwise secured by individuals, communities, corporations, or non-governmental organizations. The average extent of private reserves in this country does not exceed 100 ha and most of them are located in Cundinamarca, Cauca, and Valle del Cauca departments. With a total coverage of 94,258 ha, private reserves constitute only 0.5% of the national protected areas. We present here, two separate orchid conservation initiatives being developed in strategically selected biodiversity hotspots where populations of some endangered orchid species have been found. First, the history, achievements, future goals and needs of the current orchid conservation strategies being developed by Corporación SalvaMontes in Antioquia are shown, with details about how protecting the habitat for certain species can help protect other endangered species and, at the same time, guarantee some environmental services to the local communities. Following, we present the project of establishing a new nature reserve with a research station in Sibundoy valley (Putumayo department) by the "Biodiversitatis" Foundation in cooperation with the University of Lodz (Poland) and the CzechGlobe (Czech Republic), not only helping conservation, but also opening education and research possibilities in a region where that has not existed before. Both initiatives have habitat protection as one of their main objectives and are willing to implement or are developing *in situ* and *ex situ* conservation projects, facing a similar set of challenges and needs, and are devoted to orchid protection.

**Key words:** habitat protection, Antioquia, Putumayo, *in situ*, *ex situ*.

## Tuesday 28 May, 10:30 - 11:45

# Vanilla crop wild relatives and their microbiome: perspectives for conservation and sustainable use.

**Nicola S. Flanagan[1], María Alejandra Bedoya-Moreno[1], Zeneth García-Marulanda[1], Andrew P. Detheridge[2], Gareth W. Griffith[2], Ana Teresa Mosquera-Espinosa[1]**

1. Pontificia Universidad Javeriana, Departamento de Ciencias Naturales y Matemáticas, Calle 18# 118-250, Cali, Colombia;
2. Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom

Vanilla is one of the most economically important crops for tropical regions of low altitude. However, the vanilla crop is highly susceptible to diseases, a consequence of a narrow genetic base resulting from vegetative propagation. Crop wild relatives (CWR) represent an important component of the plant genetic resources available for genetic improvement in crop species. In Colombia 25 species of *Vanilla* are reported. Of these, 21 belong to the Neotropical ´aromatic´ clade representing the secondary gene pool for the vanilla crop. Some of these species are also promising for cultivation in their own right. An understanding of the microbial ecology of wild plants will inform the development of sustainable, eco-efficient cultivation practices for the vanilla crop. We have studied the microbiome diversity associated with plants of different *Vanilla* species in their native lowland forest habitats from several perspectives. 1. Through culture-dependent isolation we have identified the mycorrhizal fungi associated with different species. 2. Applying next generation sequencing, we have undertaken meta-barcoding of the fungal endophyte community in root and leaf tissue from wild plants. 3. We have cultured endophytic fungi from both symptomatic and asymptomatic plant tissue. 4. We have isolated phosphate-solubilizing bacteria (PSB) from the rhizosphere of wild plants. Our results provide insights into the diversity of both beneficial and potentially pathogenic microorganisms associated with *Vanilla* species in the wild, and provide a baseline from which to proceed with further research in order to evaluate the role and importance of natural microbiome constituents in strategies for both conservation and sustainable use of wild *Vanilla* species.

**Key words:** endophytic fungi, *Fusarium*, interactions, metabarcoding, orchid mycorrhizae, phosphate-solubilizing bacteria.

## Tuesday 28 May, 11:45 - 12:00

# When age old tools are still required for modern orchid conservation practices

**Hildegard Crous**

Horticulturist, Owner, Director Cape Institute of Micropropagation

Many of the Western Cape terrestrial orchids of the fynbos biome are fire dependant. Some are perennial, some bi-annual, and some even appear to have an annual growth habit. Many of the species can only be found when in flower, a short three- to four-week period when conditions are suitable. For many of the species, little if anything at all, is known about their pollinators. Some rarely produce seed. Seed survival in the soil over a period of time is another unknown, untouched subject. Additionally, most of these orchids occur in a variety of unique habitat niches which are threatened by fires, floods and human encroachment. The survival of several species hangs in the balance, and for some even, the only sure way of securing their survival is by cultivation *ex situ*. However, terrestrial orchids are often so particular in their cultural needs, that simply collecting and growing on in conventional nursery situations, mostly leads to certain death of the plant. Monitoring orchid populations over a number of years and hands-on observation skills are some of the tools required for finding the answers.

**Key words**: fynbos, observation skills, habitat niches.

## Tuesday 28 May, 12:00 - 12:15

# The Palau Orchid Conservation Initiative: an integrated approach to orchid research and management

**Benjamin J. Crain[1], Dennis Whigham[1], Melissa McCormick[1], Lawrence Zettler[2], Susan Cordell[3], Christian Giardina[3], Amanda Uowolo[3] & Nicole Hynson[4]**

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The Republic of Palau contains some of the most intact naturally forested areas in Micronesia, yet there is only rudimentary understanding of the ecology and conservation status of the ~95 orchid species that occur there. The diversity, distribution, and assemblage of orchid communities in Palau and how they are influenced by habitat characteristics, vegetation structure, symbiotic relationships, and environmental threats remains largely unknown. Accordingly, the Smithsonian's North American Orchid Conservation Center has partnered with an interdisciplinary research team to establish the Palau Orchid Conservation Initiative, whose goal is to foster comprehensive understanding of orchid ecology to improve local and global conservation strategies. Our initial objectives include quantification of orchid diversity in Palau and characterization of species' distributions to explore biogeographical questions. We subsequently aim to determine how habitat characteristics and vegetation structure influence orchid communities to understand their ecological underpinnings. Because orchids require fungal symbionts for growth, a final objective is to ascertain the availability and use of mycorrhizal fungi by Palauan orchids. Understanding controls behind variation in orchid diversity and distribution and the role of symbioses among orchid species and their habitats will allow us to build capacity for orchid conservation and restoration, including cultivation and reintroduction programs. Preliminary results point to greater orchid diversity than previously recognized, suggesting Palau is disproportionately rich for its size. Surveys of the permanent Ngardok ForestGEO plot show that multiple ecological factors, including large-scale habitat characteristics and specific vegetation attributes, heavily influence orchid communities and their structure. Furthermore, Palauan orchids form partnerships with unique fungal symbionts, which can vary among habitats, and symbiotic specialization appears to influence distribution and abundance. Collectively, our findings expand our understanding of orchid ecology, while bolstering appreciation for orchids and their habitats in Micronesia, furthering our ability to protect orchid communities and sustain a biodiverse planet.

**Key words:** orchid diversity & distribution; Forest Global Earth Observatory (ForestGeo) Network; flora of Micronesia; molecular ecology; mycorrhizal symbioses; vegetation associations and interactions

## Tuesday 28 May, 12:15 - 12:30

# Medicinal orchids of Nepal : efforts for conservation, challenges, and future directions

**Bijaya Pant**

Central Department of Botany; Tribhuvan University, Kathmandu, Nepal

Medicinal orchids of Nepal, valuable from a conservation and commercial point of view are distributed from the tropical to alpine region in the unique and highly diverse ecosystem of Nepal. Of the estimated 450 species of orchids in Nepal, 90 species are reported to be beneficial medicinal value. More species are reported to be medicinal in our recent studies. Research on medicinal benefits, and application is underway and extensive research is still further required. Medicinal orchids of Nepal are under serious threat as a result of rapid agricultural and urban development, deforestation, and illegal trade. Thus, challenges in their *in situ* conservation by habitat protection have become a serious concern. Illegal collection by rural inhabitant for trade and consumption is another serious concern. We are undertaking germplasm conservation and mass propagation of some threatened but also commercially important medicinal orchids for their *ex situ* conservation mass production. Various techniques of *in vitro* culture have been applied for the mass scale propagation of different species of medicinal orchids. Institutional collaboration has been done to establish the lab to land procedure. We have been conducting an education, awareness and orchid species restoration programme in participation with Community Forests Users Groups (CFUs) in orchid-rich areas, from which pockets illegal trade is still a practice. This initiative is expected to contribute to how local people can be encouraged to conserve *in situ* the plants that are important to them. We are also disseminating knowledge and demonstrating the local people as how their conservation efforts and cultivation of artificially propagated species are a source of long-term economic benefit and upgrade their livelihood.

**Key words:** Medicinal orchids, culture, germplasm, propagation, conservation

Tuesday 28 May, 12:30 - 12:45

## Integrating systematics and reproductive biology into African orchid conservation: a case study with the genus *Cyrtorchis* Schltr: (Angraecinae, Vandeae)

**Azandi N. Laura[1,2,3], Tariq Stévart[2,4,5], Bonaventure Sonké[1,2,4], Murielle Simo-Droissart[1], João N. M. Farminhão[2,6], Tania D'haijère[2,7], Esra Kaymak[7] & Vincent Droissart[1,2,3,4]**

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Developing conservation strategies of threatened species involves understanding their taxonomy, ecology and reproductive biology, and to integrate this knowledge into rational conservation management actions. Focusing on *Cyrtorchis*, we present here an approach that combine taxonomy and reproductive biology to develop the conservation of this angraecoid genus renowned for its challenging species delimitation problem. Indeed, its showy white long-spurred flowers, indicating a probable sphingophilous syndrome, constitute a suitable model for studies on reproductive biology of epiphytic orchids, a first step indispensable for their conservation. By using phylogenetic analyses, we tested the monophyly of 23 species hypotheses delimited using morphometrical analyses to clarify species delimitation. Based on this taxonomic background, we chose seven well-resolved species (*C. aschersonii*, *C. ringens*, *C.* cf *ringens*, *C. chailluana*, *C. letouzeyi*, *C. monteiroae* and a new species) to study, in our living collection in Yaoundé and in two natural populations, their breeding and pollination systems. A protocol involving phenological monitoring of 414 living specimens was implemented during which 330 hand-controlled pollination tests (177 self- *vs* 153 cross-pollinations) were conducted to assess factors affecting fruit set and seed viability. Pollination efficiency and reproductive success were assessed using fruit set and viable seeds production. Camera and insect light trapping were used for survey and identification of pollinators. Preliminary results show that the flowering period of 13 species occurring in Cameroon occurs from February to November, with a flowering peak during the rainy season. For the first time in Central Africa, hawkmoths were confirmed as potential pollinators of three species of *Cyrtorchis*. Seeds produced are used to supply the African orchid seed bank housed at the University of Yaoundé I which currently includes 146 specimens representing 50% of *Cyrtorchis* species.

**Key Words**: new species, pollination ecology, seed bank, sphingophily, taxonomy.

## Tuesday 28 May, 14:00 - 14:15

# Safeguarding imperiled orchid species in the southeastern United States and Caribbean

**Emily E. D. Coffey, Matt Richards & Michael Wenzel**

Atlanta Botanical Garden

Atlanta Botanical Garden works to advance the science of conservation through research collaborations and native species recovery programs. ABG's plant conservation collections and field research focus on propagation of under-represented endangered plant groups in particular on orchids and the restoration and management of their habitats. In addition to the *in situ* work the conservation horticulture team at ABG specializes in *ex situ* collections management and propagation techniques from field to nursery production of imperiled orchids. The Garden has more than 30 years of experience in the conservation and recovery of rare and threatened orchid species through propagation, collaborative restoration, and habitat management. Impacted by land conversion, overharvesting, agricultural runoff, herbicide use, climate destabilization, invasive species, and the exclusion of many processes, such as fire, many of these plant communities have been reduced to small, fragmented plots of land, and road side remnants. With little or no buffer to offset continual encroachment from human activities, wild populations are in steady decline. Additionally, with increased frequency and intensity of natural disasters impacting wild populations, rare and threatened species with narrow geographic ranges are under tremendous threat. ABG works to addresses the urgent need to protect key imperiled orchids across seven southeastern states in the US and three Caribbean Islands through *in situ* and *ex situ* conservation, development of germination and propagation protocols for target species, and seed banking. Furthermore, ABG actively engages in capacity building through workshops and *in situ* training, as well as establishment of tissue culture laboratories focusing on development of plant material for *in situ* augmentation at identified protected sites, outplanting of plants, and monitoring of natural populations.

**Key words:** orchid conservation, *in situ*, *ex situ*, southeastern US, Caribbean

## Tuesday 28 May, 14:15 - 14:30

# Strategies for orchid conservation of the Atlantic Forest in Legado das Águas, São Paulo, Brazil.

**Luciano Ramos Zandoná[1,2], Angelica Guidoni Maragni[2], Miguel Flores de Jesus[2] & Frineia Rezende da Silva[2].**

1. Legado das Águas
2. Instituto de Botânica de São Paulo

The Mata Atlântica is one of the world's top hotspots for biodiversity conservation, The Legado das Águas with 31,000 ha of conserved forest, an area corresponding to 1.5% of all Atlantic Forest native to the State of São Paulo, is the largest private reserve in Brazil. Integrating together with several Conservation Units, the largest continuous area of the most megadiverse biome and one of the most threatened on the planet. Orchidaceae are under great anthropic pressure due to collection, illegal trade, habitat destruction, pollinator reduction, being among the most illegally harvested plant families worldwide, despite current efforts to conserve them. The present work began in 2016, aiming, above all, at the knowledge and conservation of the orchids that occur in the Legacy region. *In situ* and *ex situ* conservation strategies were used, based on walking and retrieving plants in fallen trees and branches, habitat photographs, inclusion in living collections, relocation in nature, *in vitro* cultivation, reintroduction of species to the habitat and capacity of the local community for the cultivation of domestic orchids, aiming at reducing the pressure of wild plants. To date, 220 species of orchids have been identified, many of them rare and difficult to find, of which 13 are on the red list; one of them, *Octomeria estrellensis*, is considered EW (extinct in the wild). Some are maintained *ex situ*, but most are relocated *in situ*, for taxonomic and reproductive studies, and conservation education activities. To date, eight rare species have been propagated using *in vitro* techniques, producing about 10,000 seedlings that will be reintroduced into the habitat and also sold as cultivated plants. Activities developed with visitors and students, involving orchids, among them, visit to orchid and relocation of plants in nature, indicate that Orchidaceae are a large flagship family for conservation education activities.

**Key-words:** Atlantic Forest, rescue, relocation, conservation, *in situ*, *ex situ*

## Tuesday 28 May, 14:30 - 14:45

# The eastern underground orchid *Rhizanthella slateri* (Diurideae) – biology, phylogeny and conservation

**Mark Clements[1,2], Christopher Howard[1], Catherine Busby[1], Lars Nauheimer[3] & Katharina Nargar[1,4]**

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The construction of the Pacific Highway Bulahdelah Bypass project by the New South Wales, Roads and Marine Services Department provided a unique opportunity to undertake research on the biology, phylogeny and conservation of the extraordinary eastern underground orchid *Rhizanthella slateri* an Federally listed under the EPBC Act, national endangered species. All species of *Rhizanthella* are subterranean in habit making plants extremely difficult to firstly locate and then study. Our research was undertaken over a six-year period at Alum Mountain, Bulahdelah (the most well-known population of *R. slateri*) and included the excavation of a group of plants found growing in the proposed highway footprint immediately prior to commencement of construction of that part of the bypass. This rare opportunity to study whole plants *in situ* provided new data to facilitate a more complete understanding of the life cycle of this mysterious orchid. The life history, fungal relationship, potential seed vectors and phylogenetic relationships are elucidated from that study. Our phylogenomic study in Diurideae based on 64 coding plastid genes provided insights into the phylogenetic placement of Rhizanthellinae in the tribe. We also report here on the status of old sites and a new discovery of *Rhizanthella* along the east coast of Australia.

## Tuesday 28 May, 14:45 - 15:00

# Conservation of North America's native *Vanilla* species

**W. Grant Morton & Ken Cameron**

Department of Botany, University of Wisconsin-Madison, USA

More than 200 orchid species are native to the North America (north of Mexico), and more than half of these are threatened within their habitat range. Among these are six *Vanilla* species found in the state of Florida. *Vanilla planifolia* and *V. pompona* are escapes from cultivation and of little conservation concern. *Vanilla barbellata* thrives in a wide variety of habitats in Florida and throughout the Caribbean. It is a leafless species that forms large populations in the Everglades National Park where it is well protected. *Vanilla phaeantha* is a rare species that is restricted to only two sites in the state. Fortunately, one of these is the Fakahatchee Strand Preserve State Park where the populations are thriving. The situation for the remaining two species of North American *Vanilla* (*V. dilloniana* and *V. mexicana*) is far more dire. *Vanilla dilloniana* is now assumed to be entirely extirpated in the wild, and *V. mexicana* is extremely rare in Florida. After Hurricane Andrew, it was assumed to be extirpated from the USA, but has recently been rediscovered. Our research is focused on the conservation biology of these native *Vanilla* species, including studies that address how fungi can be utilized to assist in seed germination. We have isolated and identified mycorrhizae using conventional isolation techniques and ITS sequencing, and we are subsequently developing protocols for symbiotic seed germination. Fungal isolates identified as *Ceratobasidium*, *Tulasnella*, *Russula*, *Xylaria* and *Periconia* have already been documented. In addition, a microbiome meta-barcoding study is being conducted to make comparisons of microbiomes between a leafy and leafless species pair. Lastly, an investigation of existing genetic variation using next-gen GBS within and among populations in southern Florida, Puerto Rico, and additional Caribbean islands is underway to further inform conservation efforts and to develop a robust management plan for these charismatic orchids.

**Key words**: DNA, fungi, genetics, microbiome, mycorrhizae, seed

## Tuesday 28 May, 15:00 - 15:15

# Recent advances and dispelled myths: propagation, flowering and conservation of the epiphytic ghost orchid (*Dendrophylax lindenii*)

**Michael E. Kane[1], Jameson Coopman[2], Nguyen H. Hoang[3] & Paulina Quijia[1]**

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The ghost orchid, *Dendrophylax lindenii*, is a rare and endangered epiphytic leafless orchid native to south Florida and Cuba. In situ flowering and subsequent seed production have been reported to be infrequent. Both the successful asymbiotic seed culture and promotion of seed germination and early seedling development following symbiotic seed culture with an isolated ghost orchid root mycobiont have been reported. Results of the greenhouse acclimatization and field studies indicate development of a viable conservation plan is achievable. Availability of many seedling-derived ghost orchid plants provides opportunities to refine procedures for long-term plant culture maintenance *in vitro*, examination of factors influencing *ex vitro* acclimatization and out planting sustainability, and factors controlling flowering. Although highly desired, greenhouse flowering is a rare event cited to require specific environmental conditions. We found this to be inaccurate. Relationships between environmental conditions and plant size on greenhouse flowering will be discussed. Ghost orchid plants are considered difficult to maintain under greenhouse conditions and reportedly require high humidity and low air movement. In contrast, ghost orchid plants dry out during seasonal decreased precipitation and humidity in their native habitat suggesting plants actually possess some desiccation tolerance. Ghost orchid plants were evaluated for their ability to recover following desiccation stress. *In vitro*-cultured plants transferred into sterile vessels with vented closures and placed into sealed chambers maintained at 10% relative humidity demonstrated high desiccation tolerance. After 4 weeks desiccation, plant survival was 79.2% after recovery on P723 medium. Desiccated plants exhibited significant decreases in tissue water potential (-18.44 MPa), fresh mass (65.5% loss), and water content (14.2%). The plant survival observed was similar to that displayed by poikilohydric plants. This desiccation tolerance may prove beneficial for refining greenhouse culture methods and application of efficient direct field establishment of *in vitro*-derived ghost orchid plants without prior greenhouse acclimatization.

**Key words:** ghost orchid, flowering, *in vitro* culture, desiccation, acclimatization

## Tuesday 28 May, 15:15 - 15:30

# Orchid diversity: environmental drivers and consequences for orchid conservation in Mediterranean archipelagos.

**Michele Lussu, Annalena Cogoni, Pierluigi Cortis & Michela Marignani**

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Islands are a natural canvas in which evolutionary and ecological patterns have painted stunning and unique life forms. Archipelagos, their origin, history and size can be used as distinct ecological and evolutionary units. Over the course of their evolution, orchids have evolved specific ecological interactions and they are particularly sensitive to environmental alterations. Based on presence-absence data, we investigated the flora of 20 archipelagos to define drivers of orchid diversity across the Mediterranean Basin. Considering that IUCN Red List assessments are missing for many orchids and that this region is one of the most vulnerable biodiversity hotspots, characterized by a peculiar biogeography featured by long-term human presence, we also focused on the distribution of endemism. We pose the following questions: how different are the island orchid floras of each archipelago from each other and from the continental one? Can we define the main drivers that explain diversity in the islands in terms of orchids richness? We tested the relation between islands and continental floras using analysis of similarity and Jaccard's index; generalized linear models (GLM) were adopted to analyse the effects of island size, isolation, distance from the continent, human pressure and land use. We also analysed the distributional patterns of endemic entities. Preliminary results include 250 species of which 16% are single island endemisms (SIE) and only 4% are archipelago endemisms (AE). Since the Mediterranean Basin is one of the most species-rich areas, also due to its high number of islands, and is threatened by a massive anthropic presence, a discussion of the conservation status of its species is essential to promote a Mediterranean conservation network.

**Key word:** endemism, Mediterranean Basin, archipelagos, conservation network, IUCN assessment.

Tuesday 28 May, 15:30 - 15:45

## NGS-based multidisciplinary approach for the conservation of the Mediterranean *Orchis patens*

**Jacopo Calevo[1], Roberta Gargiulo[2], Juan Viruel[2], Leif Bersweden[2], Samuele Voyron[1], Michael F. Fay[2] & Mariangela Girlanda[1]**

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Effective conservation science relies on species-relevant knowledge obtained from multidisciplinary research such as population genetics, mycorrhizal associations and pollinator interactions. This research has been facilitated by the emergence of high-throughput sequencing (HTS, formerly next-generation sequencing, NGS) technologies, which enable generation of genomic data for non-model organisms. The Mediterranean *Orchis patens* is the flagship species in the European LIFE project LIFEorchids (LIFE17NAT/IT/000586, a project on the conservation of orchids and their habitats) because of an alarming decrease in the number of individuals in its populations. The phylogeographic origin of its distribution range, which shows a strong disjunction between northern Italy and Algeria and Tunisia (where today it seems to be extinct), has been discussed widely. We use comparative analysis of plastome sequences, genetic diversity and structure data from microsatellite markers, and fungal metabarcoding to better understand the causes of its fragmented distribution. These genetic results will improve the conservation assessment and management of this Mediterranean orchid species.

**Key words**: genome skimming, plastome, microsatellites, population genetics, fungal metabarcoding, LIFE project

## Tuesday 28 May, 15:45 - 16:00

# New insight into orchid conservation in the Indo-Burma Biodiversity Hotspot: a case study from *Bulbophyllum bicolor* Lindl.

**Hu Ai-Qun[1], Stephan W. Gale[2] & Gunter A. Fischer[2]**

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The natural vegetation of the Indo-Burma Biodiversity Hotspot, a hyper-diverse eco-region that spans the seasonal tropics of South China and continental Southeast Asia, has been reduced to less than 5% of its original extent. The population genetic consequences of this habitat fragmentation on the long-term survival and reproduction of native plant species are expected to be profound, especially for epiphytes, the ecological niche of which has been severely depleted. However, few studies have sought to quantify these consequences. Using the endangered tropical epiphytic or lithophytic orchid *Bulbophyllum bicolor* as a model, we integrated an examination of population genetics with SSRs and breeding system to test for the 'loss of sex' and infer likely consequences for long-term reproductive dynamics in fragmented habitats. Bagging experiments and field observations revealed *B. bicolor* to be self-incompatible and pollinator-dependent, with an absence of fruit-set over seven years. Just 22 multilocus genotypes were confirmed among all 15 extant natural populations, and 12 of the populations were found to be monoclonal; all three multiclonal populations exhibited a distinct phalanx clonal architecture. Our results suggest that all *B. bicolor* populations depend overwhelmingly on clonal growth for persistence, with a concomitant loss of sex due to an absence of pollinators and a lack of mating opportunities at virtually all sites, both of which are further entrenched by habitat fragmentation. Such cryptic life history impacts, potentially contributing to extinction debt, could be widespread among similarly fragmented, outcrossing tropical epiphytes in the Indo-Burma Biodiversity Hotspot, demanding urgent conservation attention.

**Key words:** clonal growth, extinction debt, habitat fragmentation, loss of sex

Tuesday 28 May, 16:30 - 16:45

## Restoration of orchid populations in cultural landscape: fungus availability matters

**Tamara Těšitelová[1], Hélène Vogt-Schilb[1,2], Milan Kotilínek[1], Petr Kohout[1], Pavel Sucháček[1] & Jana Jersáková[1]**

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Natural grasslands in Central Europe have suffered a dramatic biodiversity decline due to land use changes. The restoration of orchid populations is particularly difficult due to their dependence on mycorrhizal fungi for germination. We investigated edaphic conditions and diversity of orchid mycorrhizal fungi (OrM) in soil in 29 grasslands of various ages since restoration and 31 natural grasslands in the White Carpathian Mountains (Czech Republic) and in seven orchid species using next-generation sequencing. We also investigated germination abilities on a subset of restored and natural grasslands. Significant differences in the amount of nutrients and in OrM fungal diversity in soil between both grassland types were found. Fungi from the family Ceratobasidiaceae were more frequent in phosphorus-rich restored grasslands, whereas Sebacinales were more frequent in natural grasslands with higher organic matter content. The germination success of orchid species was constrained by their fungal specificity; generalists were able to germinate with a broader range of OrM fungi even in restored grasslands, whereas germination of specialists was restricted mainly to natural grasslands. In a subsequent experiment, addition of species-specific fungal inoculum into soil in restored grasslands significantly improved germination of two specialist species. Our results suggest that change in land use and edaphic factors affect indirectly the orchid recruitment by modifying fungal community in soil. The absence of mycorrhizal fungi for specialist orchid species can be overwhelmed by fungus inoculum addition, but seedling development beyond protocorm stage was not monitored.

**Key words:** arable land, restoration, mycorrhizal fungi, germination, inoculation

## Tuesday 28 May, 16:45 - 17:00

### The Million Orchid Project: a non-traditional approach to orchid conservation and restoration

**Jason L. Downing**

Fairchild Tropical Botanic Garden, Coral Gables, Florida

In 2013, Fairchild established the Million Orchid Project, now the largest and most successful public outreach program focused on orchids. The program is propagating endangered native orchids and planting them in urban areas in southern Florida and beyond. To date we have successfully planted hundreds of thousands of native orchids back into public spaces throughout South Florida with the ultimate goal of re-establishing one million plants in the region. Orchids are grown from seed in the micropropagation lab at Fairchild, with tremendous assistance from volunteers, undergraduate and graduate students, and K-12 students. The project is unique in its scale, its focus on public spaces, and its involvement of the local community from the beginning. More than 100 schools have been enlisted to grow orchids in their classrooms and surrounding neighborhoods. At its core, The Million Orchid Project is a massive living science experiment that allows us to make important discoveries about how better conserve orchids and help us develop more general strategies for rescuing rare plants within a highly developed urban environment.

**Keywords:** micropropagation, student research, restoration, outreach

## Tuesday 28 May, 17:00 - 17:15

### Survival and prosperity of common alvar orchids after habitat restoration

**Kadri Tali**

Estonian University of Life Sciences

On orchid-rich grasslands of western Estonia the main threat for most species has long been undermanagement rather than intensive management. These areas are not suitable for profitable farming and over the past 50 years have overgrown with dense juniper bush and more recently also pine forest. Project LIFE to alvars aims to restore 2500 ha of alvar grasslands in Estonia including 800 ha on Muhu island. During four years we have cleared the shrub manually and with different machinery. Now that most of the areas are under a grazing regime already Estonian Orchid Protection Club undertook an inventory of species on these severely altered grasslands, to estimate how species are doing. Individuals were mapped on older pastures, recently cleared areas and remaining dense scrublands. The results of this inventory will be discussed.

