Cymbidium Chatter

Grammatocymbidium Pakkret Adventure '4 Spikes'

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Contents

Latest News

Welcome to the second issue for 2023. Hopefully by now readers in the southern hemisphere will have plants in bloom and many more in various stages of spiking. My own collection seems to be running a little behind schedule in comparison to the past few years, perhaps due to the change from La Niña to El Niño.

Recently a new website has been launched for Australian orchid enthusiasts to facilitate the sale of plants. Membership is free and there are no fees on plant sales (payment and shipping must be arranged between buyer and seller). The site developer is actively working to improve it and add features, so I encourage readers to check it out. There are already members who have listed plants for sale and there's also a "Wanted" section where members can post their requests. Have a look at:

https://orchidmarket.net.au/

The Future of Cymbidium Intergenerics: Grammatocymbidiums

Unlike the Cattleya or Oncidium/Odontoglossum Alliances, the number of hybrid genera involving Cymbidiums is quite small. In fact, as of the final release of OrchidWiz in 2022, very few offspring had been registered for each of the hybrid genera except Grammatocymbidium:

Genus	Components	Registered Hybrids
Ansecymphyllum	Ansellia X Cymbidium X Grammatophyllum	1
Ansidium	Ansellia X Cymbidium	7
Bifrendium	Bifrenaria X Cymbidium	1
Cymaclosetum	Catasetum X Clowesia X Cymbidium	1
Cymasetum	Catasetum X Cymbidium	1
Cymbidinaea	Cymbidium X Promenaea	1
Cymphiella	Cymbidium X Eulophiella	4, incl. 3 F2s
Cyrtogramcymbidium	Cymbidium X Cyrtopodium X Grammatophyllum	1
Eulobidium	Cymbidium X Eulophia	1
Gramcymbimangis	Cymbidium X Grammangis X Grammatophyllum	1
Gramcymbiphia	Cymbidium X Eulophia X Grammatophyllum	1
Grammatocymbidium	Cymbidium X Grammatophyllum	43, incl. 10 F2s
Kalakauara	Catasetum X Clowesia X Cymbidium X Grammatophyllum scriptum	1
Phaiocymbidium	Cymbidium X Phaius	3*

* Although 3 Phaiocymbidium grexes are registered, the earliest, Chardwarense (1902), is typically considered invalid as it shows no Cymbidium traits. Don Wimber rejected it in his 1956 thesis *Cytogenetic Studies in the Genus Cymbidium*.

Much of the development with Grammatocymbidiums has occurred in the past two decades, with Kobsukh Kaenratana of Pakkret Floriculture being the predominant driving force behind them. When discussing this with him recently, Kobsukh explained:

"The quality/traits of Grammatophyllum used in breeding are:

1) heat tolerance,
2) [a] high flower count,
3) long-lasting blooms even when [the] pollen cap [is] removed,
4) ability of [the] flowers to withstand the rain, and
5) exotic upright roots.

Grcym. Pakkret Garuda 'Red'. Photo courtesy of Kobsukh Kaenratana.

Size is no longer an issue because I have made many seedlings of compact Grammatophyllum that are really compact (tiny) and bloom year-round like crazy. Grammatophyllum and Grammatocymbidium will have a great range of plant size."

The combination of the cool to warm-growing Cymbidium traits with the heat tolerance of the Grammatophyllums has produced a range of hybrids that can flower in climates varying from temperate Melbourne (I have personally grown and flowered both Pakkret Adventure and Pakkret Gargoyle outside here without heat) to the tropical Thailand. Pakkret Adventure is reported to flower twice a year in southern California as well.

Kobsukh has successfully introduced a range of colours and patterns into Grammatocymbidiums and is continuing to develop his hybrid lines. Whilst the Cymbidium and Grammatophyllum genera both share a chromosome count of 40 and similar flower morphology, they are not perfectly compatible – Kobsukh has reported that none of the F1 Grammatocymbidiums he has produced appear to have viable pollen.

Grcym. Pakkret Carnival 'Apricot', an arching to pendulous grex. Courtesy of Kobsukh Kaenratana.