**Keywords**: restoration, alvar habitats, calcareous grassland orchids

## Tuesday 28 May, 17:15 - 17:30

### Evolution of the integrated conservation strategy for fen orchid in England

**Tim Pankhurst**

Plantlife

Needs of the fen variety of fen orchid *Liparis loeselii* var. *loeselii* are being met by a partnership approach which comprises aspects of autecological research in the field, genetic work, seed-baiting, *ex-situ* cultivation and habitat restoration. The strategy is reviewed every year to maintain focus on those activities which will realise the strategic goal – to move fen orchid to the Least Concern threat level on the England Red List – in the shortest time. This approach has had considerable success and, 11 years on, that goal is in sight. The development of the strategy is charted alongside the changing status of the plant.

**Key Words:** *Liparis loeselii*, Integrated, Conservation, Strategy

Tuesday 28 May, 17:30 - 17:45

## Efforts to establish new populations of Spicer's *paphiopedilum*: a critically endangered orchid in South China

**Jiang-Yun Gao[1, *], Qiang Liu[2], Jessie Yc Hana[3], Ying Chen[3], Xu-Li Fan[3], Xi-LongWang[4] & Wen-Ke Yang[1]**

[1]Lab of Ecology and Evolutionary Biology, Yunnan University, Kunming, Yunnan 650091, China; [2]YunnanForestry Technological College, Kunming, Yunnan 650000, China; [3]Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China; [4]Tibet Plateau Institute of Biology, Lhasa 850001, China

The only population of Spicer's paphiopedilum (*Paphiopedilum spicerianum*) with 38 mature individuals was found in 2006 in South Yunnan, China. These sub-terrestrial plants grow on steep river banks, and the habitat is seriously threatened due to the surrounding monoculture of coffee plantations. This orchid is also listed as a species of Wild Plants with Extremely Small Populations in China (PSESP; State Forestry Administration of China, 2012). Based on the seeds storage, developing the propagation system via *in vitro* seed germination, monitoring on population dynamic, studies on pollination ecology and mycorrhizal fungi diversity, we conducted reintroduction and assisted colonization in June 2015 to establish new populations of this critically endangered orchid. After two years, the survival rate is significantly lower in reintroduced population than in the assisted colonized population. From 2017, most of plants flowered, but no natural fruit set was observed in the assisted colonized population. The selection of locations for new populations might be broader than initially anticipated, but for the long-term maintenance of new populations, lack of pollinators might be a great limitation. Translocation of some co-flowering plants at the same time should be considered for the successful establishment of new populations in *P. spicerianum,* especially for assisted colonization. So far, the assisted colonization is going well, but there are still many uncertain factors and difficulties in establishing a new population successfully. As a PSESP species, all seedlings come from a few parents, which could lead to a low genetic diversity of the artificial population, thus affecting the establishment and maintenance of the new populations. Since assisted colonization is outside its known original distribution range, there may not be effective pollinators in new habitats for maintaining natural reproduction, and it is not known whether seeds can germinate successfully in the new habitats. All of these points will have impacts on the establishment of self-sustaining and renewal populations.

**Key words**: assisted colonization; orchid integrated conservation; pollination ecology; reintroduction; symbiotic seed germination; Wild Plant with Extremely Small Populations (PSESP)

## Wednesday 29 May, 09:00 - 09:45

## *Plenary Lecture 2.*

### Creating sustainable conservation of orchids through sound business development - saving China's slipper orchids.

**Wenqing Perner**

Hengduan Mountains Biotechnology Ltd., Jinwan village, Chengdu, Sichuan 610203, China

The genus *Paphiopedilum* comprises approximately 130 species, with 30 in China, while genus *Cypripedium* has approximately 50 species with 30 in China. There are few temperate orchids that can match *Cypripedium* species for beauty and horticultural potential. These plants are now becoming common in the American and European marketplace. While Chinese *Paphiopedilum* species are rare and showy, have been popular for orchid collectors and also have a certain demand in the mass production market. Hengduan Mountains Biotechnology has perfected *ex situ* propagation techniques for a number of *Paphiopedilum* and *Cypripedium* species. We have marketed them since 2007, starting with flasks, but now also sell seedlings as well as mature plants. We process North American and Asian species. Hybrids are also produced. For both *Paphiopedilum* and *Cypripedium*, *in vitro* propagation involves sowing either dry seed or green pod embryos on defined media. Replated *Cypripedium* seedlings are grown in the dark at 20ºC and *Paphiopedilum* seedlings under light rack 25ºC for about 10 months. For *Cypripedium*, seedlings are deflasked, vernalized and planted out in specially prepared beds, on a carefully defined substrate mixture and grown on to maturity at our Huanglong nursery situated at 2 900 m. Average time to reach flowering after the flask is three to four years. For *Paphiopedilum*, the deflasked seedlings are planted out in a carefully defined substrate mixture in the Chengdu nursery at 500m. Through artificial propagation on slipper orchids, Hengduan Mountains Biotechnology allowed *Paphiopedilum hangianum*, *P. tranlienianum*, *P. helenae* (all CITES appendix I slipper orchids) to enter legal trade in western countries, and for 16 years the company has been doing line breeding on species and hybrids to fulfill the demand on Chinese orchids and market them worldwide while achieving the goal of conservation in a sustainable commercial way.

## Wednesday 29 May, 09:45 - 10:00

# Horticulture and biotechnology as strategies for *in-situ* and *ex-situ* orchid conservation in Colombia

**Tatiana Arias**

Center for Biological Research (CIB), Medellin, Colombia**.**

Orchids are flagship and charismatic species that can help to promote the conservation of whole Andean forests and with them ecosystem services. The lack of basic knowledge and the conservation status *in-situ* of many species is truly worrisome. Private collectors have impressive *ex-situ* living collections and abundant natural history data, but this information is not currently organized or accessible This talk aims to communicate an initiative taking place among two Colombian institutions: the Center for Biological Research and the Colombian Orchid Society. We are implementing a program for the conservation of orchids through *in-situ* and *ex-situ* conservation, integrating the use of technology with active participation of communities. We have been advancing phylogenomic studies and population genetics in groups with high endemism and diversity in Colombia (tribe Cymbideae and the genera *Lepanthes*, *Dracula* and *Masdevallia*). A virtual herbarium of living collections has been implemented together with genetic material stored at Humboldt Institute's tissue bank. At La Reserva Orquídeas in Jardín, Antioquia (tropical cloud forest) we are monitoring orchids through technology offered by a UK NGO. Lastly, we are developing different types of strategies to understand the legal and illegal orchid trade in Colombia. All these activities are being done through participatory science. Ultimately, this initiative will allow us to collect, cure, and analyze information for many orchid species in order to promote conservation and sustainable use as established in the Colombia orchid conservation strategy.

**Key words:** Orchidaceae, orchid conservation, phylogenomics, Colombia, horticulture, biodiversity

## Wednesday 29 May, 10:00 - 10:15

# Conserving the UK's Orchids at the Millennium Seed Bank (Royal Botanic Gardens, Kew)

**Jennifer L. Peach & Stephanie Miles**

Conservation Science, Royal Botanic Gardens Kew. Wakehurst Place, West Sussex RH17 6TN, United Kingdom

Native orchids are widely considered a conservation priority in the UK. As well as cultural, economic and ecological value, 19 species are identified on the vascular plant Red Data List for Great Britain. The Millennium Seed Bank (MSB) currently conserves seeds from 51 native UK orchids including 21 threatened taxa. Collections are stored at the MSB in conventional dry-cold storage (15% RH at -20°C) and have been duplicated to cryo-storage (30% RH at ~-197°C in liquid nitrogen gas vapour) since 2012. The banking of microseeds requires specialist skills, knowledge and equipment and a protocol has been implemented to maximise longevity of collections. With limited literature on orchid seed survival in long-term storage, data were collected as part of the UK Flora Project (funded by Esmée Fairbairn) to assess the viability of conserved collections and the suitability of existing protocols. Of the 137 collections conserved, 79 collections (representing 44 of the 51 taxa conserved) were sown for germination testing. Tests were sown in-vitro using BM1 or 1/2MS medium following sterilisation in 0.5% NaOCl. A cold stratification of up to 3 months was used where dormancy was expected. The majority of collections with unsuccessful tests but apparently good seed quality were assessed for viability using a fluorescein diacetate (FDA) test. Comparative testing for storage in -20°C and cryo conditions was completed for nine collections. Fifty-three collections successfully germinated with a mean result of 44% (SE=4.89%) with 19 collections germinating >50%. An additional 39 collections have shown to be viable in an FDA test with a mean viability of 43% (SE=4.76%). Results confirmed that at least one collection of the 44 taxa tested is surviving in conventional storage. The comparison of seed viability in cryo and conventional storage indicate that there is no clear preferable storage method after three years.

**Key words:** seed, microseed, seed banking, UK conservation, cryo, germination

## Wednesday 29 May, 10:15 - 10:30

# Are the dust-like seeds of orchids always dormant?

**Hugh W. Pritchard[1], Sasikarn Prasongsom[2] & Kanchit Thammasiri[2]**

[1] Royal Botanic Gardens, Kew, Wakehurst Place, UK
[2] Faculty of Science, Mahidol University, Bangkok, Thailand

Orchids have been assigned to a cluster of at least 12 families that have dust seeds and are considered to have an 'unique germination morphology and ecology, and generally have very specialized morphological (MD) or morphophysiological (MPD) dormancy'. We test this assumption, overcoming several design limitations of earlier studies, specifically that the *in vitro* germination method for orchid seeds uses pro-oxidants for disinfection and incorporates nitrate in the medium; both 'treatments' might contribute to dormancy breaking, potentially confounding judgement on the depth and nature of the dormant state. Seeds of *Dendrobium* species were sown *in vitro* and *ex vitro* and under various environmental conditions (light and dark, different temperatures) and nitrate. Germination responses to environmental and chemical stimuli will be discussed in relation to whether dust seeds constitute a dormancy class.

## Wednesday 29 May, 10:30 - 10:45

# Seed viability assessment of *Cymbidium* species and their conservation using cryopreservation

**Namrata Pradhan[1], Amir Ali Khoddamzadeh[2], Yang Mei[1], Wang Xiaoyin[1] & Uromi Manage Goodale[1]**

1. College of Forestry, Guangxi University, Nanning, Guangxi, China;
2. Department of Earth and Environment, Florida International University, Miami, Florida

Orchids are recognized worldwide for their ornamental appeal, medicinal value and ethnobotanical significance, which has lead to their over-harvest and illegal collection with detrimental effects on entire populations. Thus, there is an urgent need to develop efficient protocols for conservation of orchids. Seed cryopreservation provides a feasible option for conservation through long term seed storage and is especially useful for orchids as it can enhance the longevity of short-lived seeds and maintain their viability during storage. However, requirement for success can be species-specific. In my thesis research using six *Cymbidium* species (*C. tracyanum*, *C. cyperifolium*, *C. floribundum*, *C. kanran*, *C. goeringii* and *C. lancifolium*), I propose to: (1) determine the biophysical conditions that optimize cold stress tolerance and storage viability of seeds, (2) develop efficient seed storage protocols, and (3) investigate the effect of seed characteristics on cold stress tolerance in orchid seeds. This study will be conducted using seed material collected from the Yachang Orchid National Nature Reserve, in Leye County, Northwest Guangxi, south-western China and all experiments will be conducted at the College of Forestry, Guangxi University. Seeds of all species will be assessed to determine the following seed biophysical characteristics; seed size, morphometrics, seed moisture content, seed oil content and seed viability. Seeds will be cryopreserved at -196 $^{o}$C using three methods: (i) direct method, (ii) vitrification method and (iii) encapsulation-dehydration method with varying thawing rates. Preserved seeds will be compared with seeds stored at higher temperatures: 23, 4, -18, or -80 $^{o}$C. Viability tests and germination tests will be used to assess which experimental conditions optimize cryopreservation success. By investigating factors determining successful preservation of orchid seeds, this research will provide a strong scientific basis and technical knowledge on seed storage that needs to be urgently prioritized to conserve and continue to reap benefits from this fascinating flora.

**Key words:** orchids, conservation, physiology, seed viability, cryopreservation

## Wednesday 29 May, 10:45 - 11:00

# Rescuing orchids from logging concessionaries for rapid documentation and conservation strategy in Peninsular Malaysia

**Rusea Go, Muskhazli Mustafa, Dome Nikong & Edward Entalai**

Biology Department, Faculty of Science, Universiti Putra Malaysia, 43400 UPM SERDANG, Selangor, Malaysia

Forest destruction has always been the major factor degrading biodiversity species richness in the tropical rainforest especially biota lacking ability to adapt to environmental changes, causing their existence threatened to extinction. Since 2016, we have been venturing into rescuing orchids from active logging concessionaries in Terengganu with permission from the Forest Department of Peninsular Malaysia and Terengganu State. Rescuing orchids within a week after felling is crucial especially during the dry season. As floriculture gene pool and a potential commercial crop, and their inclusion as one of the conservation objective, detailed research on the diversity and ecology of orchids is crucial for strengthening the conservation strategy framework. Plants rescued are prepared as herbarium and living collections, in a dedicated conservatory, cultivated for further identification and phenological study. A total of 109 orchid species belonging to 39 genera were rescued, indicating high orchid density and diversity (H = 4.50 and D = 0.99), with 11 rare and four endemic species, mostly epiphytic, which is strongly correlated to the masses of felled trees, durations of exposure to the dryness stress and unfavourable soil conditions. This study recorded 38 species as new records to Terengganu; one to Malaysia, *Dendrobium agamense*, and another species as new to Peninsular Malaysia, *Crepidium oculatum*. Nine new species are to science, five were published (*Bromheadia petuangensis*, *Dendrobium ainiae*, *Dendrobium ruseae*, *Dendrobium mizanii* and *Pinalia domii*). The resilience of wild orchids towards the environmental anthropogenic disturbances was also evaluated. The wild orchids engaged two water-balance mechanisms as reactions towards the dryness-heat stress and water deficit, namely the 'drought avoidance' and 'drought escape' mechanisms. The survival rate of the rescued wild orchids was recorded at 70.6% of them flowering or fruiting within a year. Ten rare species were proposed as Threatened according to the IUCN Red List Categories and Criteria Ver.13 (March 2017). A conservation strategy was drafted for the orchids with recommendations on the sustainable practices to avoid local extinctions.

**Key words**: Orchidaceae, Terengganu, disturbed forest, species diversity, new species, new record

## Wednesday 29 May, 11:15 - 11:30

# Chikanda: Zambia's wild edible orchids

**Geophat Mpatwa[1], Nicholas Wightman[2], Royd Vinya[1], Jonathan Kendon[3] & Ruth Bone[3]**

[1]School of Natural Resources, Copperbelt University, Kitwe, Zambia;
[2]Homegarden Landscape Consultants Ltd., Zambia; [3]Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE.

Chikanda, also known as African polony, is a local Zambian food made from the tubers of several different terrestrial orchid genera. Traditionally, it was consumed by the tribes in the north east of the country, but its increase in popularity in urban centres of Zambia has seen an increase in demand which currently threatens the wild populations due to uncontrolled exploitation. Harvesting levels are unsustainable and undertaken by low income rural community members (most of which are women and girls) who are having to travel further to find enough chikanda tubers and putting their personal safety at risk. The project is seeking to reduce biodiversity loss in Zambia while supporting vulnerable rural livelihoods dependent on this resource by: i) establishing and supporting chikanda orchid cultivation methods with enhanced facilities and expertise; ii) developing and implementing community based orchid management plans to encourage sustainable harvesting; and iii) investigating and establishing alternative income strategies to support rural livelihoods in chikanda orchid harvesting areas. Field surveys and community interactions conducted during the project reveal that terrestrial orchid species in the genera *Disa*, *Brachycorythis*, *Habenaria*, *Platycoryne* and *Satyrium* are harvested for chikanda trade. The availability of these chikanda orchids in dambos (dry to wet grasslands) has reduced due to unsustainable harvesting methods. Project IUCN Red List assessing efforts have resulted in the designation of 16 species as either Data Deficient or Least Concern since little is still known about the present species distribution and population data. Our various project partners have been busy with different project activities. Copperbelt University's School of Natural Resources have been working with three rural communities in North-Western Province of Zambia to develop and implement an orchid management plan while simultaneously developing *in-vitro* seed propagation protocols. Simultaneously, Jonathan Kendon at RBG Kew's Jodrell Laboratory has also been working on *in-vitro* seed propagation protocols by both asymbiotic and symbiotic methods building on the mycorrhizal fungal isolation and culturing work by Dr. Kazutomo Yokoya during project fieldwork activities in 2017. Sanga Research and Development are conducting market survey and chain analysis work in the urban marketplaces and rural harvesting areas of Zambia. Uppsala University conducted work on the market chain from harvesters to marketeers as well as DNA analysis and databasing.

**Keywords**: chikanda, harvesting, biodiversity

## Wednesday 29 May, 11:30 - 11:45

# Propagation for conservation: edible orchids of Zambia

**Jonathan P. Kendon[1], Nicholas Wightman[2], Kazutomo Yokoya[1], Royd Vinya[3], Paul Mumba[3], Geophat Mpatwa[3] & Ruth Bone[1]**

1. Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE.
2. Home Garden, Zambia; 3School of Natural Resources, Copperbelt University, Kitwe, Zambia.

Tuberous terrestrial orchids are wild-harvested as one of the main ingredients for chikanda, a traditional food, in Zambia. Unsustainable harvesting is leading to local extinction risk. Only smuggling of wild orchid tubers across borders of countries neighbouring Zambia is currently meeting demand. A project funded by the Darwin Initiative was instigated with multiple conservation, social and awareness-raising aims, one of which was the propagation of chikanda orchids for cultivation activities. Production of propagules of the rarest species was also a project target, to lead to restoration. Identifying easy-to-cultivate species means that community groups have a route to sustainable orchid tuber harvesting, along with socio-economic benefits. Collecting trips to areas known for chikanda orchid species yielded seeds which have been used to produce propagules for cultivation purposes. Established methods were used to isolate mycorrhizal fungi from wild Zambian orchid roots to aid in the propagation process. In the first instance lab-raised orchids will be deflasked and grown *ex situ* to be used as a resource for ongoing cultivation trials. The results of lab-based propagation trials of wild-collected chikanda orchid species will be reported. These will include seed viability, germination and mycorrhizal fungus specificity. The data add to a paucity of studies of mycorrhizal fungi in Africa relative to other continents. Combining conservation assessments with seed viability and fungal specificity data can help to inform the prioritisation of conservation efforts in future.

**Key words**: chikanda, terrestrial orchid, mycorrhizal fungus, orchid seed, orchid propagation

Wednesday 29 May, 11:45 - 12:00

## Rediscovery and conservation of the rare *Octomeria estrellesis*. Production of seedlings traced with guarantee of origin, a great step in favour of conservation

**Luciano Ramos Zandoná[1,2], Angelica Guidoni Maragni[1] Frineia Rezende da Silva[1] & Miguel Flores de Jesus[1]**

1. Legado das Águas.
2. Botanical Institute of São Paulo.

Orchidaceae in Brazil currently have > 200 genera and c. 2500 species. About 130 are on the Red List and many others are extremely rare. The Legado das Águas, the largest private reserve of the Brazilian Atlantic Forest, is inserted in the municipalities of Juquiá, Miracatu and Tapiraí, with 31,000 ha - an area corresponding to 1.5% of all native Atlantic Forest in the State of São Paulo. *Octomeria estrellensis*, an endemic micro-orchid from Brazil, occurring only in the Atlantic Forest, with only three historical records in the SpeciesLink network and few specimens included in conservation collections, is a rare species, considered as EW (Extinct in Nature). During the "Legacy Orchids" project, in progress, a habitat of this species was located. 38 plants were rescued in fallen trees, 14 plants were included in the orchid and another 24 were relocated in the habitat. Habitat data as well as their location were recorded for traceability. One year later, the plants in cultivation flourished, were pollinated manually and gave rise to two fruits. The seeds grown *in vitro* produced about 1000 seedlings, which will soon be reintroduced into the habitat, sent to other living collections and also sold as cultivated domestic plants. The Legado das Águas, is now the largest *in situ* and *ex situ* genetic bank of *Octomeria estrellensis*, being the only nursery of native plants in Brazil to produce plants with traceability and guarantee of origin, thus preventing collected plants from being placed on the market, as if they were cultivated. The matrices are georeferenced from the habitat to the final stages of cultivation and growth in the nursery of seedlings and orchid, included in database, the seeds obtained, are taken to germination, the seedlings receive an identification that can be verified through a QR code.

**Key words:** Orchidaceae, Atlantic Forest, traceability, conservation, *ex situ*, *in situ*.

## Wednesday 29 May, 14:00 - 14:15 (parallel session)

### A teacher's view - thirty years of the Writhlington School Orchid Project

**Simon Pugh-Jones**

(Teacher in Charge = Writhlington School Orchid Project, Radstock, UK)

In 1991, Simon set up the WSOP as a vehicle for developing student expertise in biotechnology and enterprise. The project combines excellence in science, horticulture, enterprise and sustainable development. Highlights for the Orchid Project include horticulture awards including Gold medals at shows including two European Orchid Shows, The Cape Town Flower Show and Chelsea and Hampton Court in the UK. His students won the UK national schools science competition in 2001, 2010 and 2014 for research on tropical forests, and Simon's enterprise teams were national or regional winners in the UK Young Enterprise competition every year from 2005 to 2009. Enterprise income has funded 14 overseas students' expeditions to work with tropical communities, schools and conservation groups, in the Americas, Africa and Asia, and to innovate conservation and education capacity building. Students have set up orchid propagation laboratories in Rwanda and Laos. In Rwanda students have worked closely with schools and colleges, where projects have enhanced the science curriculum and conservation practice. Other projects include working with schools in Sikkim and, more recently, Sarawak. In the UK, his student team have led a number of orchid education initiatives to develop public displays of plants at the Eden Project, Cambridge University Botanic Garden, the Bristol Aquarium and the Bristol University Botanic Gardens. Students develop their horticultural and scientific expertise by designing, supplying and building living educational displays that engage the public in plants, tropical ecosystems and the communities that share the habitats with orchids. Simon's projects are integral to the science curriculum delivered at Writhlington School where the links and expertise developed through his projects allow students to carry out significant original research, engage in real design projects and communicate with a global community of scientists and engineers.

## Wednesday 29 May, 14:15 - 14:30 (parallel session)

### The students' experience of the Writhlington School Orchid Project

**Jess Buckle & Chloe McGiveron**

(Students at Mendip Studio School, Radstock, UK)

Jess and Chloe are sixth form students at Mendip Studio School attached to Writhlington School in Radstock, Somerset, and have been active participants in the Writhlington School Orchid Project for six and seven years respectively. The students' experience with the project is detailed, including orchid cultivation, *in-vitro* orchid propagation, tropical expeditions, working with industrial partners and exhibiting and selling plants at shows. Chloe is the propagation laboratory manager and is responsible for propagation planning, training younger students and running day-to-day operations. Jess is the glasshouse manager and is responsible for daily orchid care of > 700 orchid species, propagation, repotting and preparation of display plants and sales plants for shows. She also trains and manages younger students. Jess and Chloe took part in the 2016 expedition to Rwanda where they ran workshops in laboratory skills and orchid ecology, as well as leading groups of Rwandan students in Nyungwe National Park. Jess and Chloe have worked as consultants for project partners including the Eden Project, Bristol Aquarium and Bristol Botanic Gardens, supplying plants, installing permanent displays and learning from the staff members they work alongside. Key learning experiences for student participants are listed and discussed, including specific science, conservation, enterprise and horticulture skills, as well soft skills such as communication and team working. Student experience is broadened to encompass lessons for similar projects and key success factors to consider and develop.

Wednesday 29 May, 14:30 - 14:45 (parallel session)

## Conservation education

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Target 14 of the Global Strategy for Plant Conservation (GSPC) says "the importance of plant diversity and the need for its conservation [should be] incorporated into communication, education and public awareness programs". If we are to have any chance in succeeding in our aim of conserving as large a proportion as possible of the world's orchid biodiversity, orchid scientists need to engage with a much wider audience and make greater use of the largely untapped reservoir of expertise available, both within the amateur orchid community and, more broadly, within the population at large. A more imaginative approach is required. Each threatened orchid species has its own individual conservation story that can be told through painting, poetry and sculpture for example. Orchids such as *Oncidium alexandrae* can act as flagships for plant conservation in general, and their stories told through special educational exhibits at plant shows, through presentations, articles and books and the more conventional display boards sometimes found in botanical gardens. Today we have the opportunity, by acting as STEM (Science, Technology, Engineering and Maths) ambassadors, to inspire the younger generation in schools and universities to become more environmentally aware and, above all, provide them with pathways enabling them to become actively involved in a number of different practical projects.

**Key words:** conservation, education, STEM, GSPC

Wednesday 29 May, 14:00 - 14:15 (parallel session)

## Small populations on small islands: what chance does an orchid have?

**James D. Ackerman, Raymond L. Tremblay, Hannah Madden, Mike Bechtold & Michiel Boeken**

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Small populations are intrinsically more vulnerable to population decline and extinction. Such populations may be most susceptible when distributed on small islands, which suffer disproportionately more from human influences, both directly and indirectly. Nevertheless, small native populations that occur on multiple islands may have dispersal or life history characteristics that buffer impacts from novel disturbance regimes. We monitored three populations of the orchid *Brassavola cucullata* from Caribbean islands of Sint Eustatius and Saba and asked what the likelihood of population persistence is. Over a period of 3-4 years, we recorded growth, fruit production, herbivory, recruitment and mortality for all plants in each of our populations. Because of small sample sizes, we used the Dirichlet distribution to calculate and obtain more realistic parameters of our population projection matrix. We assessed persistence and predicted possible population changes with a mixture of traditional (lambda, elasticities) and more recent (transient dynamics and non-linear elasticities) indices. Growth, reproduction and predicted population persistence varied among years and islands. Sometimes population size was stable, whereas in other years reduction could be as high as 10%, the latter being associated with unusually hot and dry years. Transient dynamic and function analyses suggest that populations would be extremely vulnerable to reduction if small plants dominated, while populations which are dominated by large plants are not as likely to be variable in time, reduction in population density as large as 10% could still be possible. Populations of perennial plants on small islands can fluctuate substantially suggesting a degree of vulnerability. While the current situation for *B. cucullata* shows a general trajectory of decline, there are some signs of stability despite deforestation and herbivore activity. The outlook is precarious for the Saba population given the predominance of younger plants, and all three populations if spasmodic recruitment fails to occur, which may happen if disturbance regimes change and the ongoing warming and drying trends persist.

## Wednesday 29 May, 14:15 - 14:30 (parallel session)

### Irresistibly fragrant: a key innovation for the long-distance dispersal of wild *Vanilla* seeds

Adam P. Karremans[1,2*] Ruth Pillcohuarcaya[3,4] Charlotte Watteyn[5], Marco Cedeñofonseca[1,6], Isler Chinchilla[1,6], Gustavo Rojas-Alvarado[1,6], Marvin López-Morales[3], Andrewwhitworth[3,7], Bernal Rodríguez-Herrera[8], Santiago Ramírez[9] & Barbara Gravendeel[2]

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Orchidaceae are said to have the smallest seeds of all flowering plants. The seeds of orchids lack an endosperm, and most have only a very small embryo within a thin, spindle-like, transparent seed coat. This makes the orchid seed very light and ideal for wind dispersal, the generalized mechanism in the family. However, the highly appreciated aromatic seeds of *Vanilla* are unlike those of other orchids in that they are spherical, have a dark and hardened seed coat and are much larger in size. Field observations indicate that the fruits of some *Vanilla* species, like other orchids, expose the seeds by opening when ripe. Nevertheless, the seeds are not blown away by the wind or washed away by rain and do not simply drop to the ground. In fact, the seeds may remain exposed in place for weeks. Fruits of yet another *Vanilla* species do not open at all, they fall to the ground when mature without ever exposing the seeds. How are the seeds of *Vanilla* dispersing? Many hypotheses have been put forward through the years, but none has been proven. Thanks to an ongoing field study in south-west Costa Rica on the aromatic wild relatives of the commercially grown *Vanilla*, we can now demonstrate how their seeds are dispersed by animals by combining the use of camera traps and molecular identification techniques. Two different mechanisms of dispersal, employing two completely different taxonomic groups, have been discovered in these fragrant *Vanilla* species. Fragrance plays a crucial role in both and we hypothesize that animal dispersal of these fragrant fruits is a key factor in the unusually broad geographical distribution of species belonging to the genus.

**Keywords**: camera trap; fragrant fruits; long-distance dispersal; orchid seeds; *Vanilla*

## Wednesday 29 May, 14:30 - 14:45 (parallel session)

### Features of population biology of some Neottieae species in Russia

**Ekaterina Zheleznaia**

Peoples' Friendship University of Russia. Timiryazev State Biological Museum

We studied population biology and ontogenesis of *Epipactis palustris* (L.) Crantz in Moscow and Bryansk regions in 2000–18, *Epipactis papillosa* Franch. & Sav. and *Cephalanthera longibracteata* Blume in Primorsky region in 2016-18. The reproduction of long-rhizomal *E. palustris* is achieved predominantly by vegetative means – seed reproduction is not common. Short-rhizomal *E. papillosa* and *C. longibracteata de facto* do not reproduce vegetatively, but it is also difficult to find individuals of these species derived from seed in nature. Apparently, juvenile and immature stages pass very quickly in these species, during a single growth season, probably even under the upper soil horizons. Individuals of seed origin of *E.palustr is* were found only once in a swampy birch forest in *Aulacomnium palustre* "pillows" in Bryansk region. Other plants with aboveground shoots resembling those of juvenile plants, actually formed from sleeping buds on fragments of rhizomes that previously belonged to adult plants. Adult vegetative and generative plants prevail in *E. palustris* populations. Distinguishing ontogenetic stages using quantitative parameters of leaves and shoots and number of leaves is impossible for *E. papillosa* populations at Kamchatka (Vinogradova, 2012). Juvenile and immature plants were not found in *E. papillosa* populations in Primorsky region. All investigated populations were small: from three to 20 mostly generative individuals. Published data on the ontogenetic spectrum of *C. longibracteata* are contradictory and insufficient. Adult vegetative and generative plants dominate in *C. longibracteata* populations in the Primorsky region in 2016-18.

**Key words**: Neottieae, ontogenesis, population ecology, Russia

## Wednesday 29 May, 14:45 - 15:00 (parallel session)

### Demographic and genomic consequences of landscape transformation for a tropical Andean epiphytic orchid

**Nhora Helena Ospina-Calderón, Raymond L. Tremblay, Chris W. Dick, Alejandro Zuluaga, Alba Marina Torres & Nicola S. Flanagan**

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Colombia is home to > 4300 orchid species, with nearly 40% endemism. The majority of these species are found in the Northern Andes Biodiversity Hotspot. However, the diverse ecosystems in the Andean region in Colombia have undergone severe transformation, resulting in a heterogeneous mosaic composed of native forest remnants and areas under anthropogenic use. Epiphytic orchids in the subfamily Epidendroideae comprise the greatest diversity in Orchidaceae, but the impact of anthropogenic processes on the ecology and evolutionary processes in Neotropical epiphytic orchids is barely understood. In two regions of the Colombian Andes presenting contrasting patterns of landscape mosaics, we evaluated demographic and genomic structure in the endemic twig epiphyte, *Rodriguezia granadensis*, that shows floral colour polymorphism. In each region, three orchid populations were studied in continuous forest habitat, and three in areas under agricultural (coffee or pasture) production. We applied transient matrix models to determine population dynamics, and RADseq approaches to evaluate genomic diversity patterns. Population size, demographic structure and dynamics, and population genomic configuration differed between forest remnants and transformed sites. We found larger Ne, greater genetic diversity, fecundity and recruitment in forest fragments, compared to agricultural areas. Between forest and transformed sites the equivalent fruit set was seen, however in the latter recruitment and survival was extremely low. In transformed habitats orchids occurred in high density aggregations with low Ne and allelic richness. These demographic and genetic patterns clearly indicate the strong ecological and evolutionary impact of habitat transformation on these populations. This study provides baseline demographic and genomic data for continued monitoring of population demography and diversity in *R. granadensis*, as a model system with which to inform conservation and management actions for epiphytic orchids in the tropical Andes.

**Key words**: tropical epiphyte, population genomics, RADseq, demography, matrix models, habitat transformation

## Wednesday 29 May, 15:00 – 15:15 (parallel session)

### Revisiting the life cycle of Helen's bee orchid (*Ophrys helenae*) using a dynamic population model

**Martha Charitonidou* & John M. Halley**

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The life cycle of orchids is relatively well-understood qualitatively, but putting numbers on their demographic parameters remains a challenge. This study aims to improve our knowledge of orchid population dynamics: we chose *Ophrys helenae* Renz (1928) in order to acquire quantitative understanding on the life cycle, and to foresee the conservation challenges that follow. *Ophrys helenae*, commonly known as Helen's bee orchid, is a Balkan endemic species with a centre of distribution in north-western Greece, where it is locally common. An easily recognizable *Ophrys* due to its cherry-red labellum that lacks a speculum design, *O. helenae* has been identified as a shelter-mimic, in contrast to the characteristic sexually deceptive bee orchids. In recent years, its distribution seems to be active and expanding eastwards and southwards, with confirmed reports from East Attica, Corinthia and, more recently, the Isle of Lesvos. For our study, we sampled selected populations across the distribution of the species in Greece, for three consecutive years, focusing primarily on the above-ground life cycle. Our samplings revealed variability in specific demographic parameters, which was observed both between and within the selected populations of *O. helenae*. On this basis, we estimated a range for the fecundity of the species, through its variability between the selected populations. By assuming a stable population, we obtained measures of the pre-reproductive mortality of the species. Using these parameters, we constructed a dynamic population model for *O. helenae*, which also runs under scenarios of altered fecundity, mortality and environmental variability. Our findings show that population variability increases with environmental variability but is mainly driven by survival variability. Extinction probability depends on environmental variability and paradoxically rises with increasing fecundity. Our model, developed for *O. helenae*, can be extended to other species and changing climatic conditions in order to investigate coming issues in orchid conservation.

**Keywords:** population dynamics, orchids, *Ophrys*, fecundity, orchid conservation

## Thursday 30 May, 09:00 - 09:45

# *Plenary Lecture 3.*

## Illegal trade in wild orchids: an overlooked conservation challenge

**Luciano Ramos Zandoná[1,2], Jacob Phelps[1,3] & Amy Hinshley[1,4].**

1. IUCN Species Survival Commission Orchid Specialist Group - Global Trade Programme
2. Botanical Institute of São Paulo.
3. Lancaster Environment Centre, Lancaster University, UK
4. Oxford Martin School, University of Oxford, UK

Illegal wildlife trade threatens many taxa globally. Orchids are often particularly vulnerable to over-harvest because many species are slow-growing and depend on stable environmental conditions and complex ecological relationships, such as association with specific fungi and pollinators. While we may think of the large-scale commercial trade of wild orchids as a relict of Victorian horticulture, wild orchids continue to be commercially traded globally. Orchids are traded as ornamental plants, medicinal products and food, affecting thousands of species across scales. Yet, related data are often very limited due the illegal nature of trade. In 2016, the IUCN SSC Orchid Specialist Group established the Global Trade Programme, which unites the expertise of members who work on trade in an effort to promote more sustainable trade and better address illegal and unsustainable commerce. This talk will provide an overview of different categories of commercial illegal trade in wild orchids, with a particular focus on ornamental trade and examples from Brazil. It will demonstrate how much of this trade is the result of illegal harvest, meaning that it is little documented and is absent from official statistics, at the same time as being of growing conservation concern. It will present key conservation challenges and highlight priorities for addressing these challenges. These are to (1) research trade dynamics and the impacts of harvest; (2) strengthen the legal trade of orchids; (3) adopt measures to reduce illegal trade; (4) promote responsible *ex situ* and *in situ* plant germplasm banks and (5) raise the profile of orchid trade among policy makers, conservationists and the public.

**Keywords**: conservation, trade, medicine, food, ornamental plants

## Thursday 30 May, 09:45 - 10:00

# A study on wild orchid trade in a Chinese e-commerce market and its application in conservation

**Shan Wong[1, 2] & Hong Liu[1,3]**

1. Florida International University.
2. Texas Tech University.
3. Fairchild Tropical Botanic Garden.

Globally the unsustainable trade in wildlife has been recognized as a major factor of biodiversity loss. Monitoring and regulating the wildlife trade, especially that on virtual markets, has not been an easy task. In this study we carried out a survey in 2015-2016 on the most popular on-line market platform in China, i.e. Taobao.com, and determined the extent and nature of the Chinese virtual wild orchid trade. This is among the first online studies of richness in wild orchid trade. We found that approximately 100 wild-sourced orchid species were offered by over 50 online vendors. With all species pooled, a total of more than 90,000 individual plants were sold over the initial seven-month period when the sale's data were available. Four *Cymbidium* species were the top traded species in terms of volume, which reflects the high demand on *Cymbidium* spp., a group with significance in Chinese culture. The high volume and the potential for cross border trade occurring on Taobao call for stronger collaboration among government agencies, NGOs and industry nationally and internationally, and promotion of voluntary code of conducts among different stake holders to effectively curb the wild orchid trade online. Domestically China needs to strengthen its legal protection for over-exploited plants. To this end, we presented our research in a Chinese orchid conservation event organized by the National Forestry and Grassland Administration of China in summer 2018, in which government officials, researchers, NGOs and internet commerce platforms got together. With the knowledge gained from the study, we contributed to the drafting of an equivalent of code of conduct for the various orchid conservation stake holders. We also engaged a key representative from Taobao.com during the event and assisted Taobao to successfully implementing key-word triggered blocks of major wild orchid groups traded on the platform.

**Key words:** CITES; Endangered plants; Horticultural trade; Online trade; Rare plants; Wildlife trade

## Thursday 30 May, 10:00 - 10:15

# Is over-harvest a major threat to the Chinese orchids? An analysis of the Chinese Redlist

**Hong Liu[1,2]**

1. Florida International University,
2. Fairchild Tropical Botanic Garden

China completed and released online a comprehensive red list assessment for its entire known flora of higher plants in 2014 and published the assessment criteria in 2017. Among the 1502 orchids included in the assessments, 653 (43.5%) were categorized as threatened (i.e. critically endangered, endangered or vulnerable). In this study, I examined the listed criteria of these orchids to understand the role of over-harvest in driving these Chinese orchids to the brink of extinction. I found that unsustainable harvesting was listed explicitly as a reason for the orchids being classified as threatened for only 13 of the 653 species (2%). This low percentage contradicts the phenomenon that trade of wild harvested orchids is very active in China, especially species of *Dendrobium*, a major medicinal herb group. Over-harvest was listed as a factor for none of the 68 threatened *Dendrobium* species. Similarly, with only one exception, over-harvest was not listed as a threat for any of the lady's slipper orchids, a popular horticulture group (28 *Cypripedium* and 26 *Paphiopedilum* species). Interestingly, more than a quarter of the threatened *Cymbidium* species (eight out of 28 species) had an indication that over-harvest was a threat. There appears to be significant underestimation of wild-harvest as a threat for the Chinese orchids, which may hinder effective conservation strategy. Establishing and maintaining a dynamic national list of orchids in trade and their trade intensity will help to systematically correct this listing bias.

## Thursday 30 May, 10:15 - 10:30

# An assessment of medicinal orchid trade in Nepal

**Reshu Bashyal[1], Kumar Paudel[1], Amy Hinsley[2] & Jacob Phelps[3,4]**

1. Greenhood Nepal, Kathmandu, Nepal
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4. Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom

Nepal hosts numerous orchid species (n=437), including > 100 with reported medicinal properties, used in both Ayurvedic and Chinese medicinal traditions. Many species are harvested from the wild and exported to China and across South/South-East Asia, and uncontrolled harvest and trade is known to be impacting wild population across Nepal. Although all orchid species are protected in Nepal and are regulated by CITES, large-scale commercial orchid trade remains unreported and unresearched. Understanding these threats is essential to plan conservation action. This research explores Nepal's medicinal orchid trade for the first time, based on reviews of seizures, CITES records, published and unpublished literature and key informant interviews with domestic scientists, commercial horticulturalists and government agencies It provides an overview of trade, specifies gaps in the conservation of orchids and considers possible solutions, including to inform future research and conservation actions, help enforcement, and strengthen CITES implementation in Nepal.

**Keywords:** conservation, over-harvesting, seizure, traditional medicine, wildlife trade

## Thursday 30 May, 10:30 - 10:45

# Waiting for the salep storm: what future for the protected orchids of Greece?

**Martha Charitonidou[1], Kalliopi Stara[1], Konstantinos Kougioumoutzis[2] & John M. Halley[1]**

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Salep, the flour made by grinding orchid tubers into a powder, is widely used in the eastern Mediterranean area, mainly for the production of the homonymous beverage, but also as an additive in other products, such as “dondurma” ice cream. In Greece, salep is more common in the northern parts of the country, a cultural remnant of the Ottoman period. Despite the protection of Orchidaceae under Greek and international law, the demand for salep continues to drive the illegal collection of orchids in Greece. Salep is part of an emerging mass market in "traditional" or "wild" products that targets, as well as orchids, medicinal plants, aromatic herbs and edible mushrooms. Thus, the trade in salep is likely to grow, and it has been referred to as a significant threat for several orchid species in the region. Our study focuses on the current status of the orchid species that are collected for salep in north-western Greece, mainly *Dactylorhiza sambucina* (L.) Soó and *Orchis mascula* (L.) L., and possible future impacts of salep harvesting. Our research methodology includes ethnographic tools, mainly interviews with key stakeholders including collectors, as well as field sampling and population analysis. Our preliminary results indicate that the impact of traditional collection methods has been rather limited and minor. However, given the growing mass market in salep, ongoing climatic changes, and our insufficient knowledge of the orchid population dynamics, predicting future developments in population vulnerability is difficult. We discuss how similar studies can help improve conservation guidelines on the effect of collection on wild populations, as there is clearly an urgent need to find sustainable management solutions for species of commercial interest.

**Key words:** illegal trade, orchids, salep, northern Greece, orchid conservation

## Thursday 30 May, 10:45 - 11:00

# Commercial interest in endangered terrestrial orchids demonstrated by systematic analysis of patent applications using salep

**Susanne Masters [1,10], Tinde van Andel [1,2,3], Hugo de Boer [1,4,5], Reinout Heijungs [7,8] & Barbara Gravendeel [1,2,9]**

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Wild populations of threatened Mediterranean and Central Asian terrestrial orchids have a number of uses, including making salep, in which dried tubers are used as ingredients in a hot drink, consumed as a beverage and as a traditional remedy, and maraș dondurma ice-cream. Despite considerable interest in the conservation of these species, no systematic analysis of innovation and technology transfer exists for natural products harvested from wild orchids. Here, in the first systematic patent review of any wild species, we find numerous innovative industrial uses of orchid-derived salep. This recalibration of the value of salep shows starkly that, rather than being restricted to two specific products of limited regional circulation, these orchids are a rich source of unique materials with myriad applications all around the world. Noted properties of mucilaginous salep glucomannan polysaccharides led to applications as a thickening agent, in reducing water content and as an industrial colloid. Salep is potentially useful as a material, a nutraceutical, in drug delivery systems and in medicinal applications in numerous conditions. Our systematic review on salep found 244 patent applications, of which 89 were granted, spanning 163 years from 1855 to 2018. We conclude that salep is not just a product used in a limited cultural and geographical region - it is of interest to a range of commercial applications on a global scale. In order to conserve wild terrestrial orchids collected for salep, conservation science must engage with the full extent of the utility of and commercial interest in these particular orchids.

**Key words:** CITES; ethnobotany; environmental science and management; patents; systematic review; trade in endangered species

Thursday 30 May, 11:00 - 11:15

## Trade profiles of Malagasy orchids under the light of data from literature and citizen science

**Narindra Randriamialisoa & Aro Vonjy Ramarosandratana**.

University of Antananarivo, Madagascar

Madagascar orchids are practically absent from mass production but are collected by communities close to forests and then found in botanical gardens around the world as well as in private collections. Our work hypothesis is that the most traded Malagasy orchid species are also those that are rarest in nature and as a result are scarcely reported. To test this hypothesis, all data collected on orchid trade from Madagascar were cross-referenced with data reported in the scientific literature, scientific specimen export permits and those from citizen observations, which must reflect the state of populations in the wild. Between 2008 and 2014, Madagascar exported a total of 13071 orchid specimens, of which only 31% are formally identified at the species level. The genera *Angraecum*, *Aerangis*, *Aeranthes* and *Bulbophyllum* are the most traded in terms of volume. On the other hand, the most frequently requested species, *Angraecum compactum*, *Erasanthe henrici* and *Microcoelia gilpinae*, have never been observed in the wild. About 44% of transactions are local purchases made by enthusiast tourists for a commercial volume of 1889 specimens with about six specimens per person. Three of the 20 species never seen in nature are the subject of significant trade, namely *Erasanthe henrici*, *Neobathiea grandidieriana* and *Oeonia rosea*. These species should be up-listed into Appendix I of CITES. The other 17 species are not traded extensively, and their absence in the wild could indicate the destruction of their habitat. This study shows that trade data reported the presence of orchid species that are hard to find in nature and could be helpful in species management and monitoring if data sources are always reliable and accessible.

**Keywords:** conservation, database, Madagascar, Orchidaceae, trade

## Thursday 30 May, 11:15 - 11:30

# Study on edible orchids activities in the Nyika National Park and the surrounding communities with focus on drivers and consequences to find entry points for action

**Heejoo Lee**

Independent Researcher (Nyika-Vwaza Trust UK Research Grantee) / Recent graduate, MPhil Conservation Leadership, Cambridge University, UK

This research set out to understand the socioeconomic status and drivers behind collecting and trading chinaka (the delicacy dish made with edible tubers of wild orchids), Semi-structured interviews were conducted with people involved in the value chain in northern Malawi, with most focus on villagers around the Nyika National Park. Increased commercialization of chinaka in southern Africa over several decades has permeated into the communities around Nyika. For the last decade, illegal exploitation of the orchids inside Nyika Plateau has also increased. This has alarmed the law enforcement efforts in Nyika for stricter control, e.g. increased apprehensions - of "orchid poachers". The field study underway since December 2018 points to an irony in the current situation: limited resources for a comprehensive approach are leading to narrow law enforcement only on the collectors who enter the park, while leaving traders and others in the value chain in relative safety. The collectors, who are mainly villagers closest to Nyika, earn the least profit while facing the highest risk of apprehension. Meanwhile, those who bake-and-sell can make 50-100% more profit, and the traders could make 400% more profit than the collectors. As long as these actors higher in the value chain are safe from law enforcement, the demand will continue on the collectors to provide supply. Stakeholders are exploring "sustainable use", and much information and tactful approach to designing solutions are needed. First, should controlled use be tried while research for domestication at commercial-scale is taking its time? How can such schemes for controlled use be designed thinking of all the tricky challenges - such as, equity and commitment among collector communities, the co-existence of sustainably-harvested wild products and the others, and the regional-scale of trade across national boundaries? What effective campaigns to educate the consumer base - who are largely unaware - could be possible?

**Keywords**: chikanda, chinaka, edible orchids, wild plant commercial use, role of communities, illegal trade, CITES

## Thursday 30 May, 12:15 - 12:30

# From IUCN conservation assessments to seed bank: conserving threatened orchids in Africa and Madagascar

**Simon Verlynde[1,2], Murielle Simo-Droissart[3], Tariq Stévart[4,5,6], Brigitte Ramandimbisoa[7], Aina A. Razanatsima[7], Fortunat Rakotoarivony[7], Armand Randrianasolo[8], Lalao Andriamahefarivo[4,9], Nirina Rajaonarivelo[10], Félix Andriatsiferana[10], Josia Razafindramanana[10], Laura Azandi[3], Gyslène Kamdem[3], Hugh W. Pritchard[11], Tim R. Marks[11], Philipp T. Seaton[11], Bonaventure Sonké[3,4] & Vincent Droissart[3,4,5,12]**

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In the context of rapid biodiversity decline, and while *in situ* conservation should be prioritized, the threat facing by natural habitats compels us to develop accompanying *ex situ* conservation tools to adequately ensure persistence of viable populations and maintain genetic diversity. To focus our conservation efforts on the most endangered African orchids, IUCN conservation status of 175 orchid species endemic to Atlantic Central Africa (ACA) and 50 species of Madagascar were assessed and encoded on the IUCN SIS portal. We plan to pursue Red List assessments for 150 additional Malagasy species within the next two years. These conservation assessments show that > 80% of these 225 species are threatened: two might be Extinct (*Angraecopsis dolabriformis*, *Angraecum astroarche*), 24 species are Critically Endangered, 96 Endangered, 53 Vulnerable, 10 Near Threatened and only 31 qualified as Least Concern. In 2015, the first orchid seedbank of tropical Africa was established next to and linked with our shade house based in Cameroon, this year a second regional seedbank will be set up in Madagascar. These will help us in documenting the morphology and the viability, and in banking viable seeds, of about 20% of the orchid diversity of Central Africa and Madagascar (around 300 species in total). A total of 4647 manual pollination events (from 770 plants in cultivation) along with additional collections from the field have yielded 4772 mature fruits and seeds from 169 Central African orchid species, representing 38 genera of Orchidaceae. To date, we have produced seeds for 20 species assessed as threatened (CR, EN, VU).

**Key words:** Orchidaceae, Conservation, Red List, Shade house, Seed bank.

Thursday 30 May, 12:30 - 12:45

## Global conservation prioritization of Orchidaceae

**Pati Vitt[1,2], Amanda Taylor[3], Holger Kreft[3], Demetra Rakosy[4,2*], Patrick Weigelt[3], Abby Meyer[5] and Tiffany M. Knight[6, 4, 2]**

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As human pressure on biodiversity mounts, there is an increasing need to prioritize the allocation of scarce resources available for conservation. One commonly used approach to prioritization relies on the quantification of the Evolutionary Distinctness and Globally Endangered (EDGE) status of species. This metric requires a well-resolved and dated phylogeny and a high percentage of species with formal conservation assessments, which are not available for many species-rich groups in need of conservation prioritization. This was the case for Orchidaceae. Many orchid species are likely to be threatened by extinction, their populations declining due to threats such as habitat conversion, climate change and illegal trade. Yet, so far it remains difficult to assess which species would require the most urgent attention. The recent availability of a dated phylogeny for all vascular plants and a global database on orchid distributions provide the data necessary to undertake the first steps towards a global conservation prioritization of Orchidaceae. Here, we provide an overview of global patterns of species richness and endemism and present three alternative approaches to EDGE to create a list of orchid species and regions in most need of conservation prioritization: 1) the Evolutionary Distinctiveness Rarity index (EDR) based on phylogeny and standardized range size, 2) the EDR based on phylogeny and standardized by number of regions occupied, and 3) the identification of monotypic genera that occur in a single region. We find that a higher proportion of orchid species are located in a single geographical region compared to the proportion of single-region endemic species across all angiosperms. Only a small fraction of the orchid species identified as priorities for conservation have formal conservation assessments and are protected in *ex situ* collections. Our findings provide urgent recommendations for researchers, conservation professionals and curators of botanical gardens.

**Key words:** conservation, species richness, endemism, Evolutionary Distinctiveness Rarity, global assessment.

## Thursday 30 May, 12:45 - 13:00

# A continental scale analysis of threats to orchids in Australia

**Jenna Wraith[1,2] & Catherine Pickering [1,2]**

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Orchids are threatened globally, but what threatens Australian orchids, do threats co-occur and where are orchids threatened? To address these questions, we conducted a continental review of threats to orchids. First data was extracted from documents for 184 orchids listed as threatened by the Australian Government and coded into 28 categories, as only 5 Australian species occurred on the IUCN Red List. Additional data on species characteristics was obtained, and their distribution was mapped using 14,651 location records from the Atlas of Living Australia. Australian threatened orchids belong to subfamilies Orchidoideae (88%) or Epidendroideae (12%). Common threats were changing fire regimes (74% of all threatened Australian orchids), invasive species (65% of species), habitat modification (64%), grazing (63%), tourism and recreation (47%) and illegal collection (46%), which often co-occurred as threat syndromes. Most species were terrestrial, in temperate forests (96 species) and shrublands (36 species). Generalized linear models were used to assess patterns in threats to orchids using bioregions in Australia. Bioregions with less native vegetation cover were more likely to have orchids threatened by habitat modification, grazing or weeds ($p<0.05$), while bioregions with greater coverage of protected areas were more likely to contain orchids threatened by tourism and recreation, but less likely to have orchids threatened by habitat modification ($p<0.05$). Understanding these co-occurring threats and their distribution across Australia is crucial for successful management as they highlight key areas and habitats for conservation and the approach used here could be applied to other threatened species and locations. The results also highlighted that threats to orchids from climate change is not yet fully reflected in current listing documents in Australia and so may also be underestimated in other regions. Updating the IUCN Red List for Australian orchids, including greater recognition of the threat from climate change is required.

**Keywords:** Orchidaceae; threatened species; threat syndromes; conservation; threat distribution; human impacts.

Thursday 30 May, 16:30 - 16:45

# Red Listing of New Guinea Orchids

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We undertook International Union for the Conservation of Nature (IUCN) Red List assessments for almost 300 orchid species from New Guinea, or about ten percent of the total number of species known from this island. They were selected on the basis that they belonged to taxa that had been recently revised, which avoids the problem that for many orchids from New Guinea their taxonomy is poorly understood. Major issues are that New Guinea is still under-collected by botanists and the existing collections are strongly spatially biased. At first sight, one would be inclined to think that Red List assessments would be difficult to attempt under these circumstances and that most species would have to be considered Data Deficient (DD) under the IUCN criteria. However, our results show that when distribution data and knowledge of habitats, ecology and threats are taken into account, only about 24% of the assessed species are evaluated as DD ; 60% are Least Concern and 16% fall in one of the threatened categories.

## Thursday 30 May, 16:45 - 17:00

# Opportunities and pitfalls in rapid conservation assessment methods for orchids

**Barnaby E. Walker[1], Eimear Nic Lughadha[1], Cátia Canteiro[1], Helen Chadburn[1], Aaron P. Davis[1], Serene Hargreaves[1], Eve J. Lucas[1], André Schuiteman[1], Emma Williams[1], Steven P. Bachman[1], David Baines[1,2], Amy Barker[1], Andrew P. Budden[1], Julia Carretero[1], James J. Clarkson[1], Alexandra Roberts[1] & Malin Rivers[3].**

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IUCN Red List assessments are increasingly important to many conservation efforts, but currently only 7% of all vascular plants have been assessed, with orchids under-represented in that figure. Rapidly increasing the number of assessed species is vital to getting a clear picture of the threats facing plants and meeting international targets on biodiversity conservation. Assessing the status of a species can be time-consuming and may require data that is not readily available. We recently reviewed methods proposed in the literature for speeding up conservation assessments by predicting if a species is threatened or not based on information from herbarium specimens. When compared on sets of species already assessed by conventional methods, we found that these methods could correctly predict the threat status of up to 90% of our test species. However, performance on a set of orchids from New Guinea was significantly worse than average. Most methods failed to correctly predict the status of significantly > 50% of species tested, no better than guessing the threat status at random. This has given us the opportunity to explore reasons why orchids proved especially difficult for these methods. Improved understanding will allow us to refine methods and to better characterise the scale and nature of extinction risks in orchids.