Left: Grcym. Pakkret Nirvana 'Unicorn'. Right: Grcym. Lovely Melody 'Pixar'.
Below: Grcym. Pakkret Crescendo, an F2 hybrid. Photos courtesy of Kobsukh Kaenratana.

However, they are fertile as pod parents and so Kobsukh is now working with F2 Grammatocymbidiums.

Grcym. Pakkret Nebula, another F2 hybrid. Photo courtesy of Kobsukh Kaenratana.

One such hybrid available in Australia is that of Pakkret Nebula, the cross of (Pakkret Adventure X *canaliculatum*). Kobsukh explained that he made this cross using a combination of both *canaliculatum* and Alison Shaw pollen (the latter he sourced from a Victorian grower) but has never seen any seedlings that appear to be (Pakkret Adventure X Alison Shaw).

Intriguingly, Michael Coker has flowered three seedlings of this cross so far and one of the three looks just like a Pakkret Adventure. His first thought was that Pakkret Adventure must have selfed, but given the lack of viable pollen Kobsukh was sure that that was impossible.

Pakkret Adventure lookalike from a batch of Pakkret Nebula seedlings. Photos courtesy of M. Coker.

Kobsukh added: “I do not have explanation for this, and I confirm that Pakkret Adventure never produce viable pollen… It is impossible to put pollen of Pakkret Adventure together with pollen from *canaliculatum*…. It looks very much like Grcym. Pakkret Adventure but definitely not the clone I propagate for sales. The plant in the photo has a less pronounced callus ridge than my normal Pakkret Adventure.”

My only idea is that of apomixis (where a plant produces seed asexually). This is known to occur in several *Lilium* species when presented with incompatible pollen. However, in those cases it is always agamospermy – the seeds are clones of the pod parent (this is also the default definition of apomixis in the context of plants). In this case it could not be agamospermy but would be the more generalised form of apomixis. Research on this topic is limited regarding orchids, but it is thought to happen with certain Zygo crosses. Given the lack of (Pakkret Adventure X Alison Shaw) offspring, one has to wonder if the two are incompatible. Kobsukh also speculated that perhaps the Alison Shaw pollen manages to fertilise some ovules, but that the recombination process fails due to incompatibility. The large quantity of viable Pakkret Nebula embryos then provides an environment conducive to the development of these “failed-to-recombine” embryos and a small number manage to form viable seeds with two sets of identical chromosomes. Any thoughts or comments are most welcome!

One of the normal Grcym. Pakkret Nebula seedlings flowered by Michael Coker. Photos courtesy of Michael Coker.

Finally, Kobsukh shared that one of his other goals with Grammatocymbidiums is to produce a cool-growing Grammatophyllum lookalike, after being asked about it in 2017. He expects that this will require breeding them to the third generation and involve a complex combination of the cool-growing Cym species with aerial roots, traditional hot-growing Gram. species, dwarf Gram., and *Gram. stapeliiflorum*, the only highlands species of Grammatophyllum. Unfortunately, his climate restricts him from progressing this project; it would require a partnership with a grower (or growers) in a cooler climate to make it possible.

Recently Flowered Seedlings from Pierre Pujol

Once again, Pierre has provided some photos of plants he has recently flowered and some commentary to go with them. First up is a highly spotted seedling…

Pierre: *A new addition to my spotted collection – a first-bloom seedling from a cross originated by Andy Easton that I acquired at Cal-Orchid during the Santa Barbara show – Cym. ((Violet Spots x Violet King) X Paul Robeson)). Intense burgundy spots and blotches match the strong lip on standard-size flowers. It hurt my eyes just looking at it for too long! The flower shape is a bit cuppy for the US awards system. But I foresee the next generation trying to flatten it while trying to add a light yellow, orange or green base colour while still keeping the strong contrast.*

Cym. ((Violet Spots x Violet King) X Paul Robeson). A highly spotted standard from NHO breeding.

The next plant prompted some discussion between myself and Pierre over the influence of each parent, especially due to the unequal ploidy.

Left: Cym. Memoria Amelia Earhart 'Geyserland' 3n. Right: Cym. Woody Wilson 'Ann' 4n.