**Keywords:** IUCN Red List, conservation assessment, machine learning, natural history collections

## Thursday 30 May, 17:00 - 17:15

# *Habenaria occlusa*, *Eulophia schweinfurthii*; are they Rare, Extinct (EX) or Critically Endangered (CR)?

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*Habenaria occlusa* and *Eulophia schweinfurthii* are terrestrial species of Orchidaceae, growing wildly, in the Southern Highlands of Tanzania. Orchids in this area are vulnarable due to human consumption and trade, among others. We assessed the conservation status regarding the two species facing extinction jeopardy. Field surveys using modified Whittaker plots and a transect walk, ecogeorgaphical study and literature reviews were conducted, for this case. Qgis 2.18, Wallace Ver. 3 and IUCN Geocat were employed to estimate the niche, mapping and assignment of the threat category for the two species. Each of the species was only spotted once. *Habenaria occlusa* is inherently scarce and one colony was reported at Mbeya Peak along the river, the other was spotted at the river basin at Kitulo during this field work, confirming it to be a riverine species. The species is endemic to Tanzania, has not yet been assessed for the IUCN Red List or listed in its" Catalogue of Life"; the same applies to *E. schweinfurthii*. We assess the two species to be Critically Endangered using IUCN Red List categories. In light of the Critically Endangered status of the species and their rarity, urgent management actions are needed to determine population size and trends in abundance in combination with community education and awareness campaigns. We recommend to the IUCN Orchid Specialist Group to review the conservation status of these species and officially red list them, hence fostering resources for their conservation and restoration efforts.

**Key words:** *Habenaria occlusa*, *Eulophia schweinfurthii*, orchids, niche, threat, endemic, rarity

## Thursday 30 May, 17:15 - 17:30

# A proposal for the integration of seven orchid species endemics of Sardinia in the IUCN list: a tool for conservation of endangered species

**Pierluigi Cortis, Antonio De Agostini, Michele Lussu & Annalena Cogoni**

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Sardinia is the second largest island in the Mediterranean Sea (after Sicily). It is located in the west of the Italian Peninsula and to the south of the French island of Corsica. The island has an area of 241,00 $km^2$, with an elevation range from 0 to 1834 m a.s.l. Sardinia is one of the hot spots of the diversity of Mediterranean orchids, and insularity is certainly one of the factors (among the others such as the different selective pressures in the new ecological context) that has most favoured the formation of endemisms, due to the founder effect, occurring during the colonization of the island by the taxa coming from the Tuscan archipelago, Provence, Spain and North Africa. Recent investigations have made it possible to better understand what the evolutionary dynamics of orchids have been and still are, and how they can be influenced by the anthropogenic impact. The number of taxa currently present in Sardinia is not definable with certainty as it varies according to the taxonomic assessment that the various authors attribute to some of them. In the Delforge publication of 2005, 71 entities were reported for Sardinia, compared with 63 in Scrugli & Cogoni, 1998. This floristic richness is, however, subject to some threats that put at risk the conservation of taxa of taxonomic and biogeographical interest. These threats are: the loss of habitat; the trophic habits of some wild animals and farm animals bred in the wild, very abundant in the island; and the direct and indirect human pressure. Given the risk of loss of orchid biodiversity in Sardinia, this study proposes the integration into the IUCN conservation lists of seven endemic taxa of Orchidaceae (*Ophrys chestermanii*, *Ophrys conradiae*, *Ophrys exaltata* subsp. *morisii*, *Ophrys normanii*, *Ophrys ortuabis*, *Ophrys panattensis*, *Serapias nurrica*) particularly deserving of conservation efforts.

**Keywords**: endemism, endangered species, IUCN, Mediterranean basin, threats.

## Thursday 30 May, 12:15 - 12:30 (parallel session)

# Target sequence capture in orchids: developing a kit to sequence hundreds of single copy loci across all orchids

**Lauren A. Eserman[1], Shawn Thomas[2], Emily E. D. Coffey[1] & James H. Leebens-Mack[2]**

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Understanding relationships among orchid species and populations is of critical importance for orchid conservation. Target sequence capture has become a standard method for extracting hundreds of single-copy orthologous loci for phylogenomics. However, the upfront cost and time associated with the design of a target capture kit makes this method prohibitively expensive for many researchers. Therefore, we have set out to design a target capture kit to reliably sequence hundreds of orthologous loci from across all orchids. We used a published set of orthologous loci from across orchids to identify genes that were single-copy across the Orchidaceae. We were then left with 752 single-copy genes including 5490 exons. We are testing the utility of this kit to reliably capture hundreds of single-copy genes from species from most orchid subtribes. We hope that this kit will allow for greater accessibility of next-generation sequencing for orchid systematics, population and conservation genetics and, potentially, identification of poachers and other illegal orchid trade.

**Keywords**: phylogenomics; target sequence capture; next-generation sequencing

## Thursday 30 May, 12:30 - 12:45 (parallel session)

### Next-generation sequencing of orchids and their mycorrhizal fungi

**Sarah Unruh**

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The slipper orchids (Cypripedioideae) have some of the largest genomes in the orchids, which may be due to polyploidy or some other mechanism of genome evolution. We used RNA-seq data to infer a multi-locus nuclear phylogeny of Cypripedioideae and to determine whether a whole-genome duplication event (WGD) correlated with the large genome size of this subfamily. Our transcriptome phylogeny confirmed relationships published in previous studies that used fewer markers, but incorporated more taxa. We did not find a WGD event at the base of the slipper orchids; however, we did identify one on the stem lineage of Orchidaceae. Although WGD has played a role in the evolution of Orchidaceae, polyploidy does not appear to be responsible for the large genome size of slipper orchids. The conserved set of 775 largely single-copy nuclear genes identified in this study is being developed into a set of baits for targeted sequencing studies across the orchids and in specific groups. In addition to the slipper orchid study, I am using genomic data to investigate the evolutionary relationships of the fungi that form mycorrhizal relationships with Orchidaceae. I will also generate 15 reference genomes using PacBio long-read sequencing technology. Results of these studies will add to the genomic resources of mycologists and evolutionary biologists as well as help determine any genomic basis for their symbiosis with orchids.

**Keywords:** genomics, mycorrhiza, Orchidaceae, phylogenetics, polyploidy, RNA-seq

Thursday 30 May, 12:45 - 13:00 (parallel session)

## Investigating hybridization and gene flow between *Orchis militaris* and *O. purpurea*: evidence from nuclear microsatellites and geometric morphometrics

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Hybridization and introgression are widely considered to have a significant impact on plant evolution. In food-deceptive orchids of the genus *Orchis* pre-mating reproductive barriers are weak, often resulting in the build-up of large hybrid populations between certain species. However, post-mating barriers appear to be much stronger, suggesting a key role in the maintenance of species integrity. Anthropomorphic *Orchis* species are an excellent system in which to study hybridization because parents and hybrids are regularly found growing in sympatry. We developed a set of nuclear microsatellite markers which were used to characterize genetic structure in French hybrid populations of *O. militaris* and *O. purpurea*. We also studied the effects of hybridization on labellum morphology, using geometric morphometrics to quantify petal shape. Preliminary data suggest that hybrids between *O. militaris* and *O. purpurea* are fertile and regularly backcross with one or both of their parents.

**Keywords:** nuclear microsatellites, geometric morphometrics, genome skimming, hybridization, introgression, *Orchis*

## Thursday 30 May, 14:00 - 14:15 (parallel session)

# Genomic diversity in Australian orchid complexes and its implications for taxonomy and conservation

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Australia harbours a rich and highly endemic orchid flora with > 1,300 native species, of which c. 90% occur nowhere else. Many Australian orchid species possess only narrow distributions, rendering them vulnerable to threats such as climate and environmental change or habitat destruction. In fact, orchids constitute > 15% of Australia's threatened flora and over 1/3 of Australia's critically endangered plants. However, the rapid increase in orchid species recognised for Australia over the past two decades, from around 900 to > 1,300, resulted in uncertainties regarding the taxonomic and conservation status of many species, which greatly hampers effective conservation management and allocation of scarce resources. Recent advances in high-throughput DNA sequencing offer powerful genomic approaches to obtain unprecedented insights into the genetic diversity of orchids, facilitating the re-assessment of the taxonomic and conservation status of rare and threatened orchids. Here we present the results of four case studies in Australian orchid species complexes based on double-digest restriction-site-associated DNA sequencing (ddRADseq): 1) of two highly endemic epiphyte species of Australia's Wet Tropics mountain top forests (*Bulbophyllum lageniforme* and *B. newportii*) to assess their genetic diversity to inform *in-situ* and *ex-situ* conservation strategies in the face of future climate change; 2) in the *Corybas aconitiflorus* complex, which includes the nationally threatened species *Corybas dowlingii*, to elucidate species delimitation and its implications for conservation; 3) in the *Thelymitra fuscolutea* complex, which includes two endangered and three rare species, to clarify species delimitation; and 4) in a hybrid zone between the threatened species *Caladenia arenaria* and *Caladenia concinna* to assess levels of genetic admixture in the population to inform conservation strategies for *Caladenia arenaria*. Our case studies show that ddRAD sequencing is an effective tool to obtain robust genomic data to inform orchid taxonomy and conservation.

**Keywords**: *Bulbophyllum*, *Caladenia*, *Corybas*, conservation genomics, high-throughput sequencing, *Thelymitra*

Thursday 30 May, 14:15 - 14:30 (parallel session)

## Utility of DNA barcodes and stable carbon isotope signatures on Philippine orchid taxonomy and ecology

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Molecular and ecophysiology profiles of at least 80 species in 32 genera across three subfamilies of Philippine orchids were documented to provide evidence towards the understanding of Philippine orchid classification. Orchids were collected from various regions of the country, and their vouchers processed and deposited at De La Salle University Herbarium. Fresh leaf samples were then stored in a low temperature facility at the Molecular Science Unit Laboratory of DLSU. Total DNA was extracted and processed using standard protocol and the cpDNA regions, i.e. *rbcL*, *matK* and *trnH-psbA*, were PCR-amplified, sequenced and analyzed for their utility in studying species variation and relationships. The same plant materials were processed and analyzed for carbon isotope compositions ($\delta^{13}C$) to provide information on the possible photosynthetic pathways. Results of this study show that *rbcL* and *matK* are the more useful barcodes based on robustness in PCR amplification and sequence alignment, gene length, ability to identify or define interspecific sequence differences in species-rich genera, i.e. *Eria*, *Bulbophyllum*, *Dendrobium* and *Dendrochilum*, ability to distinguish intraspecific sequence differences, and nucleotide diversity or divergence. Moreover, results of the stable carbon isotope analysis show that most of the Philippine orchids sampled are representative of $C_3$ plants (isotope ratios -22.40 to -38.44), with a few as facultative CAM (-12.76 to -17.89), obligate CAM and $C_4$ plants (-12.76 to -13.83). A cluster analysis also shows that the orchids can be grouped as ground-$C_3$ plants, epiphytic-$C_3$ plants, epiphytic-obligate CAM plants, and epiphytic-facultative CAM or $C_4$ plants. Though classification based on photosynthetic pathways and life forms does not provide definite correlation to the systematic classification of orchids, it can however provide insights on the ecology and adaptive capability of the species towards climatic changes over time.

**Keywords:** DNA barcoding, $\delta^{13}C$ isotope, photosynthetic pathways, life forms, orchid classification, climatic change

## Thursday 30 May, 14:30 - 14:45 (parallel session)

### New evidence of hybridization in *Platanthera* species

**Marilin Mõtlep, Kadri Tali, Aigi Ilves & Tiiu Kull**

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Studies focusing on hybrid zones contribute to better understanding of speciation processes. Hybridization between *Platanthera bifolia* and *P. chlorantha* (Orchidaceae) has been often reported in previous studies. We conducted a crossing experiment in four populations in 2015 and 2016 in Estonia and afterwards determined fruit set and assessed seed quality to find out if there are any post-mating barriers between the sister species and germinated hybrids *in vitro* to see how hybrids differ from their parental species in molecular traits. We also measured morphological traits of the study species and their putative hybrids in ten sympatric populations and two allopatric populations and collected leaf samples for AFLP analysis. We tracked stained pollinia in two sympatric sites in 2017 to find out if pollen flow occurs between the study species. The crossing experiment revealed no post-mating isolation between the *Platanthera* species; the barrier could exist at the F1 level, but this was not tested in our study. Hybrids with known parents had intermediate molecular traits and so did the putative hybrids. We detected pollen flow between the study species. Our results showed weak reproductive isolation between *P. bifolia* and *P. chlorantha* and may refer to the early stages of speciation of the species.

**Keywords**: reproductive isolation, putative hybrid, AFLP, artificial crossing, *in vitro* germination, pollen tracking

Thursday 30 May, 14:45 - 15:00 (parallel session)

## Chemical identification promotes the conservation of cryptic sexually-deceptive orchid taxa

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Cryptic species pose a challenge for conservation practitioners, as they cannot be readily distinguished morphologically. Further, the splitting of single nominal species into several newly recognised cryptic species typically decreases the population sizes and ranges of the newly recognised species, and thus may increase their extinction risk. There is potentially a high incidence of cryptic taxa in sexually deceptive orchids. Multiple ecologically discrete cryptic entities have been identified, many of which may meet the IUCN criteria for listing as endangered, but measures to formally recognise or conserve them have been limited. A key limitation is that conservation practitioners require an effective method of identifying morphologically cryptic taxa, which are typically recognised by their use of species-specific pollinators through infield pollinator baiting with picked flowers. Unfortunately, pollinator baiting involves a large time investment and picking fresh flowers - not ideal for potentially rare taxa. In the Australian orchid *Drakaea livida*, traditional pollinator baiting revealed the presence of three cryptic ecotypes attracting different pollinators. Subsequent chemical analyses of *D. livida* labella using gas chromatography–mass spectrometry (GC-MS) identified ecotype-diagnostic compounds that correlated with the attraction of specific pollinator species. Over 300 floral extracts were screened for ecotype-diagnostic compounds to investigate the population size and geographical range of the ecotypes: data critical to effective conservation measures. It was found that labellum extracts of pollinated flowers could be used in place of fresh flowers, thereby not affecting floral reproductive success. Compounds could be readily detected in the GC-MS data without the determination of their chemical structures. Given the importance of unique floral chemistry in sexually deceptive orchids, it is likely that this novel straightforward chemical analysis methodology will be a useful tool for conservation practitioners that can be applied to other taxonomically challenging sexually deceptive orchid taxa.

**Key words:** cryptic taxa, sexual deception, orchid, chemical analysis, conservation

Thursday 30 May, 15:00 - 15:15 (parallel session)

## True staminodes of Cypripedioideae

**Marpha Telepova-Texier , Victoria Kharchenko**

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Establishing the true nature of the elements of the flower, in particular the staminode, is important for understanding the trends of adaptations (Ronse de Craene & Smets 2001). The structure of the flower of Cypripediodeae is described in varying ways by different authors (Hooker 1823, Brown 1831, Eichler 1875, Darwin 1908, Cribb 1998, Prenner *et al.* 2010). This is due to the fact that in the course of floral analysis authors attached importance to different arguments. To test the homology hypotheses, Patterson (1982) proposed using three tests: similarity, conjunction and congruence. Based on their use, we believe that the position and morphology of the petaloid lobe whorl indicates its similarity with petals more, than with stamens. And following Hooker (1823), we believe that Cypripedioideae, form two petals: a lip and, opposite this, a petaloid lobe. In contrast, Brown took this perianth element to be a staminode, as in many classical monographs. Our research suggests that in the flower of all Cypripedioideae, two staminodes form over each group of stamens, but previous authors did not attach any importance to them. Therefore, staminodes and the petaloid lobe protect the reproductive system of the flower from the negative effects of the environment. According to the test for conjunction and congruence, two different transformational series, that lead to the formation of two types of staminodes in one flower cannot exist or must be genetically fixed (Wagner *et al*., 2003; Angelini & Kaufman 2005). A change in flower symmetry from polysymmetric to monosymmetric is usually accompanied by the accretion of elements preceding their reduction (Meeuse 1966) and, therefore, one side of the flower slows down in its development relative to the opposite side. Such changes are well seen in the flowers of Cypripedioideae.

**Key words:** Cypripedioideae, flower, petal, petaloid lobe, staminode, stamen

## Thursday 30 May, 15:45 - 16:00 (parallel session)

# The demography of orchids: life history, population dynamics and global rarity

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Orchids are fascinating, charismatic flowering plants with unusual life histories and peculiar dependencies on pollinators and mycorrhizal fungi. Most orchids are rare and therefore of conservation concern. We review and summarize the state of knowledge on their demography, including both their life histories and their population dynamics. Most orchids have complex life cycles involving prolonged periods of seed germination and recurrent occurrence of dormancy. Costs of reproduction appear to be common, as do other costs, most notably costs of sprouting, growth and vegetative dormancy. Population growth rates assessed using matrix population models varied between 0.50 and 2.92 (average growth rate: 0.983 ± 0.026), suggesting that most species are either slightly declining or have a stable population size. Although vital rates may differ strongly from one year to the next and may be highly correlated, the few available studies using stochastic analyses showed that vital rate correlations probably have little effect on the stochastic population dynamics of orchids. Our overview further indicates that most orchid populations involved in demographic studies were much larger than the average orchid population (mean sample size 351 ± 61), possibly leading to biased insights into orchid demography. Future research should therefore develop and apply novel analytical tools to estimate the status of small orchid populations with limited or few transitions in some vital rates. Assessing the impact of individual heterogeneity on orchid population dynamics could lead to improved estimates of important population demographic parameters such as the population growth rate or net reproductive rate. Finally, more research is needed to assess how orchid populations respond to global environmental change and whether they are able to adapt to changing environmental conditions.

**Keywords:** conservation, average populations, population density, biotic interactions, individual heterogeneity, evolution

## Thursday 30 May, 16:00 - 16:15 (parallel session)

# Inherently at risk? Does morphological variation predict conservation status?

**Raymond L. Tremblay, Anthony Soto & Valeria Meléndez-Hebib**

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The cause of rarity and conservation status of species has mostly been attributed to anthropogenic influence and humans have been shown to be the main cause of the likely demise of species threat. Here we evaluate an additional confounding factor which could in part explain the possible rarity and conservation status of some species. We hypothesize that species that inherently have low morphological variation would more likely be included in the red list. Based on the under the assumptions that low variation is likely to be correlated with reduced ecological width and biogeographical distribution consequently reducing niche breath and ultimately increase the likelihood of being included in the red list. To explore this hypothesis, we estimated the within-species morphological variation of Australian orchids and used the conservation status as published by Backhouse (2007) for each species. Using generalized linear model analysis, our result show that species with differential Red List threat level (threatened, critical, rare, vulnerable, endangered and extinct) have different morphological variation associated with some of the variables studied.

**Keywords**: morphological variation, Red List, predicting extinction, Australian orchids

Thursday 30 May, 16:15 - 16:30 (parallel session)

## Impact of root system type and elevation on the richness and distribution of orchid flora in the Czech Republic

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Understanding diversity patterns along elevational gradient and the effect of global change on abundance, distribution patterns and survival of species, especially of the most endangered ones, are two highly discussed topics in biodiversity research. Here we compare the distribution patterns of orchid taxa in six floristic areas of the Czech Republic and explore specific trends in the data within each of these areas. We use data on 69 terrestrial orchid species and subspecies recorded in the Czech Republic and classify them according to their root system into three categories – rhizomatous, intermediate and tuberous orchids. Using available distributional data on these species and interpolate them in 100-m elevational intervals along the entire elevational gradient of each floristic area. Six OMI analyses were performed to calculate the niche breadth of the orchid species studied. Using regression techniques, we explored how orchid species richness and mean niche breadth depend on elevation for each of the three orchid categories separately. Most of the coefficients of determination in these regressions were high and statistically significant. The most widely distributed orchid group in the Czech Republic were the rhizomatous orchids, followed by intermediate and tuberous ones. Both species richness and mean niche breadth have shown different trends between the orchid groups studied. These patterns are probably strongly influenced by the orography of the country, in combination with the distribution of different habitat types in the six floristic areas of the country.

**Keywords:** diversity, elevation, orchid distribution, phytogeographical area, mean niche breadth.

Thursday 30 May, 16:30 - 16:45 (parallel session)

## Habitat and phorophyte comparisons of the mule-ear orchid, *Trichocentrum undulatum* between the core and edge distribution: a Cuba and southern Florida story

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The Florida state-listed endangered mule-ear orchid, *Trichocentrum undulatum*, is an epiphytic orchid that is endemic to the Caribbean region. Southern Florida of the USA is the northern limit of the species distribution, with only one surviving population, in the Everglades National Park. We know of only one phorophyte host, i.e. the buttonwood, for this orchid in southern Florida. The habitat is threatened by sea-level rise due to its coastal proximity. We studied the species' ecology in neighbouring Cuba and compared it to that in southern Florida. We found a high density of *T. undulatum* in Cuba throughout the country in various habitats. We were able to document > 70 phorophyte species in the wild and a high proportion of terrestrially bound individuals. With the continuing threats of habitat loss due to sea-level rise and detrimental plant-animal interactions, the future of the mule-ear orchid population found in the coastal regions of Florida is grim. It may be wise to implement reintroductions and artificially diversify the phorophyte hosts of the orchid in southern Florida. The knowledge gained by comparisons between core and edge distributions for the species may aid in future restoration and conservation activities by providing baseline information.

**Key words:** Caribbean islands; endangered species; island biodiversity; orchid habitat; plant ecology; plant conservation

## Thursday 30 May, 16:45 - 17:00 (parallel session)

# *Tridactyle* (Orchidaceae): a story of speciation and colonisation in São Tomé and Príncipe.

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Despite many zoological biogeographic studies on the islands of the Gulf of Guinea, the mechanisms of diversification are still poorly understood, and almost no studies have been conducted on the origin of the flora. Here, we used the genus *Tridactyle* (Orchidaceae) (50 spp.) as a model to understand the story of colonization of epiphytic orchids. This genus is only found in continental Africa and São Tomé and Príncipe and has a very high rate of endemicity on these islands. Resolving phylogenetic relationships allowed to investigate how species diversity has evolved on this archipelago and, more precisely, we tested three main colonisation scenarios that could explain the diversity of *Tridactyle*: i) a scenario of colonisation without speciation, ii) adaptative radiation and iii) a simultaneous colonisation by taxonomically and/or ecologically linked organisms. In this study, we used one nuclear maker PhyC (c. 900 pb), sequencing of the plastome (pDNA: c. 160,000 bp), ribosomal DNA (rDNA: c. 5900 bp) and habitat characteristics for each species (41 species), which provided: (i) a phylogenetic relationships tree between species and estimated the divergence period between the main lineages, and (ii) reconstructed ancestral states regarding biome preferences. Preliminary results suggested than the adaptative radiation was the most probable scenario, with one species, *Tridactyle tridactylites*, colonizing both islands and differentiated with elevation and habitats.

## Thursday 30 May, 17:00 - 17:15 (parallel session)

# Preliminary floristic checklist of Orchids flora of Gashaka Gumti national park, Nigeria

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Gashaka Gumti National Park (GGNP) is considered to be rich in biodiversity on account of the distinct vegetation types, ranging from lowland forest, savanna woodland, swamp, to high altitude luxuriant montane forest. It is Nigeria's largest national park and protected ecosystem covering a land mass of 6731 $km^2$. The park harbours an enormous proportion of fauna and flora, resulting from the altitudinal gradient, climatic conditions and its strategic location at the interface adjoining the western Cameroon highland. However, the flora of this area is poorly documented, with obvious paucity of information on Orchidaceae, probably due to botanical sampling biases. We carried out elaborate field exploration and reviewed previous reports on the flora of GGNP. Our results recorded 67 species (84%) of epiphytes and 13 species (16%) of terrestrial orchids. Genera with the highest number of species were *Bulbophyllum* (15 species) and *Polystachya* (ten species), while *Habenaria* was the richest terrestrial genus with five species. *Angraecum gabonense*, *Bulbophyllum renkinianum*, *Tridactyle tridentata* and *Rhipidoglossum polydactyla* are newly recorded for Nigeria. *Angraecopsis elliptica*, *Bulbophyllum calvum* and *Holothrix aphylla* are among the rarest orchid species in Nigeria. The highest species number was recorded in the lowland forest of the southern sector, particularly Kwano, with 42 species, 29 species in the montane forest, five species in the savanna woodland, and an additional four taxa in the Lowland gallery forest. The number of taxa recorded from GGNP alone represents about 26 % of the combined orchid flora of Nigeria reported in previous studies. Our study identified recent transhumance to the enclave of the park (leading to grazing), and unabated illegal logging of *Pterocarpus erinaceus* and *Afzelia africana* as the major noticeable threats to the continual existence of orchid species in GGNP. Therefore, further inventories with rigorous techniques such as tree climbing and living specimen cultivation are warranted for robust conservation of the orchid flora of GGNP.

**Key words:** Chabbal Hendu, Kwano, Montane vegetation; Gumti sector, Nigeria

## Thursday 30 May, 17:15 - 17:30 (parallel session)

# Orchids from Galápagos Islands

**Galo Jarrín**

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Charles Darwin, almost 200 hundred years ago said about the flora of the Galápagos Islands: "All of the plants have a wretched, weedy appearance, and I did not see one beautiful flower. When on shore I proceeded to botanize and obtained ten different flowers; but such insignificant, ugly little flowers…. (Darwin diary, September 1835). This is the image of the Galápagos orchids, most of them inconspicuous terrestrial plants, even to the people living in the islands; orchids are ignored, even worse in the continent. There are 16 native species, five are endemics, one of the endemics is recorded by the Red Book of IUCN as a critically threatened, collecting only in Santa Cruz island. The information, scientific literature on Galápagos orchids is limited. With the deep conviction that study orchids in the islands can reflect novelties for science, and the importance of conserving endemic plants, we travelled to Galápagos, specifically to Santa Cruz island, where *Cyclopogon werffii*, the lost orchid was discovered. Surely, the terrestrial orchids from Galápagos should be lived in the actual agricultural zone of the island, for that, with the help of a photographic catalogue; we interview people living in farms around Bella Vista and Santa Rosa, the humid, highland of the island. Apparently, terrestrial orchids, most of them endemics, do not live anymore in this zone; probably they went up to the more wild areas in the top of the island. Now, we are focusing on finding any specimen of *C. werffii* in order to save the species and verify the endemism. Orchids from Galápagos Islands are threatened by lack of knowledge about them, climatic change, increase of agriculture and livestock, urbanization, invasive plants and animals. Interest in wildlife in Galápagos is focusing more on fauna; we call on the international orchideologist community to enable study of the orchids there and them to conserve them for the future.

**Keywords:** Galápagos, orchids, ignorance, endemics, conservation, extinction

## Saturday 1 June, 09:00 - 09:45

## *Plenary Lecture 4.*

### How future proof are our orchids? Orchid conservation in the genomics era

**Barbara Gravendeel[1,2,3]**

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Everywhere in the world, we modify landscapes to meet our increasing needs for natural resources. The expanding human footprint drives global change, which is most pronounced in the urban environment, a rapidly increasing new biome, that replaces natural areas in which many wild orchid species used to occur. Temperatures in cities are significantly higher and urban populations of plants and animals are much more fragmented because they are separated by buildings and roads. An increasing number of studies show that several wild species not only survive but thrive in the city biome because they adapted to this new environment. Currently, six exotic plant species establish themselves in the Dutch city biome every year. Other examples include certain pollinator species, of which a higher number nowadays lives in city gardens, parks and allotments as compared with neighbouring farmlands. Genomic and other technologies could support conservation efforts by using predictive models to help build natural communities in cities that are resilient to global change. The latest genomic techniques allow researchers to answer questions that are very different as compared with a decade ago as our capacity to examine evolutionary mechanisms increases with the amount of genome data available. In this lecture, I will give examples of new insights applicable to orchid conservation that could be obtained with such data.

## Saturday 1 June, 09:45 - 10:15 (parallel session)

# Origins of allotetraploids in *Dactylorhiza* (Orchidaceae): insights from coalescent and maximum likelihood analyses of RADseq data.

**Marie K. Brandrud[1], Mark W. Chase[2,3], Mikael Hedrén[4] & Ovidiu Paun[1]**

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*Dactylorhiza* (Orchidaceae: Orchidoideae) is much affected by allopolyploid speciation and reticulate phylogenetic relationships. Although in most cases polyploids are immediately isolated from diploid relatives, sets of sibling allopolyploids often hybridize with each other, thereby increasing the complexity in an already challenging situation. Here we use variation at tens of thousands of genomic positions uncovered by RADseq to unravel the convoluted evolutionary history of *Dactylorhiza*. We first investigate circumscription and relationships of diploid species in the genus using coalescent and maximum likelihood methods, and then group 16 allotetraploids by maximum affiliation to their putative parental diploids, implementing a method based on genotype likelihoods. The direction of hybrid crosses is inferred for each allotetraploid using information from maternally inherited plastid RADseq loci. Starting from age estimates of parental taxa, the relative ages of these allotetraploid entities are inferred by quantifying their genetic similarity to the diploids and numbers of private alleles compared with sibling allotetraploids. Whereas north-western Europe is dominated by young allotetraploids of postglacial origins, comparatively older allotetraploids are distributed further south, where climatic conditions remained relatively stable during the Pleistocene glaciations. Our bioinformatics approach should prove effective for the study of other naturally occurring, non-model, polyploid plant complexes.

**Keywords :** allopolyploidy, genotype-free inference, phylogenomics, RADseq, reticulate evolution, speciation.

## Saturday 1 June, 10:15 - 10:30 (parallel session)

# Evolutionary diversification of *Lepanthes* (Pleurothallidinae): a hyper-diverse Neotropical orchid lineage

**Diego Bogarín[1,2,3], Oscar A. Pérez-Escobar[4], Franco Pupulin[1,4], Erik Smets[3,6,7] & Barbara Gravendeel[3,5,6]**

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*Lepanthes* is one of the six most diverse genera of angiosperms in the Neotropics containing more than 1125 species. The genus ranges from Mexico and the Antilles to Peru and Bolivia, with few species in the Guianas and Brazil. The greatest diversity is concentrated in the Andes and southern Central America. The origin of its extraordinary species diversity has been attributed to the epiphytic habitat, genetic drift, pollination mechanisms, orogenic processes, past climatic fluctuations, and/or key innovations such as colonization (extrinsic) or trait evolution (intrinsic). The individual influence of all these factors on the diversification of this most species-rich Neotropical orchid lineage has not yet been evaluated. Here, we used *Lepanthes* as a model to investigate the evolutionary processes that promoted species diversifications. To test hypotheses about the main drivers behind the evolution of these miniature orchids, we improved the taxonomy of the group by combining morphological characters with solid, densely sampled phylogenies, demonstrated the utility of multi-locus datasets for the resolution of species complexes derived from recent-rapid diversifications, described a new pollination system in the group, identified morphological characters associated with similar pollination mechanisms and discussed the impact of orogenic processes (formation of the Andes and Central America) on speciation. With the new insights obtained, conservation practices can be improved to protect not only these orchids but also their associated pollinators and hosts to climate change in especially cold, high-elevation areas where they are most diverse.

**Keywords:** biogeography, character evolution, phylogenetics, pollination, montane forest

## Saturday 1 June, 10:30 - 10:45 (parallel session)

# Population genetics of *Cypripedium calceolus* L. and implications for conservation

**Roberta Gargiulo**

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Understanding the eco-evolutionary mechanisms behind population survival is the first step towards the effective conservation of populations. Analysis of genetic variation in the temperate orchid *Cypripedium calceolus* has revealed a diversity higher than expected and unpredicted patterns of differentiation. Although the gene pools observed today can be explained by post-glacial colonisation from ancient refugia, migration patterns and their relationships with the persistence of genetic diversity still need to be clarified. Starting from this background and the widespread observation that clonal reproduction plays a major role in the long-term survival of the species, we have formulated hypotheses regarding the phylogeography and the evolutionary history of *C. calceolus*. These hypotheses may be relevant to all temperate orchids with a dual system of reproduction. We plan to employ a population genomic approach to look at the signature of neutral and non-neutral evolutionary forces on *C. calceolus* populations.

**Keywords**: lady's slipper orchid, population genetics, phylogeography, clonal propagation

## Saturday 1 June, 10:45 - 11:00 (parallel session)

# Applying the RAD-seq to test the hybrid speciation of *Paphiopedilum wenshanense*

**Hong Jiang[1]†, Bing-Hong Huang[2]†, Ji Jun Kong[1],Mei Hua[1], Zhen Yong Xiang[1], Pei-Chun Liao[2]* & Yung-I Lee[3]**

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*Paphiopedilum wenshanense* ($2n = 2x = 26$) has been considered as a natural hybrid species due to the intermediate floral characters between P. bellatulum ($2n = 2x = 26$) and P. concolor ($2n = 2x = 26$). According to our field investigations, these species are allopatrically distributed in Yunnan, China. *Paphiopedilum bellatulum* is found in the western Yunnan near 1500 m, *P. concolor* is distributed in the south-eastern Yunnan at around 500 m, and *P. wenshanense* is distributed from central to south-eastern Yunnan at around 1500 m. In south-eastern Yunnan, the geographical distribution of *P. wenshanense* and *P. concolor* are not overlapped. In this study, we used restriction-site associated DNA sequencing (RAD-seq) to generate thousands of single-nucleotide polymorphisms (SNPs) to verify the hypothesis of hybrid speciation in *P. wenshanense,* and test whether the genome introgression is associated with the geographical distribution. The genome-wide scans of SNPs reveal an evidence of adaptive introgression between *P. wenshanense* and *P. concolor* in contact zones, while *P. bellatulum* did not involve in any hybridization event with *P. wenshanense* and *P. concolor*. Our study demonstrates that the RAD-seq can generate sufficient molecular markers for investigating the speciation and hybridization issues in *Paphiopedilum* species with large genome.

**Keywords:** *Paphiopedilum*, restriction-site associated DNA sequencing (RAD-seq), hybrid speciation, single-nucleotide polymorphisms (SNPs)

## Saturday 1 June, 11:00 - 11:15 (parallel session)

# The impact of chromosome number changes on the diversification of angraecoids in tropical Africa (Epidendroideae: Vandeae: Angraecinae)

**João N. M. Farminhão[1,2], Pierre Meerts[1,2], Simon Verlynde[3,4], Esra Kaymak[5], Vincent Droissart[1,6,7,8], Murielle Simo-Droissart[8] & Tariq Stévart[1,7,9]**

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The impact of the widespread occurrence of polyploidisation and descending dysploidy on lineage diversification has never been explicitly tested, to our knowledge, in Orchidaceae. Angraecoids (Vandeae: Angraecinae) present a diverse range of chromosome numbers which makes them a good study system to understand karyotype evolution and its role in cladogenesis. Within Angraecinae, two major clades are recognised: one mostly confined to Madagascar, where only $x = 19$ is reported; and the Afroneotropical clade, which apparently presents a rare example of ascending dysploidy in the orchid family, with c. 90% of its species inferred to have $x = 25$. In this study, we aimed to trace the evolution of the chromosome number in the Afroneotropical clade and to test, for the first time, the likely impact of chromosome changes on diversification in Orchidaceae. By using a near-comprehensive phylogenetic tree of angraecoids at the genus level, including 280 species, we mapped the chromosome counts of 116 species in 35 genera. Accordingly, we inferred the ancestral haploid number of most Afroneotropical angraecoid genera, starting from $x = 19$, and we identified a total of three first step ascending dysploid events in *Conchograecum* ($x = 21$), *Dendrophylax* ($x = 22$) and in the 'Aerangidinae' ($x = 25$); and a single first-step descending dysploidy occurrence in *Calyptrochilum* (x = 17). Within the Aerangidinae clade nine independent second step descending dysploid events were identified, which define new generic and suprageneric synapomorphies, namely in *Ancistrorhynchus* and *Microcoelia* ($x = 24$); and in the *Cyrtorchis-Tridactyle* clade ($x = 23$). Furthermore, two secondary and one tertiary dysploid events were identified in *Aerangis* and *Summerhayesia*, respectively. Finally, nine neopolyploid events were identified in eight genera. These findings have allowed us to test whether there was a significant contribution of chromosome number evolution to the radiation of angraecoids in tropical Africa.

**Keywords**: Africa, *Angraecum*, character evolution, dysploidy, phylogeny

## Saturday 1 June, 09:45 - 10:15 (parallel session)

# The use of stable isotope natural abundance ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) to elucidate orchid mycorrhizal nutrition: an update with perspectives for orchid conservation concepts

**Gerhard Gebauer**

BayCEER - Laboratory of Isotope Biogeochemistry, University of Bayreuth, Germany

In Orchidaceae, the usually mutualistic mycorrhizal symbiosis has been modified in several directions. In nature, all orchids start into their life by completely relying on their mycorrhizal fungi as a nutrient source. This unique, initially mycoheterotrophic nutrition provides a predisposition to remain using the fungal partners as nutrient suppliers throughout the entire life cycle. In fact, > 200 orchid species are known to survive achlorophyllous in a fully mycoheterotrophic nutrition mode. These orchids thrive in light-limited forest-ground habitats and use fungi simultaneously forming ectomycorrhizas with trees or wood or litter-decomposing fungi as mycorrhizal hosts. Initially and fully mycoheterotrophic orchids bear the isotopic composition of their host fungi and are thus isotopically distinguished from accompanying autotrophic plants. Due to an isotopic positioning between autotrophic and mycoheterotrophic plants, a steadily increasing number of adult chlorophyllous orchids is identified as gaining carbon simultaneously from two sources, own photosynthesis and fungal hosts. This mixotrophic mode of nutrition is known as partial mycoheterotrophy. Fungal partners of partially mycoheterotrophic orchids can be either ectomycorrhizal fungi or saprotrophic fungi of the ubiquitous rhizoctonia group or both. Fully and partially mycoheterotrophic orchids associated with ectomycorrhizal fungi become dependent on forest trees to support their fungal hosts, but they become simultaneously independent from light supply for photosynthesis. In contrast, partially mycoheterotrophic orchids mycorrhizal with rhizoctonia fungi still require sufficient light supply for their survival. In-depth knowledge on the fungal partners and the mode of nutrition of each individual orchid species is of essential importance to develop improved orchid conservation concepts. Rather crucial for conservation concepts is the co-occurrence of orchid species with different nutritional requirements and fungal partners within micro-habitats. My presentation will highlight examples for co-occurring orchids with different nutritional strategies, thus requiring different conservation concepts from Central European forests and Western Australian shrubland.

**Keywords:** conservation, mycorrhiza, mycoheterotrophy, orchid nutrition, stable isotopes

## Saturday 1 June, 10:15 - 10:30 (parallel session)

# Ecology Informing Orchid Conservation Translocations: the ménage à trois of orchid, fungi and pollinator

**Noushka Reiter[1, 2]**

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Globally, plant species are under threat, with a fifth of all plant species facing extinction. Australia has one of the worst records globally for plant and animal extinctions. There are over 1800 species of mostly endemic orchids found in Australia, the main diversity of which are terrestrial species in the temperate south. In Australia, orchids are over-represented among threatened species, with > 17% of all plant species that are threatened in Australia being orchids. The Orchid Conservation Program at the Royal Botanic Gardens Victoria currently works with > 30 endangered orchid species. Together with program partners we undertake seed collection, mycorrhizal and pollination studies, threat abatement and conservation translocations. Conservation translocation is often the most effective option to boost wild numbers sufficiently in order to create self-sustaining populations. While reviews of the literature suggest conservation translocations often have limited success, they can be highly successful when the orchid's full life cycle is understood. Using examples from our program I demonstrate that successful large scale conservation translocations are underpinned by an understanding of both the mycorrhizal and pollinator associations and in the case of *Caladenia colorata* has led to a self-sustaining population with consistent recruitment. Mycorrhizal associations can be broad or specific, and in some cases can change with habitat, highlighting the need to propagate plants with fungal species that suit the translocation site. In orchids, pollination strategies are often highly specialised meaning that pollinator availability should be considered when selecting recipient sites e.g. the sexually deceptive *Caladenia hastata* has a highly specialised pollinator system and an extremely restricted distribution of the pollinator. Some food rewarding systems (e.g. *Caladenia colorata* and *Caladenia versicolor*) have pollinators that are highly specific, although widespread in distribution. Further in some rewarding and generalist systems, e.g. *C. arenaria*, care is needed to avoid hybridisation at conservation translocation sites.

**Keywords:** conservation, translocation, mycorrhizae, pollination

## Saturday 1 June, 10:30 - 10:45 (parallel session)

# Exploring orchid-mycorrhizal interactions in New Zealand to understand hot-spots and guide conservation actions

**Carlos A. Lehnebach[1], Lara Shepherd[1], Karin van der Walt[2], Jennifer Alderton-Moss[2, 3]**

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Orchid-mycorrhizal interactions have been little studied in New Zealand (NZ), and not much is known about what the fungal groups are involved, levels of specialisation or how orchid fungal preferences influence their distribution and local abundance. This lack of knowledge has created a significant gap in conservation efforts. We aim to uncover the fungal preferences of sympatric orchids with distinct habit (terrestrial versus epiphytic) and trophic strategies (photosynthetic versus myco-heterotrophic) co-occurring under southern beech forest. To this end we have used next-generation sequencing technologies and total root DNA to characterise the mycorrhizal fungal diversity found in ten orchid genera (*Acianthus*, *Caladenia*, *Chiloglottis*, *Corybas*, *Cyrtostylis*, *Drymoanthus*, *Earina*, *Gastrodia*, *Pterostylis* and *Thelymitra*). Also, we have isolated fungal pelotons from those species in genera that contain threatened taxa and used them for DNA identification and symbiotic seed germination experiments using different media and seed sterilisation methods. Asymbiotic germination methods were explored for epiphytic species. So far, we have identified 14 fungal taxa across six orchid genera (two Ascomycota and 12 Basidiomycota), but we have been unable to isolate fungal DNA from epiphytic orchid species. Our preliminary results for terrestrial orchids suggest sympatric species (two or three orchid species per site) with divergent mycorrhizal associations. As for germination experiments (set up three months ago) only *Corybas cheesemanii* has germinated (currently at stage 3) asymbiotically on ½ strength Muarshige & Skoog basal medium and Knudson C medium. Pelotons from *Pterostylis cardiostigma* isolated and cultured in fungal isolation medium have remained unchanged, whereas those from the control species *Spiranthes australis* have grown steadily.

**Keywords:** epiphytic orchids, myco-heterotrophy, next generation sequencing, seed germination, seed storage, terrestrial orchids.

Saturday 1 June, 10:45 - 11:00 (parallel session)

## Diversity of orchid mycorrhizal fungi from endemic and widespread orchids native to the Republic of Palau in Micronesia (Western Pacific)

**Melissa K. McCormick[1], Benjamin J. Crain[1], Lawrence W. Zettler[2] & Dennis F. Whigham[1]**

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Islands are model systems for biodiversity studies, but the factors that influence the diversity and distribution of island inhabitants are not always well understood. Colonization of islands has long been considered a function of dispersal ability, yet orchids tend to be disproportionately underrepresented relative to what might be expected on the basis of the extensive dispersal ability of their dust-like seeds. Symbioses are rarely considered when trying to discern the mechanisms that drive biodiversity patterns on the planet. While a lack of pollinators may impact distribution of many orchids, orchids also need to form associations with fungi to survive and reproduce, specific orchid-fungal partnerships, i.e. employing only one species of fungus versus partnering with diverse species, may control the distribution, abundance and overall patterns of orchid biodiversity. For orchids on islands, there is likely to be a distinction between species that dispersed to an island and those that evolved and became endemic species once they arrived. A large proportion of long-term island species have specialized relationships with symbionts, perhaps evolved long after colonization as an adaptation to the low diversity of potential symbiotic partners. We used DNA sequencing to compare the diversity of mycorrhizal fungi in the genera *Tulasnella, Ceratobasidium* and *Serendipita* that were associated with 25 of the 90 native orchids found in the Republic of Palau, including 15 endemic or near endemic species and ten that were widespread. Samples for each species were collected from similarly distant sites in Palau to the extent possible. We found that the widespread species disproportionately associated with diverse fungi, while endemic species predominantly associated with specific fungi. This suggests that orchids that associate with diverse fungi are more likely to be find appropriate mycorrhizal fungi and successfully colonize distant islands, but they may evolve over time to specialize on fungi that are particularly beneficial.

**Keywords:** *Ceratobasidium, Tulasnella,* mycorrhiza, Orchidaceae, epiphytic, specificity

## Saturday 1 June, 11:00 - 11:15 (parallel session)

# Isolation and identification of fungal endophytes from orchids native to the Republic of Palau in Micronesia (Western Pacific)

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Despite its small land area (535 km$^2$), the Republic of Palau in the Western Pacific harbours an unusually large number of native orchid species (90), of which 30 are endemic. In 2017, the Palau Orchid Conservation Initiative, spearheaded by the North American Orchid Conservation Center (NAOCC), was established to study the mechanisms behind orchid diversity in this forested archipelago. The project collaborators include the U.S. Forest Service, University of Hawaii Manoa, Palau Division of Forestry and Ngardok Nature Reserve. Illinois College's role in this three-year collaboration consisted of the collection and long-distance (> 13,000 km) transport of orchid root samples into the United States leading to fungal isolation, molecular identification and storage (cryopreservation). An eventual goal is to propagate Palauan orchids for conservation and/or horticulture using fungi assignable to the rhizoctonia complex (e.g. *Ceratobasidium*, *Tulasnella*, *Serendipita*). Roots of epiphytic and terrestrial orchids spanning different growth stages were targeted during three trips to Palau, and on the third trip (October 2018) mature capsules were also collected for symbiotic germination trials. To date (March 2019), 63 fungal endophytes were isolated in pure culture, and many were deposited into UAMH for safekeeping. Among the fungi acquired and verified by molecular methods include members of Ceratobasidiaceae from *Bulbophyllum clandestinum*, *Phreatia palawensis* and *Moerenhoutia hosokawae*. Most of the endophytes were assignable to Tulasnellaceae (e.g. *Tulasnella*, *Epulorhiza*), and these were recovered from roots of *Crepidium setipes*, *Diennia volkensii*, *M. hosokawae* and *Pseuderia micronesiaca*. Roots of the leafless endemic, *Taeniophyllum palawense*, harboured fungi provisionally identified as *Tulasnella* and *Serendipita*. To our knowledge, this is the first report documenting orchid endophytes from Micronesia. These fungi will be used in future restoration efforts, and exploring questions aimed at understanding orchid diversity, distribution and abundance.

**Keywords:** *Ceratobasidium*, *Tulasnella*, tropical, Orchidaceae, epiphytic, conservation

## Saturday 1 June, 11:45 - 12:00 (parallel session)

# The symbiotic germination mechanism of the Chinese traditional medicinal plant *Dendrobium officinale* (Orchidaceae) inoculated with *Tulasnella* sp.

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Mycorrhizal fungi colonize orchid seeds and induce the germination. This so-called symbiotic germination is a critical developmental process in the lifecycle of all orchid species. However, little is known about the molecular changes taking place during seed germination, especially symbiotic germination. *Dendrobium officinale* is an endangered epiphytic orchid, which is widely used in traditional Chinese medicine in China. In previous studies, we have screened the fungi (*Tulasnella* sp.) promoting germination of *D. officinale* and examined the ultrastructural changes accompanying symbiotic germination. Here, we summarized the recent advance on mycorrhizal diversity, mutualism molecular mechanism and mycorrhizal fungi application on *D. officinale* in our lab and emphasized the molecular and metabolic change during interaction between *D. officinale* and their fungal partner using the comparative transcriptomic and proteomic analysis. In addition, we discussed the possible role of plant hormone in symbiotic germination of *D. officinale*. Phytohormone quantification revealed plant hormone accumulation in the protocorms of *D. officinale* infected by fungi. Exogenous GA treatment or adding GA inhibitor can inhabit mycorrhizal formation and decrease seed germination rate in symbiotic germination assay. Our study will contribute to a better understanding of the orchid seed development and biology.

**Keywords:** *Dendrobium officinale*, mycorrhizal fungi, seed germination, gibberellin, signal pathway.

Saturday 1 June, 12:00 - 12:15 (parallel session)

## *In vitro* seed germination of native Ecuadorian orchids

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Protocols for seed germination and seedling development were developed for native Ecuadorian orchid species. Mature seeds were collected from native populations in the north-western Andes in Ecuador. Putative mycorrhizal fungi were isolated from roots of orchid seedlings and mature plants *in situ*. Seeds and pure fungal cultures were transported to the laboratory located at the University of Florida, Gainesville, Florida, USA. *Ceratobasidium* and *Tulasnella* strains were identified using ribosomal DNA internal transcribed spacer (ITS) amplification and Sanger sequencing. Four asymbiotic orchid seed germination media: Knudson-C (KD-C), Vacin & Went modified orchid medium (VW), ½ strength Murashige & Skoog (½MS) and Phytotechlonogy orchid seed sowing medium (P723) were examined for their effectiveness in promoting seed germination and protocorm development of *Dracula felix, Maxillaria porrecta* and *Polystachya concreta* in both 0/24 h and 12/12 h light/dark photoperiod at 23 ºC. Effects of temperature (23 ºC, 18 ºC and 23 ºC Light /18 ºC Dark) were also evaluated with *D. felix* and *M. porrecta*. Germination occurred regardless of medium, photoperiod and temperature treatments. However, advance seedling development of *D. felix* only occurred on VW and ½MS in the 12/12 h light/dark photoperiod either at 18 ºC or 23 ºC light /18 ºC dark. Seedling development in *M. porrecta* was observed on ½MS and P723 regardless of photoperiod and temperature treatment. *Polystachya concreta* seeds attained seedling development stage in all media, except KN-C, in the 12/12 h light/dark photoperiod. Protocorm like bodies were observed on 0/24 h light/dark photoperiod. Effects of putative mycorrhizal fungi isolated from native populations on seed germination and seedling development were also examined. Results of this study will aid in the development of efficient seed germination protocols for the conservation of native orchids from Ecuador.

**Keywords:** orchid, seed germination, native, Ecuador, *Dracula*, orchid mycorrhiza

Saturday 1 June, 12:15 - 12:30 (parallel session)

## Orchid mycorrhizae research in southern Ecuador

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Ecuador has one of the highest orchid species diversity on the planet, and therefore orchid research here is among global conservation priorities. In Neotropical mountain rainforests, the epiphytic orchids dominate, and the knowledge of their life cycle is of high interest. The roots of epiphytic orchids harbour various fungal taxa including mycorrhizal (MF) and non-mycorrhizal associates. MF are the essential component of orchid life cycle improving nutrient uptake at the germination and adult stage. Within the framework of the two-year PROMETEO project, we investigated orchid root-associated fungi including their i) community diversity, ii) long-term storage and iii) bacterial associates. Using a culture-dependent approach we revealed high diversity and dominance of non-mycorrhizal associates in orchid roots. Four strains of potentially MF from Tulasnellaceae and Ceratobasidiaceae were obtained, and Ceratobasidiaceae were isolated into culture in Ecuador for the first time. Subsequently, the potential MF were subjected to a cryopreservation experiment examining the effectivity of several cryomethods. Among others, we found that fungus identity had a crucial role for MF long-term cryostorage. Further, we uncovered high diversity of bacteria associated with *Serendipita* sp., a potential MF of the orchid species *Stanhopea connata*. Along with *in situ* protection of valuable habitats, detailed investigation of orchid mycorrhizae and their *ex situ* preservation is the crucial tool for future conservation of Orchidaceae in the Neotropics.

**Keywords**: bacterial associates, cryopreservation, epiphytic orchids, fungal isolation, Neotropics, orchid mycorrhizae.

## Saturday 1 June, 12:30 - 12:45 (parallel session)

### The mycorrhizal communities of four co-occurring *Habenaria* species affected by the distribution of mycorrhizal fungi and available nutrients in the soil

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Because orchids depend fully or partially on mycorrhizal fungi for carbon and other essential resources, differences in the mycorrhizal communities associating with orchids can be expected to mediate the abundance, spatial distribution and coexistence of terrestrial orchids in natural communities. In order to investigate if the local-scale orchids distribution limited by the distribution and abundance of orchid mycorrhizal fungi, four co-occurring *Habenaria* species in a small-scale habitat were conducted in Yunnan province of China. We assessed the mycorrhizal communities both in the plant roots and in the soil using next-generation sequencing technology (NGS). We also collected the soil at the sites of each samples and analyzed for total carbon and nitrogen, pH and organic matter. The results showed that significant differences in mycorrhizal communities in roots were observed among species, whereas strong clustering and significant segregation characterized the spatial distribution of the four *Habenaria* species. The variation in OUT composition of mycorrhizal fungi among species was explained mainly by mycorrhizal fungi in the soil. Soil nutrients had significant effect on OUT richness both in the roots and the soil. The results indicated that interspecific differences in mycorrhizal composition can arise due to geographical pattern of distribution of orchid mycorrhizal fungi in the soil with difference nutrient availabilities. Our results suggest that mycorrhizal associations are important factor driving small-scale habitat heterogeneity among terrestrial orchid species and may therefor contribution to orchid coexistence.

## Saturday 1 June, 12:45 - 13:00 (parallel session)

# Two New approaches to acquiring the elusive mycorrhizal associate of the endangered Hawaiian bog orchid, *Peristylus holochila*

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The Hawaiian bog orchid, *Peristylus holochila* (Hbd.) N.Halle [synonym *Platanthera holochila* (Hbd.) Krzl.] is the rarest of three orchid species endemic to the Hawaiian archipelago and is currently listed as a U.S. Federally endangered species. Fewer than 50 individual plants remain in small isolated populations on three islands (Kauai, Maui, Molokai), and only one remains on Kauai. Not only is this terrestrial species critically endangered and protected by law, it remains vulnerable to wild pigs, invasive plants and unscrupulous collectors, and it is difficult to propagate from seed. In 2002, *in vitro* symbiotic seed germination was successfully applied to *P. holochila* for this first time using a mycorrhizal fungus originally acquired from Florida. This fungus [*Tulasnella calospora* (Boud.) Juel (synonym *Epulorhiza repens*), UAMH 9824] was chosen for its proven track record of facilitating seed germination of a wide range of North American orchids. Despite having practical merit for the immediate recovery of *P. holochila*, concerns were raised about using a fungus of Florida origin to propagate seedlings destined for release in Hawaii. Efforts then shifted to acquiring the natural mycorrhizal associate(s) of *P. holochila* for this purpose. Two fungal endophytes (*Tulasnella* sp.), isolated from *P. holochila* protocorms on Molokai, were later tested but neither facilitated germination *in vitro*. The decision was then made to germinate seeds asymbiotically which was successful, but a slow (3.1 years) and cumbersome process with high seedling mortality. Given that *T. calospora* is a ubiquitous species, we are now exploring whether the Florida fungus is already present in Hawaii using modern molecular approaches. One such fungus has been identified (HM230649.1) with an 89% identity match. In addition, we are attempting to isolate fungal endophytes from naturalized orchids in Hawaii (e.g. *Polystachya concreta*), i.e. using roots of non-native orchids as 'environmental filters' for acquiring suitable Hawaiian fungi.

**Keywords:** invasive species, *Tulasnella*, tropical, Orchidaceae, terrestrial, conservation

## Saturday 1 June, 14:00 - 14:15 (parallel session)

### Effects of mycorrhizal fungi on seed germination and growth of an endangered medicinal orchid, *Dendrobium officinale*

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Mycorrhizal fungi are essential for seed germination of orchids under natural conditions. Nonetheless, in recent years, the germination of orchids' seeds without fungal symbionts has been successfully and widely applied in many orchids such as *Dendrobium officinale*. A large-scale commercial planting (without mycorrhizal fungi) of *D. officinale* is taking place in several places. However, because absence symbiotic relationships with mycorrhizal fungus in seedlings upon transplantation, which leads to the slow growth and high mortality. *Dendrobium officinale* is an endangered medicinal plant under key protection in China. It has been popular both as a traditional medicine and food supplement for 2000 years. As consumers prefer wild-collected *D. officinale* material, its wild populations have been over-exploited to the point of local extirpation. Therefore, the bionic cultivation is an important way to alleviate pressure on wild populations. In order to achieve lasting conservation benefits, the most critical point is to obtain compatible fungi for symbiotic seed germination. In most reported researches, germination-enhancing fungi have been isolated from roots, but it is a complicated and time-consuming work to find compatible fungi from the high diversity of root-associated fungi. We isolated fungi from natural protocorms and obtained seven fungal isolates, including *Tulasnella calospora* TYKX, *Tulasnella calospora* TYGU, *Tulasnella* sp. TYEU, *Tulasnella* sp. TYKM, *Sebacina* sp. TYFJ and *Sebacina* sp. TY1, of which TYFJ is the most efficient at supporting seed germination up to the seedling stage. At 60 days of incubation, 51.44 ± 6.58% of seeds developed into seedlings in TYFJ treatment, with fewer or no seedlings in other treatments. Our results indicated that orchids may need different fungal partners for seed symbiotic germination vs. adult plant development. The fungal resource will be of great importance for the large-scale cultivation of *D. officinale* plants using symbiotic germination technology and can enhance restoration efforts and its conservation in the future.

**Keywords:** *Dendrobium officinale*, mycorrhizal fungi, bionic cultivation, symbiotic seed germination, orchid conservation

Saturday 1 June, 14:15 - 14:30 (parallel session)

# Effect of mycorrhizal fungi on seed development of *Rhynchostylis gigantea* (Lindl.) Ridl.

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*Rhynchostylis gigantea* (Lindl.) has beautiful flowers, making it suitable as a pot plant. In this study, mycorrhizal fungi from *R. gigantea* were investigated and isolated. The results revealed that eight fungal isolates were isolated from roots of the orchid. Culture broth of fungal isolates was used to analyze seed germination and protocorms development of *R. gigantea* seeds. Fungal isolate 1 on seed germination and seedling development showed significant different ($p \leq 0.05$) to the control and had the highest percentage of seed germination (92.83%) and size of protocorms (0.16 mm) after two weeks of culture. The fungal isolates were analyzed for their ability to produce indole-3-acetic acid (IAA) after culture on Czapek medium supplemented with 2 mg/mL L-tryptophan. The results showed that eight fungal isolates were found to contain IAA in the range 0.52-2.54 μg/mL. The results implied that mycorrhizal fungi from *R. gigantea* might be useful for propagation of species of *Rhynchostylis* in the future.

**Keywords:** *Rhynchostylis gigantea*, mycorrhizal fungi, seedling development

## Saturday 1 June, 14:30 - 14:45 (parallel session)

# *Prasophyllum* conservation – investigating the causes of poor germination in a genus of threatened Australian orchids

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The orchid flora of Australia has one of the highest extinction rates in the world. Epitomising this trend is *Prasophyllum*, a large genus of terrestrial orchids containing 140 species from southern Australia with 39 species currently listed as threatened. Complicating conservation efforts, symbiotic and asymbiotic methods usually fail to germinate *Prasophyllum* seed for reasons not yet understood. Our study aims to investigate three factors that may be the cause of poor seed germination: 1) seed viability through a study comparing pollination treatments followed by tetrazolium staining; 2) mycorrhizal fungi associations, encompassing specificity, differences between adult plants and protocorms, seasonal turnover of fungal communities, and the effect of habitat; and 3) the effect of nutrient composition in symbiotic and asymbiotic germination media. Tetrazolium staining showed that viability across three *Prasophyllum* species ranged from 12-43%, even in an apomictic species with polyembryonic seed. Preliminary findings indicate that *Prasophyllum* are usually mycorrhizal generalists that associate with Ceratobasidiaceae fungi. The Ceratobasidiaceae OTUs used by *Prasophyllum* are closely related to pathogenic fungal species, and many of them have pancontinental distributions. Sequencing of fungal isolates showed that closely related *Prasophyllum* species often share the same fungal OTUs. In germination trials, the composition of nutrient media significantly affected germination. A broad-spectrum trial of asymbiotic media showed that *Prasophyllum* will only germinate on very low nutrient media. In contrast, the symbiotic media trial found that higher nutrient modified oats medium resulted in the highest symbiotic germination rates with two out of three isolates, with standard oatmeal agar not able to support symbiotic germination. The finding that the nutrient composition of symbiotic germination media has a large effect on germination has broad applications, particularly for *ex-situ* conservation programs. This PhD project provides a valuable case study on how to investigate the causes of poor germination across a genus of threatened orchids.

## Saturday 1 June, 14:45 - 15:00 (parallel session)

# Route to recovery- use of symbiotic system for the yellow early marsh orchid using mycorrhizal fungus from a closely related species

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Yellow early marsh-orchid (*Dactylorhiza incarnata* subsp *ochroleuca*) is a species of central and north-western Europe and Scandinavia, found in periodically inundated calcareous fens. Latest records show six UK populations, one with 10-20 plants, two sites with two plants and two sites where one or two plants occasionally appear. Asymbiotic seed germination is sporadic and very slow, and understanding the mycorrhizal partner to initiate seed germination is of paramount importance. One mycorrhizal fungal isolate obtained from *Dactylorhiza fuchsii* from a wet meadow was found to be a compatible fungus for successful seed germination. For the first time in Britain symbiotically produced seedlings could be used for assisted colonisation/reintroduction to help recover this species in its natural habitat.

**Keywords:** mycorrhiza, symbiotic, assisted colonisation, reintroduction

Saturday 1 June, 11:45 - 12:00 (parallel session)

## Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid

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While there is increasing recognition of Batesian floral mimicry in plants, there are few confirmed cases where mimicry involves more than one model species. Here, we test for pollination by mimicry in *Diuris* (Orchidaceae), a genus hypothesized to attract pollinators via mimicry of a range of co-occurring pea plants (Faboideae). Observations of pollinator behaviour were made for *Diuris brumalis* using arrays of orchid flowers. An analysis of floral traits in the co-flowering community and spectral reflectance measurements were undertaken to test if *D. brumalis* and the pea plants showed strong similarity and were likely to be perceived as the same by bees. Pollen removal and fruit set was recorded at 18 sites over two years to test if fitness of *D. brumalis* increased with the abundance of the model species. *Diuris brumalis* shares the pollinator species *Trichococolletes capillosus* and *T. leucogenys* (Hymenoptera: Colletidae) with co-flowering Faboideae from the genus *Daviesia*. On *D. brumalis*, *Trichocolletes* exhibited the same stereotyped food-foraging and mate-patrolling behaviour that they exhibit with *Daviesia*. *Diuris* and pea plants showed strong morphological similarity compared to the co-flowering plant community, while the spectral reflectance of *Diuris* was similar to *Daviesia* spp. Fruit set and pollen removal of *D. brumalis* was highest at sites with a greater number of *Daviesia* flowers. We conclude that *D. brumalis* is pollinated by mimicry of co-occurring congeneric members of Faboideae. Evidence for mimicry of multiple models, all of which share pollinator species, suggests that this may represent a guild mimicry system. Interestingly, *D. brumalis* belongs to a complex of species with similar floral traits, suggesting that this represents a useful system for investigating speciation in lineages that employ mimicry of food plants.

**Keywords:** Orchidaceae, Faboideae, Colletidae, mimicry, pollinator behaviour, plant fitness.

## Saturday 1 June, 12:00 - 12:15 (parallel session)

# Pollination by sexual deception in Pterostylis (Orchidaceae)

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Pollination by sexual deception, whereby flowers mimic female insects to attract conspecific males as pollinators, is a prime example of deceptive pollination with a highly specialised plant-pollinator interaction. Knowledge of pollinator species and abundance is important in the conservation of threatened sexually deceptive orchids, as many species rely on only one species of insect for pollination. *Pterostylis* (Orchidaceae: Cranichidae) is a diverse, Australasian orchid genus with > 270 described species including 28 nationally threatened taxa in Australia. The flowers have a unique floral morphology with an intricate trap system designed to force dipteran visitors to remove pollen on escape from the flower. Recently, sexual deception of male fungus gnats (Diptera: Mycetophilidae, Keroplatidae) has been confirmed in five species of *Pterostylis*. Using the pollinator baiting method, the aim of this study is to investigate whether sexual deception is widespread in *Pterostylis*. We present confirmation of pollination by sexual deception in *Pterostylis cycnocephala*, which deceives male fungus gnats from the family Sciaridae, a new pollinator family for *Pterostylis*. In addition, seven other species of *Pterostylis* were found to sexually attract male fungus gnats and remove orchid pollinia, although pollen deposition has not yet been observed in these. In contrast, *P. furva* flowers emit a strong, foul odour and attracted several species of scuttle flies (Diptera: Phoridae) which did not exhibit sexual behaviour. These results add to previous studies and anecdotal observations, and together suggest that sexual deception may be widespread and common in *Pterostylis*. Based on these results, 26 out of 28 nationally threatened Australian species may be sexually deceptive and therefore reliant on one or few species of pollinator. In order to conserve threatened *Pterostylis* species, future work needs to focus on understanding the distribution and abundance of these pollinators in the landscape.

**Keywords:** *Pterostylis*, sexual deception, pollination, conservation, fungus gnat

## Saturday 1 June, 12:15 - 12:30 (parallel session)

# Evolution and development of pollination related floral traits in *Phalaenopsis*

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A major key innovation during the evolution of flowering plants is the transfer of pollen by pollinators from one flower to another. In Orchidaceae, this involves a very precise placement of pollen on specific body parts of pollinators that ensures reproductive isolation and species radiations. The variation in shape and size of different floral organs of orchid flowers is related to differently sized pollinators. In terms of evolution and development, the homology of these floral organs is complex as it involves duplication and modification of different developmental genes that gained novel expression domains. An example of such a group of modified genes is the MADS-box genes that have a crucial role in floral organ development. We performed a combination of micromorphological (SEM and micro 3d-CT scanning) and molecular techniques (transcriptome analysis) to understand the evolution and development of callus, stelidia and gynostemium foot, three highly modified floral organs in orchids involved in pollinator attraction. We collected early stage and mature tissue samples from flowers of the bee-pollinated *Phalaensopsis equestris* (PE) and *P. pulcherrima* (PP), two species that differ significantly in terms of floral morphology: PE has a large callus but short stelidia and gynostemium foot, whereas PE has a small callus but long stelidia and pronounced gynostemium foot. Results of the SEM analysis show that the stelidia develops quite early, whereas the callus and gynostemium-foot develop much later. The micro 3D-CT scans, in combination with differential expression analysis, show that the callus and stelidia are derived from staminodia, whereas the column foot has a mixed sepaloid-petaloid-staminodal origin. We currently investigate whether differences in floral morphology between the two species of *Phalaenopsis* are correlated with differential expression of developmental genes. If this is indeed the case, this could provide a genetic base for attracting differently sized bee species as pollinator.

**Keywords:** callus, pollination, gynostemium foot, stelidia, transcriptomics

## Saturday 1 June, 12:30 - 12:45 (parallel session)

# First report of *Papilio* pollination in angraecoid orchids

**Thierry Pailler**

Peuplement Végétaux et Bioagresseurs en Milieu Tropical, CIRAD / Université de La Réunion.

Orchidaceae have a wide range of pollinators and exhibit pollination systems among the most diverse, specialized and complex of all angiosperms. Since Darwin's observations on members of the genus *Angraecum* from Madagascar, angraecoid epiphytic orchids have been much celebrated for providing the most extreme adaptations to large-moth pollination, as in *Angraecum sesquipedale* with a nectar spur 30 cm long, which involves highly specialized hawkmoth pollination systems. More recently in the Mascarene archipelago (Reunion, Mauritius and Rodrigues) angraecoid orchids have been found to be pollinated by song-birds (Aves) or even crickets (Orthoptera). An investigation of the pollination biology of *Neobathiea hirtula*, an endemic species of Madagascar and the Comoros, displaying typical flowers for angraecoid orchids (white and medium-size, and medium-spurred) were conducted. Direct observations and video recordings were used to identified natural pollinators. Pollinator-linked floral traits, namely spur length and nectar column height, were investigated and compared to the tongue length of pollinator species. Pollinator efficiency (pollen removal and pollen deposition) were quantified in natural field conditions during the 2018 flowering season (February). *Neobathiea hirtula* requires pollinators to set fruit. Two pollinator species from the genus *Papilio* (*P. demodocus* and *P. epiphorbas*) were observed removing pollinia and also with pollinia on the base of their tongue. These butterflies are day foragers and visit one to two flowers per inflorescence before leaving the plant for a long flight to another flowering individual. At the Kirindi site, 47.12 % of the flowers had their pollinia removed and 23.70 % had pollen loaded on their stigma. Our observation of *Papilio* pollination in *Neobathiea* is the first report of butterfly pollination in angraecoid orchids.

**Keywords**: *Papilio*, *Neobathiea*, angraecoids, butterflies, pollination.

## Saturday 1 June, 14:00 - 14:15 (parallel session)

# Relationships between environmental factors, life history strategies, and the richness and distribution of orchid species

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Concerns regarding the long-term viability of threatened and endangered plant species are increasingly warranted given the potential impacts of climate change and habitat fragmentation. Understanding the abundance and distribution patterns of species is therefore one of the key goals of biogeography and macroecology. Various approaches are used to tackle this task. The first choice are the species distribution models, which enable prediction of the occurrence of a species based on a set of GPS coordinates of known sites and a set of biotic and abiotic characteristics of these sites. The output of these models is a map of the potential distribution of the species, where the likelihood of its occurrence (a number between zero and one) is depicted in the same way as, e.g., altitude on classical maps. An alternative is analysis of the relationship between species richness (or plant abundance) and various environmental factors. An additional dimension is the life history of the species or group of species, like root system, pollination strategy etc. Here we compare these approaches and look for patterns appearing, when their combination is considered. We use Orchidaceae as a model group, as they are the largest and most diverse family of flowering plants, currently facing unprecedented risks of extinction. Despite substantial conservation emphasis on rare orchids, populations continue to decline. Hence, understanding of factors determining their abundance and distribution is of primary importance.

**Keywords:** diversity, orchid species richness, environmental factors, species distribution models.

## Saturday 1 June, 14:15 - 14:30 (parallel session)

# Phorophytes in epiphytic orchid ecology: a case of mutual exclusion?

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In the management of areas protected for epiphytic orchid conservation it is important to identify the preferred phorophyte species, so that special consideration may be taken to preserve them. There seem to be such frequent patterns of preference/avoidance in orchid-phorophyte relationships, that orchid-rich habitats should probably to a large extent be defined by their tree species composition. A survey of possible cases of narrow tree-orchid specificity revealed a great diversity among specialized orchid hosts, but also a strong representation of certain families of trees. This field clearly requires much more study. Basic curiosity, however, also requires that we go beyond the name of a phorophyte taxon. At present we know very little about what makes epiphytes establish on certain species of phorophytes while being absent on other tree species nearby. This question becomes more puzzling when the phorophyte species preferred by one orchid species is avoided by another. We explored a case of apparent mutual exclusion among two orchid species colonizing differential hosts within the same area. The usual approach towards explaining tree-epiphyte relationships is to correlate presence/absence with data on phorophyte features, such as bark pH, rugosity and water holding capacity. We attempted to compensate for the age and growth dynamics of the tree by analysing several heights to get a glimpse of age-related gradients in features.

**Keywords:** bark features; orchid habitat; species interaction; woody plant analysis

## Saturday 1 June, 14:30 - 14:45 (parallel session)

# Biotic and abiotic factors affecting phorophyte preference in epiphytic orchids: a pilot study from southern China.

**Lorenzo Pecoraro[1, 2], Hanne N. Rasmussen[3], Finn N. Rasmussen[4], Sofia I.F. Gomes[5], Vincent S.F.T. Merckx[5] & Lei Cai[2]**

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Epiphytic orchids exhibit varying degrees of phorophyte tree specificity. Some orchid species show a strong phorophyte preference, while other species associate with a broad range of tree hosts. Although trees that carry epiphytic orchids play a crucial role in orchid life cycle, little is known about this phorophyte-epiphyte relationship. We performed a pilot study to investigate why epiphytic orchids prefer or avoid certain phorophyte trees. We selected two orchid species, *Panisea uniflora* and *Bulbophyllum odoratissimum* co-occurring in a forest habitat in southern China. *Panisea uniflora* was found to grow on *Quercus yiwuensis*, but avoided *Pistacia weinmannifolia* trees. The latter phorophyte host carried *B. odoratissimum* that was never found to colonize *Q. yiwuensis*. We also selected *Beilschmiedia percoriacea* as a neutral tree, carrying several epiphytes, but neither *B. odoratissimum* nor *P. uniflora*. We analysed a number of biotic and abiotic environmental factors potentially influencing the relationship between studied orchids and trees, including bark and orchid root fungal diversity. We hypothesised that the tree host specialization of the studied orchids was influenced by the presence of orchid mycorrhizal fungi, which were in turn biased toward particular tree species. Both morphological and molecular culture-based methods, combined with metagenomic analyses, were used to assess fungal communities associated with studied orchids and trees. A total of 232 fungal species in 72 genera were isolated from bark samples. Ascomycetous fungi belonging to *Cladosporium*, *Cyphellophora*, *Fusicolla*, *Penicillium*, *Pestalotiopsis* and *Trichoderma* were dominant. The highest fungal diversity was observed on neutral trees with 71 taxa, followed by P. *weinmannifolia* trees with 47, and *Q. yiwuensis* with 31. Metagenomic analysis confirmed the presence of significantly different fungal communities on the three investigated tree species. Different fungi were also found associated with the two analysed orchid species. Fungal diversity, influenced by bark features, may affect tree selection by epiphytic orchids.

**Keywords**: epiphytic orchids, phorophyte trees, fungal communities, orchid mycorrhizal fungi, bark.

# *7TH INTERNATIONAL ORCHID CONSERVATION CONGRESS*

## *Posters*

# P1. Extremes of forest-urban gradient offer some refuge for alien orchid invasion

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Urbanization decreases the abundance of native species, which may enable exotics to experience enemy release in urban areas, enhancing their invasive capacity. The invasive autogamous orchid, *Spathoglottis plicata*, acquires some biotic resistance in Puerto Rico from a native orchid weevil specialist, *Stethobaris polita*. The distribution of *S. polita* along an urbanization gradient may affect the distribution of *S. plicata* and its future spread. To determine the effect of land cover on both species and the impact of *S. polita* on the spread of *S. plicata*, we modeled the distribution of the interaction between the two species and additionally assessed weevil abundance and the damage they do in different land cover types and localities. Land cover was the most important predictor of distribution for both species. *Spathoglottis plicata* occurs in forests, pastures, and urban areas; however, *S. polita* is largely absent from urban areas, including urban forests, and along rivers within wet forests. This distribution likely reflects the dispersal ability of *S. plicata*, in addition to potential human intervention, and the inability of *S. polita* to penetrate or become established within the urban matrix. Thus, within the invasive range of *S. plicata* in Puerto Rico, geographical heterogeneity in acquired interactions is expected to result in higher seed production within areas of enemy release. The orchid is likely to spread more rapidly in urban forests, as well as along forest rivers, where *S. polita* is sparse.

**Keywords:** invasion barriers, biotic resistance, urban ecology, Orchidaceae, weevils, GIS

# P2. Attachment forces of herbivorous snails on leaves of terrestrial and epiphytic orchids

**Richa Kusuma Wati[1,2], Barbara Gravendeel[1,3,4], Rogier R. van Vugt[5], & Arie van der Meijden[1,3]**

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Plants protect themselves against herbivores with various structures embedded in their epidermis. Examples of such structures are epicuticular hairs, waxes, glands and glandular trichomes. Mechanical interactions of epicuticular hairs include piercing and physical impedance of herbivore locomotion. Epicuticular waxes consist of tiny crystals. These wax crystals break off when herbivores step on them and contaminate body parts of herbivores, in this way reducing the contact area and inhibiting attachment. Epicuticular glands and glandular trichomes can secrete and inject toxic compounds and form an effective defensive strategy against herbivores as well. Although mostly known for their spectacular floral diversity, orchids are also characterized by a substantial leaf anatomical diversity. Despite the large variation in epi- and subcuticular protective structures, though, orchids suffer from herbivore damage, both in the wild as well as in cultivation, hampering *in situ* and *ex situ* conservation. Invasive and exotic herbivorous snails are among the most common orchid pests and terrestrial orchids seem to suffer considerably more from snails than epiphytic species. To answer the intriguing question whether terrestrial and epiphytic orchids protect themselves differently against herbivorous snails, we investigate the adhesive forces of gastropod mantle feed of two very common orchid pests. By analysing the adhesion of small Subulinidae and larger Pleurodontidae snails with a 'bug centrifuge', we study how adhesive forces are related to the presence of epicuticular hairs, waxes, glands and glandular trichomes on the leaves of four different orchid species, two epiphytic ones and two terrestrial species. The acceleration (*g-force*) at which the snails are released during the experiments are used as a proxy for adhesion. Anatomical details of the epi- and subcuticular protective structures present on and in the orchid leaves investigated are studied with scanning electron microscopy (SEM) and light microscopy (LM). Our study provides more insight in the various ways by which orchids protect themselves against herbivorous snails. This knowledge can help improve the conservation of endangered orchid species.

**Keywords**: Bug centrifuge, epicuticular, orchid herbivores, snails

## P3. Community orchid bank

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Thailand has high species diversity of the orchid, totalling 1,176 species, but many species have become threatened due to over-collection as well as habitat destruction. From a conservation point of view, we should particularly focus on the species that are utilized commercially, especially the ones that are desired for ornamental purposes, such as *Vanda coerulea* (blue vanda), *Rhychostylis gigantea* and *Paphiopedilum* spp. etc. With that strategy, *ex-situ* and *in-situ* conservation are urgently needed. However, in Thailand, the orchid conservation project seems not to be succeeding without community involvement and other stakeholders such as government and private sectors and scientists. A good example is the blue vanda project initiated by Queen Sirikit Botanic Garden (QSBG) and the sub-district administrative organization since 2008, encouraging local people to conserve this threatened orchid species by means of propagation, reintroduction and an educational orchid sightseeing program - altogether resulting in additional income to local people. Furthermore, the community supports *ex-situ* conservation by growing the orchid in private homes. There are 65% (n=74) of all houses growing blue vanda in their homes, and 57% of all houses have mother plants totalling 262 individuals which could be used to produce seeds by hand pollination. Then some of the orchid pods are sent to Queen Sirikit Botanic Garden's laboratory and Khlong Pai Plant Genetic Conservation Center as well as their local laboratory to produce more seedlings. The owners of the mother plants receive seedlings to compensate for growing or sell for extra-income like an interest. In this case, it has shown that the natural resources could be kept by local people which they shall get benefit latter with assisting by local government, scientific institutes and fund organizations.

**Keywords**: Orchid conservation, Community orchid bank, blue vanda

## P4. Comparative seed germination and seedling development of two species of *Habenaria* from northern Thailand

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*Habenaria rhodocheila* and *H. janellehayneiana* are terrestrial orchids with showy flowers. The decline of this wild orchid population due to over-collection and deforestation is a major concern. Moreover, the germination of seeds under natural conditions is very low, leading to the decrease of the natural population. The objective of research was to investigate seed germination and seedling development of both species under *in vitro* conditions. Asymbiotic seed germination was evaluated on various cultured media including ½VW, VW, ½MS, MS, and OMA media for 16 weeks. The result showed that *H. rhodocheila* seeds germinated after 2 weeks of culture (23.52 %) on ½VW medium, and the highest percentage of seed germination was observed after 11 weeks of culture (66.67%) with five developmental stages of protocorms. Seeds of *H. janellehayneiana* showed sign of germination after 4 weeks of culture (2.60%) on OMA medium, and there were three developmental stages of protocorms after 16 weeks of culture. The results indicated that differences in germination and protocorm development between these two *Habenaria* species exist. Further research on symbiotic seed germination is required to promote seed germination and facilitate seedling establishment.

## P5. Assessment of diversity, distribution and conservation status of the orchids of Assam and their *ex-situ* conservation at the botanic garden of BARC, Trombay, Mumbai, India

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Due to continuous habitat fragmentation by indiscriminate felling of forest trees, clearance of forest cover for expansion of agricultural land, encroachment, illegal trade for medicinal use and various other anthropogenic activities, the orchid flora of Assam has become highly threatened. The present study was aimed to make a comprehensive account on the orchid flora of state, which will throw light on its diversity, distribution, habitat specificity and rarity, conservation status of individual orchid species and *ex-situ* conservation of the some of the highly threatened orchid species at the botanic garden of BARC, Trombay. In the current census, we have found 482 taxa (477 species and five varieties) belonging to 109 genera occurring in the state. Among them, 279 taxa are epiphytes, 136 taxa are terrestrial, 51 species are either epiphytes or lithophytes, eight species are saprophytes, five species are either terrestrial or lithophytes and three species are lithophytes. Out of the total, one is probably Extinct, 11 are Critically Endangered, 36 are Endangered, 115 are Vulnerable, 122 are Near Threatened and 166 are categorized as Least Concern. Further, due to lack of data conservation status of 31 species remains Data Deficient. Out of 162 species of threatened orchids, about 50 species are already conserved at the botanic garden of BARC. We hope that the study can serve as a resource document for the conservation of orchid flora of the state, help in the preparation of recovery and threat abatement plans and act as a source of information in increasing public awareness on the conservation issues and a focus for research planning.

## P6. Long-term conservation of Malagasy orchids

Vincent Droissart[1,2,3], Brigitte Ramandimbisoa[2], Lalao Andriamahefarivo[2], Hugh Pritchard[4], Simon Verlynde[2,5,6] & Tariq Stévart [2,3,7]

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More than 1000 orchid species occur in Madagascar which represents ~ 7% of Malagasy flora, a large part of it being threatened due to rapid habitat destruction. Intensive field surveys conducted in Madagascar by the Missouri Botanical Garden have brought > 250 orchid species into cultivation using a network of four orchid shade houses. Building on these living collections and with a support from the National Geographic Society, we propose to develop an integrated approach to long-term conservation of Malagasy orchids involving field surveys, *ex situ* collection (living plant and seed bank) and IUCN assessments. Our project objectives perfectly align with six of the 13 Conservation Plans endorsed by IUCN/SSC Orchid Specialist Group. Specifically, we plan to bank seeds of 15% of Madagascar's orchid diversity (ca. 150 species), focusing on those that are the most threatened. A full set of seeds will be stored in Madagascar, and a duplicate set will be conserved at the Royal Botanic Gardens, Kew, UK. Data collected during the project will serve to assess the risk of extinction of ~200 orchids and to update the IUCN Red List (https://www.iucnredlist.org/). The results will be disseminated through popular and scientific papers, the Catalogue of the Plants of Madagascar (http://www.tropicos.org/Project/Madagascar) and portals related to orchids (e.g. http://www.osssu.org/).

**Keywords:** *ex situ* conservation, IUCN Red List, Madagascar, Orchidaceae, seed bank, shadehouse

# P7. Historical utilization of *Vanilla* in northern South America and prospects as a novel non-timber forest product

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Vanilla is one of the most economically important crops for low altitude humid tropical regions. Mexico is widely considered the cradle for vanilla domestication, and a large body of historical texts documents the importance of vanilla in the country both pre- and post-European contact. Cultivated *Vanilla planifolia* belongs to the so-called "aromatic" clade, containing 47 species; 42 are native to South America. Colombia is a significant center of *Vanilla* diversity, with 24 species in the aromatic clade, five of which are endemic. Nonetheless, vanilla is barely cultivated in the country today. We explored the economic prospects for native South American *Vanilla* species by reviewing botanical and historical records of the use and commerce of vanilla. We focused on the region equivalent to the Viceroyalty of Nueva Granada prior to 1819, and during the 19th and 20th Century in Colombia. We compiled substantial accounts of the traditional use of vanilla by indigenous communities across equatorial America, from the early 17th Century onwards. In Colombia, ethnic communities used vanilla as a fragrance or body perfume (e.g. guayaberos ethnic group, Orinoco) and others as a magic amulet (e.g. afro-descendant communities, Chocó; Putumayo). Documents record the commercialization of wild-collected vanilla from the region of Nueva Granada and Portuguese colonies to Europe during the 18th Century. During the 19th Century, several enterprises were established in Colombia for the cultivation and commercialization of vanilla, such as *"Empresa de Vainilla"* in Antioquia. In the early 20th Century, a number of Colombian researchers detailed aspects of cultivation, natural and artificial pollination, profit and trade of vanilla in Colombia. Our review illuminates the hitherto unrecognized historical economic importance of vanilla in northern South America. It also provides a prospective for present and future enterprises in vanilla cultivation and an incentive for conservation of the natural *Vanilla* resources in the region.

**Keywords:** aromatic clade, cultivation, domestication, Nueva Granada, traditional use.

## P8. Orchid Recovery Program, Illinois College. – viability of orchid endophytes in prolonged cool (4-6º C) storage for conservation purposes

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Orchid conservation in this 'age of extinction' will likely depend on a blend of three actions to be successful: 1) design and management of natural reserves, taking into account the specialized needs of orchids; 2) establishment of *ex situ* seed and mycorrhiza banks for orchids under immediate threat, and 3) development of techniques for orchid restoration. In action two, the 'specialized needs' includes abiotic and biotic factors, the latter of which involves pollinators and mycorrhizal fungi to ensure fruit set and seed germination, respectively. Cryopreservation is often employed by international culture collections (e.g. UAMH in Canada) to preserve important strains of fungi indefinitely and is preferable to continuous subculturing and/or cool storage (i.e. refrigeration). For some researchers, however, cryopreservation may not be a practical option. In this study, we assessed the survival of 100+ orchid endophytes collected in North America that were stored in refrigeration (4-6º C) for several years. The majority of the fungi were basidiomycetes assignable to *Ceratobasidium* and *Tulasnella*. Using agar slants within screw-cap tubes, cultures were maintained on two different media (oat meal agar and potato dextrose agar). In general, *Tulasnella* strains remained viable for longer periods in cool storage than those assignable to *Ceratobasidium*. For orchids that utilize *Ceratobasidium* to complete their life cycles (e.g. *Platanthera leucophaea*, *Dendrophylax lindenii*), new storage techniques and/or more frequent subculturing may be necessary to maintain the important strains of this genus in a viable state, as found in a subsequent germination ability test.

## P9. European alpine species, *Chamorchis alpina,* threatened in the future?

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*Chamorchis alpina* is a high mountain species distributed in northern and alpine Europe. In the Alps and Carpathians, it grows usually above 2000 m a.s.l., but in Scandinavia its populations are found in lower areas. While it is classified by the IUCN Red List of Threatened Species as a taxon of least concern (LC), it is endangered in Poland and Finland and vulnerable in Slovakia and France. The aim of the present study was to evaluate the possible impact of the future climate changes on the distribution of the suitable niches of *C. alpina* using ecological niche modeling (ENM) approach. Probable modifications of the geographical ranges of *Dasytes alpigradus, Formica lemani* and *Leptothorax acervorum* were visualized to assess the possible negative effect of the global warming on the pollinators of the studied orchid. Furthermore, the glacial potential range of *C. alpina* was reconstructed to evaluate a general direction of its suitable niches alterations. To reduce sampling bias, only 184 georeferenced localities of the initially gathered 5432 records were used in the analyses. Twelve bioclimatic variables served as input data. The impact of the global warming on habitats of the studied orchid was evaluated using four Representative Concentration Pathway scenarios for 2070 - RCP2.6, RCP4.5, RCP6, and RCP8.5. Conducted analyses indicated that during the Last Glacial Maximum habitats suitable for *C. alpina* were broadly distributed in Central and Western Europe. As a result of the predicted climate changes the coverage of suitable niches of *C. alpina* will decrease. The most significant habitat loss will be observed in RCP8.5 with the total range contraction of almost 250000 $km^2$. Climate changes will not significantly disturb distribution of suitable climatic niches of all studied pollinators, therefore their availability should not negatively affect populations of *C. alpina.*

**Keywords**: alpine species, Chamorchis, climate change, ENM, global warming

## P10. Assessing species delimitations in the south-western Australian *Thelymitra fuscolutea* complex

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The sun orchids (*Thelymitra*, Diurideae) are, with 120 species, the fourth largest orchid genus in Australia. A third of the species in the genus are listed as rare or threatened in different states and ten species are listed as threatened nationally. Many species have small geographic ranges, and are threatened by habitat loss and environmental change. Despite substantial taxonomic work in *Thelymitra* over the past few decades, many uncertainties persist regarding species delimitation and previous molecular phylogenetic studies based on Sanger sequencing were unable to resolve relationships among closely related *Thelymitra* species. The south-western Australian *Thelymitra fuscolutea* complex is of high priority for conservation. It consists of seven species, five of which are rare [*T. dedmaniarum* (endangered), *T. magnifica* (priority flora), *T. jacksonii* (priority flora), *T. stellata* (priority flora), *T. yorkensis*], and two are widespread (*T. benthamiana*, *T. fuscolutea*). One additional form is recognized as putative new species (*T.* sp. Ongerup). To clarify taxonomic concepts and assess species delimitations in this complex, we carried out double-digest restriction-site-associated DNA (ddRAD) sequencing. We performed molecular phylogenetic and genetic admixture analyses to assess genetic structure and diversity within the complex. Our results showed that the two widespread species both formed well-defined clades. However, the small-ranged and rare species displayed varied levels of genetic admixture. Three clades were found that have a uniform genotype, but seven accessions from three species displayed admixture from multiple genotypes indicating gene flow between populations. The putative new species displayed a similar genotype to another species of the complex and hence does not merit recognition at species level. We demonstrated that the application of ddRAD sequencing facilitated assessment of species boundaries in the *T. fuscolutea* clade and allowed novel insights into the phylogenetic relationships and genetic structure to inform taxonomic revision and improve conservation strategies.

## P11. Towards a national centre for orchid conservation in Italy

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The Tuscia Germplasm Bank (TGB) is a research facility devoted especially on issues related to the conservation of terrestrial orchids in Italy: *in situ-ex situ* conservation, red listing, viability testing, and reproduction biology (fruit-set studies, *in vitro* asymbiotic germination and propagation). With about 250 orchid taxa and 90 endemics, Italy hosts one of the richest orchid flora in Europe. Currently, about 80 taxa belonging to 18 genera, 32% of the Italian orchids, are preserved in TGB, for a total of 260 seed accessions collected in 11 out of 20 administrative regions. Particularly, the TGB banked 12% of the Italian endemics (mainly in the genus *Ophrys*) and 25% of the threatened taxa, 29% of the Critically Endangered taxa (e.g. *Dactylorhiza elata* subsp. *sesquipedalis* and *Platanthera kuenkelei* subsp. *kuenkelei*) and 40% of the Endangered ones (e.g. *Anacamptis palustris*, *Epipactis placentina*, *Limodorum trabutianum*, *Orchis mascula* subsp. *ichnusae*). The knowledge and experience gained over the last 10 years have allowed the TGB to overcome the local level, providing a key contribution toward the establishment of a national orchid conservation centre, in collaboration with RIBES, the Italian network of seed-banks. A hub aimed at the development and implementation of a national strategy, with the ultimate goal of ensuring effective conservation of the Italian orchid flora, meeting the international goals of the EU Biodiversity Strategy to 2020 to averting global biodiversity loss: (1) defining a priority list to ensure the long-term conservation and protection of all the threatened taxa and the Italian endemics which are under the full national responsibility; (2) increasing and coordinating seed-banking throughout Italy; (3) improving the quality of the germplasm reserves; (4) coordinating a safety-duplicate program to secure accessions; (5) developing species-specific propagation protocols; (6) supporting *in situ* conservation activities (translocations, reinforcements, etc.) to restore native orchid populations.

**Keywords:** conservation planning; endemics; seed-banking; terrestrial orchids; threatened species

## P12. LC-MS/MS and molecular networking: A promising approach for chemical composition evaluation of orchids extract - Case study with *Gastrodia elata*

**Quentin Favre-Godal[1,2], Lorène Gourguillon[1], Ludivine Riffault-Valois[2], Aurélie Urbain[2], Sonia Lordel[2] & Patrick Choisy[1]**

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Due to the high ornamental and medicinal value of Orchidaceae, it is desirable to quickly evaluate their chemical composition for quality or research purposes. We propose for orchid extracts the use of an integrated strategy combining High Resolution Mass Spectrometry (HRMS) and a computational approach with molecular networking increasingly used in the phytochemistry/metabolomic field, to rapidly identify secondary metabolites from untargeted MS/MS analysis. We applied this strategy to *Gastrodia elata* root aqueous extract, a saprophytic perennial herb in Orchidaceae. To analyze the extract, an analytical method using Ultra Performance Liquid Chromatography - Electrospray Ionization – Quadrupole Time of Flight device (UPLC-ESI-qTOF) was developed. MS/MS data were then submitted to Global Natural Products Social Molecular Networking (GNPS) platform online after pre-treatment. Molecular networks were finally visualized. This representation assisted the identification of various adducts and alike compounds by establishing clusters according to the fragmentation similarities. Thanks to this approach, ten molecules were putatively identified based on HRMS and MS/MS spectra. Among them, five gastrodin derivatives, parishin B, C and E, had their identities confirmed by comparison with commercial standards. Despite the numerous studies already achieved on orchid extracts characterization, there is a lack of experimental MS fragmentation patterns. The MS/MS data obtained here comes to fill partially these missing data and will be included in the GNPS public database to be shared with the Orchidaceae phytochemistry researcher community.

**Keywords**: *Gastrodia elata*; dereplication; mass spectrometry; molecular networking; GNPS

## P13. Review on orchids and their mycorrhizal fungi: how do they establish a mutualistic relation instead of a competition?

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Orchids are associated with diverse fungal taxa, including diverse non-mycorrhizal endophytic fungi and mycorrhiza. Orchid mycorrhiza fungi (OMF) are required at least for one life stage and bring several fitness advantages to the plant. Therefor a better understanding of the role of mycorrhizal fungi on seed germination and further growth is needed to develop orchid conservation models. To capture the complexity of OM interactions, significant genomic, transcriptomic, and proteomics studies have been performed, reveling partly the role of each partner. In this poster review, the early stage of the establishment of orchid and mycorrhizal fungus relationship will be described, with special emphasis on chemical communication and defense mechanisms. Based on the recent finding on OM and their similarities with arbuscular mycorrhiza (AM) (Miura et al. 2018), we suggest a putative model representing the different strategies that OM fungi might employ to establish this symbiosis. It is hypothesized, in regards to the work of Kohler et al. (2015), Chagas et al. (2018), Miura et al. (2018) and Yuan et al. (2018) that in response to plant molecule signals such as strigolactones and flavonoids, OM fungus will secrete chitin oligomers activating the Common Symbiosis Genes (CSGs) and elicitors to downregulate and evade the plant defense. As a result, more metabolomic studies with special attention to each partner contribution are finally encouraged to provide evidence to these assumptions and better understand OM to help orchid conservation.

**Keywords**: orchid mycorrhiza; fungi; symbiosis.

# P14. Volunteers help unravelling the secrets of a 200-year-old European Orchid collection at the Museum of New Zealand Te Papa Tongarewa.

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In a trip to Great Britain between 1875 & 1876, Sir James Hector (Director of the Colonial Museum, Te Papa's predecessor) purchased 28,000 herbarium specimens from the British Museum. The aim was to build a reference collection to assist New Zealand botanists with the identification of naturalised plants and the description of new species. It is believed this purchase combined the collections of Silvanus Thompson, his wife Bridget Thompson and British botanist John G. Baker, who was taught by Silvanus. Later on, Baker became the herbarium keeper at the Royal Botanic Gardens Kew (1890–1899). Records at Te Papa regarding this transaction, its content and the origin of the specimens are far from complete. Only its arrival in 1877 is acknowledged in the museum's records along with the statement "…*specimens have remained in its original zinc-lined packing because of poor environmental conditions and lack of botanical staff*". About 140 years later, thanks to the hard work of volunteers, we have learnt a bit more about the orchids in this collection. There are about 400 orchid specimens, representing 26 genera. Specimens were collected between 1773 and 1870 in places such as Austria, Algeria, England, France, Germany, Norway, Switzerland and Russia. Some of the main collectors are Jacques Clarion (1779–1844), Claude Jordan (1814–1897), Karl Zeyher (1799–1858), Gaston Genevier (1830–1880) and Benjamin Carrington (1827–1893), among many others. However, we are still far from fully understanding what we have. For instance, we need help decoding handwritten notes, confirming species identification and updating the taxonomy of challenging groups (e.g. *Ophrys*). Towards this end our volunteers have digitized and imaged the entire orchid collection. Images are available via museum's web site Collection Online (https://collections.tepapa.govt.nz/). Please have a look and let us know if you can help us doing some detective work.

## P15. Carbon utilization patterns and enzymatic activities of orchid mycorrhizal fungi

**Alžběta Novotná[1], Sophie Mennicken[1], Tamara Těšitelová[1], Helene Vogt-Schilb[1,2], Jiří Bárta[1], Bertrand Schatz[2] & Jana Jersáková[1]**

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Co-occurring orchid species tend to associate with distinct mycorrhizal communities, which supposedly help orchids to effectively partition limited resources. Orchid mycorrhizal fungi (OrM) from the polyphyletic rhizoctonia group are generally considered as soil saprophytes, but their ability to utilize various nutrient sources and the subsequent secretion of extracellular enzymes have been little studied. To understand if OrM fungi indeed provide resource partitioning to orchids, we used the semi-quantitative tests to investigate the carbon utilization and the enzymatic activity of OrM fungi associated with18 orchid species from four sites in the Czech Republic and southern France. A pilot study of eight fungal isolates (genera *Ceratobasidium, Tulasnella, Sebacina*) obtained from four orchid species from a Czech site showed substantial differences in the secretion of several hydrolytic enzymes. Our poster will present an investigation of 32 OrM taxa for their enzymatic activity and carbon utilization patterns.

**Keywords**: carbon utilization; enzymatic activity; fungal functional trait; orchid mycorrhiza.

## P16. Endemic epiphytic orchids of the Brazilian Atlantic Rainforest: distribution and conservation status

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The Atlantic Rainforest (ARF) phytogeographic domain currently occupies 15% of the Brazilian territory and is home to 35% (ca. 15,600 spp.) of all angiosperms in the country. Even after the devastation of about 90% of its original cover and about 120 million people living there, this biome remains an important biodiversity center of endemic species. Around 1,550 species of Orchidaceae occur in the ARF, of which 652 spp. are endemic epiphytes from this domain. The objective of this work was to verify the distribution of these endemic species across this phytogeographic domain and verify the current conservation status of each one. Through the collection of geographic occurrences, bibliographical data and consultation of herbaria, we summarize 15,543 collections records for epiphytic orchids endemic to the ARF. The results of the occurrences plotted in 1°degree quadrants indicate that the highest species richness ranges are in São Paulo, Rio de Janeiro and Espírito Santo states, with 162-201 endemic species per quadrant. 71% of the species have 20 or fewer records in the collections, and 20% species are known from only one locality. Regarding conservation status, we verified that 78.8% (514 spp.) of species have not had their status assessed. Among the species already analyzed (138 spp.), 11% (73 spp.) are categorized as DD, LC or NT, 23 (3.5%) VU, 23 (3.5%) EN, and 19 (2.9%) CR. Faced with this alarming condition of this hotspot domain, we believe that it is urgent to join a multidisciplinary approach to protect these species. At the first moment, our results support taxonomic studies to verify species circumscription and their distribution, and sampling design for phylogenetic and phylogeographic studies. Combining the detailed knowledge about occurrence with physiological and ecological studies concerning local distribution of individuals we will be able to predict where and which is the best practice to reintroduce new individuals in degraded areas.

**Keywords**: biodiversity, conservation status, endemism, Neotropics, species richness

# P17. Evolutionary and ecological studies in *Dryadella* Luer (Pleurothallidinae, Orchidaceae) as a model for conservation of rare and endemic micro-orchids from Atlantic Forest

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Because of reduction of native areas in Brazilian Atlantic Forest, populations of several plant species are fragmented with definitive loss of genetic variability. The combined study on evolution, biology and demography are ideal for assessing the current situation of populations, to understand the more complex scenery of rare species, and detect cases where their maintenance may be compromised. *Dryadella* comprises about 50 epiphytic species, some of which are endemic and highly endangered in Atlantic Forest. *Dryadella zebrina*, here informally recognized as a species complex, includes *D. zebrina*, *D. edwallii* and *D. liliputiana*. The complex presents highly variable morphotypes in terms of floral size and colouring pattern in petals and sepals, which overlap in geographic distribution. This project aims to carry out evolutionary and ecological studies at two temporal scales and in complementary approaches to understand: (1) historical processes of diversification of these lineages (2) contemporary ecological processes influencing the potential for maintaining of relict populations in their natural habitats. 137 individuals from 33 populations from Northeast to South Brazil were sampled. 66 sequences from *rpL*32-*trnL* and *psbD-trnT* cpDNA regions were obtained for the first evaluation of molecular variability and lineages structure. Genotyping tests for 57 individuals with ten microsatellite primers were performed showing high polymorphism and the possibility of dealing with a polyploid complex. 72 individuals were marked in two populations and have been monitoring in relation to size, the number of leaves, flowers, and fruits for a long-term demographical experiment. Although the study is still in the initial phase, we intend that at the end of its execution we can: (1) define the current conservation status of remaining populations; (2) make projections of demographic dynamics for estimation of probability of local extinctions; and (3) establish criteria and recommendations for management and possible reintroduction of individuals in nature.

**Keywords**: conservation genetics, endangered species, evolution, population ecology, species complex

## P18. The biogeographic patterns of the *Difforme* group of *Epidendrum* (Laelinae-Orchidaceae) revealed an Atlantic Forest - Amazon connectivity during the Pleistocene.

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The biogeographic relationship between the Amazon (AM) and Atlantic forest (AT) floras has been extensively discussed in the last century. The recent incorporation of molecular clock methods has enabled estimating divergence times and biogeographical events across lineages. Ancestral connectivity between these two ecosystems, which likely occurred during the last glacial cycle, explains the existence of sister species in both areas. These Neotropical regions shelter the species-rich genus *Epidendrum* (Laelinae, Orchidaceae), with over 1,500 species for which the phylogenetic relationships are not well known. The most comprehensive taxonomic circumscription is that of Eric Hágsater, who recognized around 100 species in the widespread *Difforme* group of *Epidendrum*. However, species boundaries remain unclear for many of them, such as between the eight species which grow in Brazil. Two of them occur exclusively in AM and the remaining six grow in AT. We aim to reconstruct the biogeographic patterns of the Brazilian *Difforme* species using phylogenetic reconstructions based on two nuclear and six plastid regions. Our results suggest a recent diversification which likely occurred after the Late Pliocene and highlight the role of the AM-AT connectivity throughout the Quaternary glaciations in the evolution of this Neotropical group.

**Keywords**: Brazil, dating, molecular clock, Neotropics.

## P19. *In vitro* fungal specificity of orchids from two species-rich Czech sites

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Co-occurring orchids tend to associate with distinct mycorrhizal communities in order to decrease competition for limited nutrients. Such local specificity might be driven either by local ecological conditions or by physiological preferences of orchids for a specific mycorrhizal fungus. Here we germinated symbiotically *in vitro* conditions seeds of five and nine orchid species with eight and 12 mycorrhizal fungi obtained from two Czech sites, respectively. The used rhizoctonia fungi (assigned to operational taxonomic units) comprised ten OTUs from Tulasnellaceae, four from Cerobasidiaceae and two from Serendipitaceae. Seed germination rate and protocorm development after three months of incubation will be presented.

**Keywords**: germination, mycorrhizal fungi, orchid, seed, specificity

## P20. Youth Orchid Conservation Brigade of Southeast Mexico

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The Youth Orchid Conservation Brigade of Southeast Mexico, formed in 2017, consists of a group of talented and dedicated young people who have received training in orchid ecology, conservation and sustainable management. These young people work with the media and in their local rural communities, involving the whole population in raising awareness, training, and carrying out activities in favour of the conservation of orchids and their environments. The group now meets once or twice a year, in different parts of Southeast Mexico, to offer training, motivation and practical experience. Deforestation, illegal extraction, fungicide application and the destruction of ecosystems and biodiversity-friendly agroecosystems, fuelled by over-population, poverty and misguided or corrupt politics are the principal causes of orchid extinction in Southeast Mexico, which includes the two most biodiverse states in the country (Chiapas and Oaxaca). The generation and sharing of knowledge concerning the presence, distribution, conservation and legal status, ecological requirements and sustainable management of plant species, along with the training and motivation of young people, community leaders and inhabitants of protected areas, will increase our chances of preserving not only the plants, but also their ecosystems for the next generations. The Youth Orchid Conservation Brigade forms part of an Integrated Strategy for the Restoration and Conservation of Epiphytic Orchidaeae in the Southeast of Mexico, ongoing for over 20 years. Work includes a diversity of research projects and the setting up of restoration sites and legal sustainable cultivation units in rural communities. Of great importance is our attempt to motivate a return to the cultivation of coffee and cocoa beneath a diversity of native shade trees, wherein orchids and other epiphytes, and a high proportion of other elements of native biodiversity, can coexist with human economic activity. We propose to market the products as “CaféOrquídea” and “CacaoOrquídea”.

**Keywords**: rural communities, sustainable management, epiphytic orchids, youth participation, restoration sites.

# P21. Drivers of Population Dynamics in the Federally-Listed Threatened Orchid, *Isotria medeoloides*

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*Isotria medeoloides* (small whorled pogonia) is a Federally threatened species that is endemic to eastern North America. Efforts to develop protocols to propagate the species have not been successful because it is non-clonal, cannot be propagated asexually, and seeds have not been successfully germinated. Conservation efforts, beyond long-term monitoring of known populations, have mostly focused on increasing light availability in areas where plants occur. Canopy thinning experiments in two sites have demonstrated that increased light availability resulted in higher growth rates, increased flowering, and seed production. Light availability is not, however, the only factor that influences orchid population dynamics because protocorms of all orchids and dormant plants of many orchids are non-photosynthetic and obtain all of the required resources from mycorrhizal fungi. However, little is known about how the abundance of mycorrhizal fungi affects orchid populations. Mapping and monitoring *I. medeoloides* plants in several sites have shown that dormancy is common, and plants reappear after remaining underground for more than a decade. We hypothesized that the distribution, abundance, growth and emergence from dormancy of individuals in *I. medeoloides* populations would be driven by the distribution and abundance of mycorrhizal fungi, in combination with light availability. We combined the use of specific PCR primers for quantitative real-time PCR and spatially nested soil sampling with measurements of light availability in three distinct sites. We found that the distribution of *I. medeoloides* in mapped populations and the emergence of plants from dormancy were related to the distribution and abundance of mycorrhizal fungi in the soil. Plant growth during the growing season and the likelihood of flowering the subsequent year were more affected by light availability. Results of our ongoing research on this threatened species indicate that factors that influence the abundance of mycorrhizal fungi are as important as light availability for population dynamics.

**Keywords**: mycorrhizal fungus abundance, Orchidaceae, *Isotria*, light

## P22. Understanding of complex diversity in Pleurothallidinae as the way to effective conservation

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Orchidaceae subtribe Pleurothallidinae are a young group distributed in tropical America only. However, this group intensively diversified into the largest subtribe in orchids now comprising more than 18 % of orchid species. The overall diversity and processes driving this enormous diversification are still poorly understood. Whole-genome changes and chromosome rearrangements are supposed to play important role in plant diversification, but only little is known about genomes of Pleurothallidinae. Therefore, our study is aimed to trace the evolution of Pleurothallidinae genomes. We employed Hyb-Seq sequencing, flow cytometry and karyology to get insight into phylogenetic relationships within Pleurothallidinae, nuclear genome sizes and endoreplication patterns, and chromosome numbers and rearrangements, respectively. Our NGS-based phylogeny mostly supports the current taxonomic division, but also indicates reticulate evolution in some lineages. Nuclear genome size (1C-value) ranges from 0.22 to 5.41 pg and chromosome numbers span from 12 to > 60; however, the link between these traits is not straightforward because of diverse accumulation of various TEs. Approximately one half of the investigated species exhibit progressively partial endoreplication, a unique phenomenon ascribed to orchids, and the minimum proportion of replicated genome is about 19%. Our results indicate that the hyperdiverse morphological variation in Pleurothallidinae might stem from their extremely diverse genomes. This also represents an extra level of plant diversity which deserves special protection.

**Keywords**: diversity, chromosome, evolution, genome, next-generation sequencing, Pleurothallidinae

## P23. Genomics of orchid mycorrhizal fungi

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Strange things can happen when organisms form tight interactions, defined as symbioses. Fungi and algae pair to form lichens, and protists and bacteria can team up with termites so the insect can break down wood. One of the more inexplicable symbioses involves orchids and fungi. Other plants give beneficial root fungi sugar they made from photosynthesis and receive limiting nutrients like nitrogen. Orchids, however, require sugar from fungi so their seeds can grow and develop. What do the fungi get in return? Why do these fungi participate in this symbiosis? I am using a genomics approach to study the fungi involved in this symbiosis. To do this I have written a funded Community Sequencing Project through the Joint Genome Institute to "resequence" the 35 fungal isolates in my collection and generate 15 reference genomes using PacBio sequencing technology. Within this set of 15 genomes, there are fungi that facilitate orchid germination and isolates that have been isolated from protocorms but do not support germination *in vitro*. Sequencing data are still forthcoming, but analyses using preliminary data provide insight into the evolutionary history of these fungi, genomic features and the evolution of gene families. These various approaches will inform each other and generate hypotheses about the functionality of this unique symbiosis. Knowledge of fungal relatedness and symbiotic capabilities could translate to concrete management efforts to save endangered orchids.

**Keywords:** evolution, genomics, mycorrhizae, Orchidaceae, *Rhizoctonia*, symbiosis

## P24. Guatemala: orchids in danger

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Guatemala is a relatively small country in term of size, but its geographical and biological diversity is enormous. It is estimated that about 10,804 species of vascular plants can be found there, many of them are endemic. Biodiversity richness of Orchidaceae in Guatemala is an attribute that a few countries have, but for many years it has been poorly studied. It ranges from a little more than 100 species mentioned by Bateman (1843), 527 species reported by Ames & Correll (1952-1953), 800 species proposed by Archila (1992) and 1237 proposed also by him in 2014. In the most comprehensive work published by Archila and co-authors in 2018, the presence of 223 genera and over 1200 species has been reported in this megadiverse country. Unfortunately, Guatemala faces significant trans-national and national threats to biodiversity. These include direct and indirect impacts from climate change and illegal trade, as well as the indirect impacts of the illegal trade in drugs. Factors that contribute to orchid loss in Guatemala are, for instance, changes in the land use (deforestation for agriculture, timber production, conversion of mixed forests to conifer plantations), reduction of the cloud cover resulting from global warming, disappearance of pollinators, collectors and street vendors, as well as herbicide use that affects both pollinators and populations of the terrestrial orchids. The situation is further complicated by the significant poverty level of many Guatemalan residents.

**Keywords**: Central America, illegal trade, species loss, orchid flora

## P25. Exploring endohyphal bacteria communities within orchid-fungus network

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Plant-associated fungi often harbour endohyphal bacteria (EHB), i.e. symbiotic bacteria living inside fungal cells, but their ecological role in the plant-fungus network is largely unexplored. EHB could be involved in the production of growth factors stimulating fungus growth and may play a role in phosphate acquisition and transport. Orchids are strongly dependent on mycorrhizal fungi during germination and subsequent growth, but the presence of EHB in orchid mycorrhizal fungi and their symbiotic role has so far been ignored. Here, we fill this gap by high-throughput sequencing to identify the EHB present in 65 rhizoctonia fungal strains from 10 orchid species growing in the Czech Republic. Our preliminary results on the EHB community composition revealed at the phylum level, that Proteobacteria were dominant in all orchid taxa (on average 72% of the community composition), followed by Actinobacteria (10%), Acidobacteria (8%) and Firmicutes (5%). These phyla are well known from plant rhizosphere and associations with arbuscular mycorrhizal fungi. Our poster will present detailed information on differences in EHB community between orchid species and between fungal strains from the families Ceratobasidiaceae, Tulasnellaceae and Serendipitaceae. Through this study, the identification of EHB and their association specificity with mycorrhizal fungi, could enable to underly the role of EHB in orchid resource acquisition and orchid coexistence.

**Keywords:** endohyphal bacteria, orchid coexistence, high-throughput sequencing, rhizoctonia fungi, tripartite network

# P26. Mycorrhizal interactions in Orchidaceae: a database to study ecology and evolution

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All orchids are initially mycoheterotrophic during germination forming exceptional mycorrhizas with c. 25000 species of free-living basidiomycete and ascomycete fungi, known as orchid mycorrhiza (ORM). The evolution of ORM from ancestral arbuscular mycorrhizal ancestors has most likely occurred in the most recent common ancestor of Orchidaceae, c. 90 million years ago. This transition is linked to niche expansions and radiations, which in synchrony with the development of specialized pollination syndromes, has likely promoted speciation in the largest family of plants on earth. Traditionally orchids were considered to interact largely, if not only, with members of the 'Rhizoctonia' complex. Molecular identification methods have demonstrated that this assemblage consists of fungal taxa of three distinct lineages: Ceratobasidiaceae, Tulasnellaceae and Sebacinales. These techniques also revealed many other fungal lineages that are potentially capable of forming ORM. In addition, severally partially and fully mycoheterotrophic species of Orchidaceae have been found to associate with ectomycorrhizal and wood and litter-decaying fungi, suggesting that mycorrhizal interactions in Orchidaceae are evolutionary dynamic and potentially linked to niche shifts. However, we currently lack a comprehensive overview of the diversity of fungal taxa that engage in mycorrhizal interactions with orchids. Here, we present a database of mycorrhizal interactions in Orchidaceae, based on an exhaustive inventory of studies that have used sequencing the ITS region to identify potential ORM fungi. We will use this database to infer the evolutionary dynamics of the ORM interaction in the framework of plant and fungal evolution. Specifically, we are interested in how mycorrhizal shifts are potentially linked to plant trophic mode shifts – from initial mycoheterotrophy to full mycoheterotrophy – and whether these shifts parallel in time, space, and climatic niches.

**Keywords:** orchid mycorrhiza, mycoheterotrophy, interactions, mutualism, ITS

## P27. Adaptation and Conservation in *Myrmecophila*

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Identifying the factors that impact the host tree attachment in epiphytic orchids are the keys to successfully establish a viable and long-term *ex situ* collections in botanic garden settings. Fairchild Tropical Botanic Garden is the home of many endangered orchids and the availability of tropical host trees provides great potential for building *ex situ* collections for orchid conservation. However, long periods of drought and strong hurricanes make it very challenging for the plant species to adapt. Seedlings cultivated symbiotically are especially prone to desiccation and mortality after out planting. This dynamic environment may favor species and/ or genotypes that can quickly acclimate and rapidly attach. Many orchids have low natural reproduction rates. Out of their native ranges, orchids may lack pollinators and appropriate mycorrhizal fungi necessary for germination and seedling growth. Understanding the best attachments methods for outplant survival growth are critical to creating the best practices for orchid introductions and *ex situ* conservation efforts which is most important part of the plant conversation at botanic gardens. In this study, the best attachment protocols to different species of host tree, different orientations, and different heights using *Myrmecophila* hybrid orchids were studied. Finally, quantify establishment success using root counts, leaf counts, attachment rates, and acquisition of mycorrhizal fungi were recorder throughout the study.

**Keywords**: orchids, *ex situ* conservation, global warming, South Florida, botanic garden

# P28. Application of organic amendments in tissue culture of South Floridian endangered orchids

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Due to their tiny, dust-like seeds, which do not contain food reserves, orchids require assistance from species-specific mycorrhizal fungi that form a symbiotic relationship with the orchids, in which they provide nutrients for germination and embryo development. Because of this specialized relationship with fungi, germination rates in nature are exceedingly low. Using *in-vitro* micropropagation methods in which artificial growth mediums provide the necessary nutrients, thus, eliminating the need for the fungi symbiont, germination rates can reach nearly 100%. However, the nutrient requirements for each orchid varies among species. *Epidendrum nocturnum* is an imperiled, native to South Florida, epiphytic orchid species, and the focus of this study. The seeds of *E. nocturnum* were tested with five different treatments, each with ten replications, and seed technology tests including the coefficient of velocity of germination, germination index, germination rate index, the mean of germination time, the final germination percentage, germination speed, and mean daily germination and plantlet vigor assessment were compared across each treatment. Murashige and Skoog media (MS) supplemented with charcoal and agar was used as a basal media (BM) for all treatments (C: Control – only BM, $T_1$: BM + 10 g/L banana powder, $T_2$: BM + 10 mL/L coconut water, $T_3$: BM + 5 g/L banana powder + 5 mL/L coconut water, $T_4$: BM + 10 g/L potato dextrose). Results showed a significant increase in the final germination percentage by 17% and 15% when using potato dextrose ($T_4$: 10 g/L) and banana powder ($T_1$: 10 g/L), respectively. In addition, analysis of plantlet vigor assessments showed 40% greater length and 31% greater dry weight in plantlets when using media amended with 10 g/L banana powder ($T_1$). The aim of this study was to use biotechnological conservation methods to develop a protocol for fast *in-vitro* multiplication of *E. nocturnum* using organic amendments in the growth media.

**Keywords**: orchids, conservation horticulture, *Epidendrum nocturnum*, *in-vitro* micropropagation

# P29. Seed viability assessment of *Cymbidium* species and their conservation using cryopreservation

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Orchids are recognized worldwide for their ornamental appeal, medicinal value and ethnobotanical significance, which has lead to their over harvest and illegal collection with detrimental effects to entire populations. Thus, there is an urgent need to develop efficient protocols for conservation of orchids. Seed cryopreservation provides a feasible option for conservation through long term seed storage and is especially useful for orchids as it can enhance the longevity of short-lived seeds and maintain their viability during storage. However, requirement for success can be species-specific. In my thesis research using six *Cymbidium* species (*Cymbidium tracyanum*, *Cymbidium cyperifolium*, *Cymbidium floribundum*, *Cymbidium kanran*, *Cymbidium goeringii* and *Cymbidium lancifolium*), I propose to: (1) determine the biophysical conditions that optimize cold stress tolerance and storage viability of seeds, (2) develop efficient seed storage protocols and (3) investigate the effect of seed characteristics on cold stress tolerance in orchid seeds. This study will be conducted using seed material collected from the Yachang Orchid National Nature Reserve, in Leye County, Northwest Guangxi, Southwestern China and all experiments will be conducted at College of Forestry, Guangxi University. All species' seeds will be assessed to determine the following seed biophysical characteristics; seed size, morphometrics, seed moisture content, seed oil content and seed viability. Seeds will be cryopreserved at -196 $^{o}$C using three methods: (i) direct method, (ii) vitrification method and (iii) encapsulation-dehydration method with varying thawing rates. Preserved seeds will be compared with seeds stored at higher temperatures: 23, 4, -18, or -80 $^{o}$C. Both viability tests and germination tests will be used to assess which experimental conditions optimize cryopreservation success. By investigating factors determining successful preservation of orchid seeds, this research will provide a strong scientific basis and technical knowledge on seed storage that needs to be urgently prioritized to conserve and continue to reap benefits from this fascinating flora.

**Keywords**: orchids, conservation, physiology, seed viability, cryopreservation

## P30. Convergent evolution of orchid and pyroloid protocorm.

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Seeds of mycoheterotrophic and mixotrophic plants tend to be very reduced in structure and also possess limited reserves. This is the case of orchids and pyroloids. Despite their great phylogenetic distance (monocots and dicots, respectively), their ecology and anatomy including seed morphology is very similar. Using our novel protocol for axenic *in vitro* cultivation of pyroloids, we were able to study ontogenetic development from seeds to plants. Seeds of both the above-mentioned groups contain globular living undifferentiated embryo surrounded by a dead testa. Gemination starts with enlarging of the embryo, still with no signs of differentiated organs. Enlarging is isodiametric in orchids resulting in spherical structure, while it is polar in pyroloids resulting in elongated structure. Later on, the shoot apical meristem develops in orchids while the root apical meristem develops in pyroloids. In the case of orchids, the spherical structure is called a protocorm because it precedes formation of the cormus. In the case of the pyroloids, the elongated structure is much smaller; nevertheless it precedes formation of cormus as in orchids. We therefore propose to call both these structures protocorms. The ontogenetic development in both plant groups share similarities – they lack a differentiated embryo and undergo post-germination protocorm development which forms only one meristem pole. This indicates convergent evolution between orchids and pyroloids. The loss of one meristematic pole could be caused by inability of tiny seeds with limited reserves to form more organs, and/or formation of both organs is useless when a mycorrhiza feeds the plant. A third option is that the formation of mycorrhizal tissue in one protocorm half prevents formation of meristem. We hypothesise that germination into a protocorm could be a general feature of mycoheterotrophic plants with dust seeds, but further study is necessary.

**Keywords**: *in vitro*, germination, protocorm, pyroloids, orchids, mycoheterotrophy

## P31. Aspects of the population structure of *Quisqueya ekmanii*, a rare orchid of the Dominican Republic

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Jardín Botánico Nacional Dr. Rafael Ma. Moscoso

The four species from *Quisqueya* Dod genus are endemic to the Dominican Republic, and they are listed as critically endangered in the Red List of the Vascular Flora of the Dominican Republic. *Quisqueya ekmanii* has the best-known spatial distribution to date; it has only been observed growing on top of rocks in *Pinus occidentalis* forest, in the Sierra de Bahoruco National Park, at an altitude from 1,900 to 2,000 m a.s.l. The purpose of this research is to accomplish the first study the ecology and population structure of the species. Two field expeditions were made, to record the life stages of each plant (immature or adults), population density and if they grew alone or in groups. The sampled area consisted of two parcels of 100 m² each. A total of 394 plants were recorded, with a density of 1.97 plants/m², all of them growing in groups. This study is a small close-up of the population ecology of *Q. ekmanii*; it is essential to accomplish this type of studies to understand the behavior of the plant community in the long term. Further information will be obtained, and it will be used to create *in situ* and *ex situ* conservation plans for this species.

**Keywords**: *Quisqueya ekmanii*, Orchidaceae, ecology, conservation

# P32. Growing indigenous community knowledge & know-how for orchid conservation and use in ecotourism in the Northwest Amazon, Colombia

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Guainía, known as La Tierra de Muchas Aguas (the Land of Many Waters) is located in the transition zone between the Amazonian forests and the plains of the Orinoco biome on the western edge of the pre-Cambrian Guyana Shield geological formation. The region presents a heterogeneous mosaic of ecosystems, including seasonally flooded forests, tierra-firme forest, catingas, white sand savannahs and tepuy granite outcrops. The majority of the population pertains to indigenous communities, principally the Puinave, Kurripaco, Sikuani and Piapoco, who live along the river tributaries of the ´Fluvial Star´ of Inírida, declared a Ramsar wetland conservation area in 2014. Ecotourism interest in the region is increasing, with the biodiversity offering important opportunities for enhancing community livelihoods through wildlife observation, although the region is little studied. Here, in a participatory manner with members of two indigenous communities in Guainia, Caranacoa on the Inírida River, and Chaquita on the Atabapo River, and the student study group ´Bioetno´ in the SENA regional further education college, we documented the orchid diversity in the two communities. Orchid observation paths were designed by the communities, and fallen epiphytic orchids were collected to establish an orchid garden in each community. A photographic record was compiled and a visual guide was developed for species identification, published in both Spanish and the native languages of Puinave and Kurripaco. In total, 72 species were recorded, of which 60% are new reports for the department of Guainía. The genus *Epidendrum* was the most diverse, with eight species, followed by *Catasetum* with five species. Species with high value for ecotourism activities were documented, including *Aganisia cyanae*, *Cattleya violacea, Caularthron bicornutum*, *Cycnoches chlorochilon* and *Scuticaria steelei.* This is an ongoing initiative for community capacity-building in the inventorying, identification, and eco-touristic observation of orchid diversity in community territories.

**Keywords:** ecotourism, Northwest Amazon, orchid diversity, participatory research

## P33. Assessing the evolutionary significant units for the conservation of *Cypripedium macranthos* by the population genetic analysis

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*Cypripedium macranthos* is widespread in Taiwan, China, Korea, Japan and Russia. Because of the horticultural value, some small populations of *C. macranthos* are becoming endangered due to illegal harvesting or habitat loss. Study of genetic diversity among populations across different countries is required for determining the evolutionary significant units (ESUs) and management units (MUs) for future conservation plans. Because of the limitations caused by a low number of microsatellite markers in this large-genome species (1C = 37.4 pg), six cpDNA markers, including *accD* and the intergenic spacers, i.e. *atpF-atpH*, *atpI-atpH*, *psbA-trnH*, *rpL32F-trnL(UAG)* and *trnL-trnF*, were used to identify plastid DNA haplotypes. By associating the phylogenetic clades of these haplotypes with geographic distribution, we found that two island populations, Taiwan and Hokkaido, form two separate clusters from other populations. In contrast to these island populations, populations on the Asian continent do not show distinct geographic structures. This study indicates that the edge populations, Taiwan and Hokkaido, are two unique ESUs, while continental populations should be also considered as different MUs for conservation.

**Keywords:** *Cypripedium macranthos*, evolutionary significant units, genome size, haplotypes, population genetics

## P34. Diversity, distribution and abundance of orchids of the Nyika National Park, Malawi

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Nyika National Park is the largest protected area in Malawi and contains numerous edible and non-edible orchid species. This study determined orchid species diversity, distribution and abundance in areas within the park frequently targeted by collectors for the edible tubers. For diversity of orchids, a series of 20x50m sample plots were established. Diversity of orchids for each site was calculated using Simpson's diversity index, while abundance was calculated as the total number of individual orchids found per hectare. We compared diversity and abundance of orchid species using the Kruskal-Wallis H test. Assessment of the distribution pattern of the edible orchid species was done by recording site names and describing the nature of the habitat and vegetation from these sites. The information gathered assisted to map those areas targeted by orchid gatherers. In addition, soil samples were also collected from each plot to determine soil physiochemical properties, including soil carbon, pH, organic matter, phosphorous, magnesium, potassium, calcium, nitrogen and iron. Principal component analysis was used to show distribution of species. Canonical correspondence analysis was used to show relationships of orchid species data and the soil environmental variables in the collection sites. Out of 54 orchid species recorded, 43 were identified as "edible" gathered for chinaka/chikande trade by surrounding communities. The edible orchids belong to five genera; *Disa*, *Satyrium*, *Habenaria*, *Brachycorythis* and *Neobolusia*. The non-edible orchid genera recorded were *Roeperocharis*, *Brownleea*, *Cynorkis*, *Disperis* and *Eulophia*. Results revealed an increase of 23 more edible orchid species targeted by communities in the national park than previously reported. The ecological habitats for these terrestrial edible orchids are montane grasslands; montane wetlands, pine plantation, evergreen forests and miombo woodlands. The montane wetlands and grasslands had the highest species diversity than the rest. In terms of orchid abundance, the pine plantation (3600 stems/ha from low orchid diversity) and the montane wetlands (2360 stems/ha) were the highest, while miombo woodland (390/ha) had the least. Variation in abundance and species richness according to this study is ascribed to dissimilarities in ecological characteristics partly influenced by soil physiochemical properties in occurrence vegetation types. The pine plantation has shown to have negatively affected diversity of orchid species in Nyika national park. In view of this, the montane wetlands and grasslands will require special attention from the Department of National Parks and Wildlife to protect the rare and/or endangered orchid species. Owing to the high orchid species diversity and abundance in the plateau grasslands and wetlands as opposed to the miombo woodlands which are closer to surrounding villages, the plateau grasslands and wetlands are prone to further community exploitation pressure. Therefore, seed banking and germination studies of keystone species of the Nyika national park are urgent priorities to ensure community and other stakeholders' involvement in conservation and future restoration/domestication programmes.

**Keywords***:* edible orchids, diversity, abundance, soil properties

## P35. Relationships of *Liparis* species in Russia based on nuclear and plastid sequences phylogenetic analysis

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Six rare species of *Liparis* are known in Russia: *L. loeselii* (L.) Rich., *L. japonica* (Miq.) Maxim., *L. krameri* Franch. et Savat., *L. kumokiri* F. Maek., *L. makinoana* Schlechter and *L. sachalinensis* Nakai). *Liparis* is one of the most complicated genera of Orchidaceae, in spite the fact that numerous researches were dedicated to the systematics of this genus. Numerous morphological features – such as size of perianth parts, shape of lip, length of inflorescence and other – are usually used in *Liparis* species delimitation. However, intraspecific variability of these features makes *Liparis* species difficult for delimitation. Taking this fact into consideration, the number of *Liparis* species in flora of Russia turns out to be an open-ended question. We analysed sequences of nuclear (nrITS) and chloroplast (*trnL-F* spacer; *psbA-trnH* spacer; partial *ycf1b* sequence) DNA in order to discover additional molecular features applicable for delimitation of *Liparis* species. 37 accessions of six species of *Liparis* distributed in Russia were involved in analysis, including critical specimen from Kunashir (*Liparis* sp. nov.?). The final set contained 52 accessions. The results of nrITS analysis show that representatives of Far East populations form three well-supported clades. A critical specimen from Kunashir occupies its own isolated position and has specific nucleotide substitutions. Furthermore, the results of analysis indicate paraphyly of genus *Liparis*. Results of chloroplast *trnL-F* and *psbA-trnH* spacer analyses are coherent with results of nrITS analysis. The results of *ycf1b* analysis did not reveal specific substitutions or indels for the studied species. This fact eliminates ycf1b marker from the group of potential molecular-diagnostic features.

**Keywords:** *Liparis*, rare species, molecular-diagnostic

# P36. The Wild Orchid Association of Portugal (AOSP)'s contribution to the first Red List of the Vascular Plants of mainland Portugal

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The Wild Orchid Association of Portugal (AOSP) is dedicated to the study, conservation and popularization of native orchids in Portugal. Its research activities, especially in the last ten years, have contributed to improve the knowledge of the distribution and conservation status of the country's orchid flora. The recognition of these exhaustive field surveys led the Botanical Society of Portugal (SPBotânica) to invite AOSP to participate in the redaction of the first Red List of Vascular Plants of mainland Portugal. A total of 49 species and three subspecies of orchids was considered, following the species treatment of Flora Iberica. Fifteen species were evaluated using the IUCN criteria and categories. *Epipactis palustris* was assessed as Regionally Extinct, and *Anacamptis collina*, *Neotinea ustulata* and *Orchis provincialis* fell in the Critically Endangered category. Seven species are evaluated as Endangered, namely, *Anacamptis laxiflora*, *Cephalanthera rubra*, *Dactylorhiza insularis*, *Epipactis fageticola*, *Gymnadenia conopsea*, *Platanthera bifolia* and *Serapias perez-chiscanoi*. *Neottia nidus-avis* and *Spiranthes aestivalis* are assessed as Vulnerable. Finally, *Dactylorhiza elata* and *Dactylorhiza sulphurea* were evaluated as Near Threatened. The Red List of Vascular Plants of mainland Portugal will play an important role in raising awareness about the imperiled state of part of the country's native orchid flora and reinforce the urgent need to define and implement relevant conservation actions.

**Keywords**: Europe, ENGO, regional Red Listing, Mediterranean.

# P37. Application of biofertilizers in the *in vitro* propagation of *Cattleya labiata* Lindl.

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*Cattleya labiata* Lindl. is a Vulnerable orchid (VU), due to collection for commercial purposes and the reduction of its habitat. *In vitro* propagation methods like the thin cell layer technique (TCL) are efficient for the mass production of orchid seedlings. *In vitro* techniques use growth regulators in culture media, some of which are very expensive, so the use of biofertilizers may be an alternative to replace them, reducing the cost of seedling production. The objective of this study is to evaluate the effect of microalgae and growth regulators on *in vitro* propagation of *C. labiata*. Mature seeds were cultivated on MS, MS/2 (half strength) and WPM media, with and without the addition of activated charcoal (CA). For the TCL technique, transverse and longitudinal sections of the protocorms (3 months old) were inoculated in culture medium MS/2 containing different concentrations (0, 0,5, 1 and 4 gL-1) of extract and biomass of two microalgae (*Messastrum gracile* and *Chlorella vulgaris*) and of different concentrations (0, 0,5, 1 and 4 mgL-1) of plant growth regulators such as ZEA, BAP and TDZ. The best responses of asymbiotic germination occurred in the MS/2 medium supplemented with 2 gL-1 of CA (86,5% of germinated seeds and 27% of seedling formation, after 112 days of cultivation). PLBs occurred in the fourth week after inoculation; currently after 12 weeks many PLBs already have radicles. The TCL provided no significant difference between the section, concentrations of plant regulators and the concentration of microalgae extracts and biomass in the formation of PLBs, this supposedly occurs by the presence of zeatin in the composition of the microalgae that play a physiological role similar to the regulators tested for PLBs. It can be concluded that there are more sustainable alternatives such as the use of microalgae for the production of *C. labiata* seedlings.

**Keywords**: thin cell layer, microalgae, micropropagation.

# P38. More than twenty years of orchid *ex situ* conservation in Africa and Madagascar

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Orchid conservation represents a significant challenge in Tropical Africa and Madagascar, areas in which habitats are facing increasing pressure from agriculture, mining and logging activities. In these regions, a large part of the epiphyte flora is composed of orchids, most of which, when lacking flowers when encountered in the field, are impossible to identify. However, accurate identification is essential for developing conservation strategies since about 69% of tropical African and Malagasy orchids are potentially threatened. To overcome this identification problem, a shadehouse cultivation system was developed in São Tomé in 1997 and has now been extended across Continental Africa and Madagascar, providing a powerful tool for conducting thorough orchid inventories since most epiphytes can easily be brought into cultivation and grown to produce fertile, identifiable material. The network currently counts 12 shadehouses in six countries and, to date, 32,013 living orchids collected in the field have been grown, representing about 500 species, from which > 23,764 herbarium specimens have been collected, most associated with silica gel-preserved material and photos. This material has been used to describe 41 previously unknown species and to assess the conservation status of hundreds of species in three key areas (West Africa, Central Africa and Madagascar), while also leading to the publication of 44 articles in taxonomy, phylogeny, floristics and for the general public. Initially designed to facilitate orchid inventory and identification, the shadehouses now contribute to orchid conservation by protecting plants in safe, controlled conditions, producing material for seeds banking. They also offer valuable training opportunities for young botanists, thereby contributing to in-country capacity building. Key factors that explain the success of this shadehouse network and its long-term survival are the support provided by reliable local partners, the involvement of young botanists and students, and the development of new techniques to grow thousands of living orchids successfully and cost-effectively.

**Keywords**: Orchidaceae, epiphyte, Africa, Madagascar, *ex situ* conservation, shade house

## P39. Orchid conservation initiatives in Malaysia

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Orchids totalling close to 4000 species in Malaysia are one of the most diverse and most widespread family here had gained momentum in recognition among policy makers and guardians of the forest as one with the profile that fits and can benefit plant conservation on a broad scale in recent years. Listed not only as conservation indicator but also as priority germplasm for sustainable floriculture industry in the country, a milestone that could safeguard wild orchids from verge of extinction in the natural habitat. Through our 30 years of studying orchids in the wild, we understand more about the distribution, rarity, threats and extinction of orchids than ever before, and we have the scientific tools to address many of the problems; however many species face daily threats including habitat loss and unsustainable exploitation mainly via Internet trade. Prior to executing workable conservation plan, various research institutions working closely with Forestry Departments in Malaysia to first inventory and document the orchid species richness in the country. Selangor, Sarawak and Perlis Forestry Department in collaboration with Universiti Putra Malaysia Serdang have published seven orchid books that cover various habitat types. Selangor Forestry Department is leading on publishing biodiversity data in form of books for its various ecotourism's sites and State Parks, and has published two books on orchids. Sarawak state has published one on the limestone orchids, and Perlis, the first to embark on the feat, published one in 2010 and is currently preparing a new book that includes other flagship wild flowers. Realizing the importance of documenting its biodiversity wealth, Malaysia has developed an information system that would be a one-stop retrieval point or repository for biodiversity facts and as a part of the commitments to CBD to facilitate reporting and the transfer of biological diversity and conservation-related information both nationally and internationally.

**Keywords**: orchid books, database, species, documentations, policy, extinction

## P40. Conservation status of Madagascan orchids

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Orchids are the largest plant family in Madagascar with nearly 1000 species, of which over 90% are endemic. We have undertaken preliminary IUCN Red List assessments of all species that do not have published or draft assessments. The Global Strategy for Plant Conservation (GSPC) Target 2 calls for an assessment of conservation status of all known plant species by 2020. Madagascar is still far for reaching this goal, and only 186 orchid species are currently listed in the IUCN website. Kew Madagascar is aiming to complete assessments of all orchids for the IUCN Red List and to update data on distribution and threats for existing assesments, leading to a national strategy for orchid conservation. Our assessments are based on analysing herbarium collection using the GeoCAT tool developed by RBG Kew (www.geocat.kew.org), records in the literature and field surveys. Our work indicates that over 76% of species are threatened (Critically Endangered, Endangered or Vulnerable). The majority of species are threatened by habitat loss. The assessments will help researchers, decision makers and park managers to prioritise species for conservation and hopefully help to avoid extinctions.

**Keywords**: Orchidaceae, IUCN Red List, conservation status, Madagascar.

## P41. Unravelling the CITES regulations that enable legal trade of orchids

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