Pierre: *What happens when crossing a standard-size flower with Mem. Amelia Earhart? Here are two examples of offspring from my cross (Mem. Amelia Earhart 'Geyserland' x Woody Wilson 'Ann'). This*

cross was brainstormed to produce a Cabaret-type flower (Woody Wilson 'Ann' X Vogelsang) made by the late L. Batchman. The goal was to combine the standard round shape but soft spike of WW with an MAE influence.

The yellow and apricot cultivars exhibited below produced 9.5 cm flowers strongly influenced by WW, while other siblings exhibited the right pigment overlay from the MAE phenotype. I used the MAE cultivar 'Geyserland', a putative 3n plant, which could explain the more decisive influence of the 4n Woody Wilson parent in the offspring. Note that the flowers in the second blooming season were significantly larger and of better form than in the first bloom, which makes it more difficult to select plants to keep during the first blooming season.

The size of the blooms is the result of the potential combinations of genes from each parent. Based on its ancestry, WW should provide two sets of genes that produce standards to its progeny. MAE, however, is the cross between a small standard (Hazel Tyers) and a miniature species (*devonianum*). The triploid form, at most, could only pass on one set of genes for a miniature. Hence the smallest flowers expected from this cross would be a combination of (standard + standard + mini) or (standard + standard + small std + mini), whilst MAE 3n would be (small std + small std + mini). This explains why the cross is consistently larger than MAE.

Pierre also asked what the number of chromosomes of the seedlings are in these types of crosses (3n x 4n). This was briefly touched on in my article on Ploidy in Issue 40 (December 2022), but I expanded upon it in my discussion with Pierre. In my experience, triploid pod parents only produce a small number of seedlings (and that's if the cross is successful, which they often aren't). The pod parent runs into a problem when it tries to produce the ovule, as 3 sets of chromosomes don't neatly split in half! My understanding is that you will always get aneuploids and 3ns in the offspring, with the even-ploidy seedlings being the same as the pollen parent, i.e.:

- 3n x 2n = 2n, 3n and aneuploid offspring
- 3n x 4n = 3n, 4n and aneuploid offspring

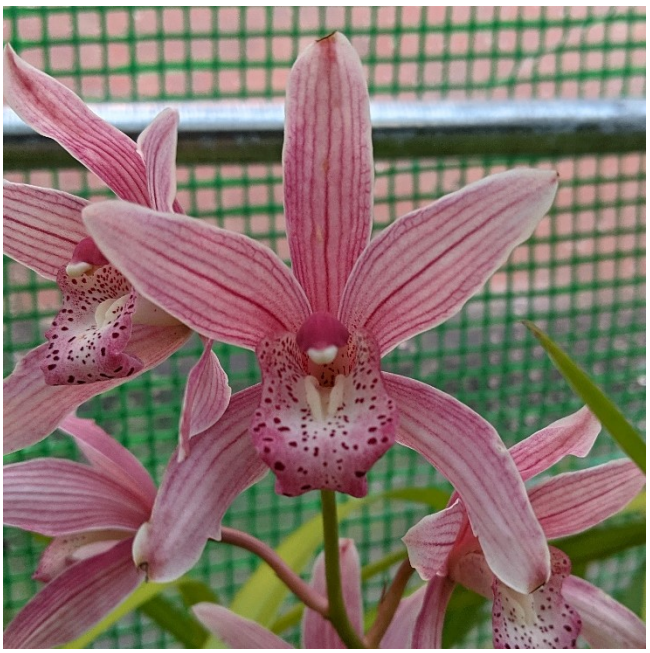
I have about 8 or 9 seedlings surviving from a 3n x 2n cross and once they're large enough I indeed to do some stomatal guard cell measurements to estimate the ploidy. My hunch is that the couple of poor growers (and the other 3 that have died) were aneuploids, as there were only 12 seedlings to start with from flask.

Finally, the last seedling Pierre shared was another that prompted some discussion and thought:

Pierre: *While it took its time before the first bloom, it was worth the wait! Cym. Sinseiden (*sinense *x* seidenfadenii*) 'Raspberry', a seedling from a cross made by Kevin Hill and a lucky pick from a plant table at a local orchid meeting, when it was just a tiny single bulb plantlet. First inflorescence with fifteen, eight cm wide flowers, on one erect spike. Clean stripes of raspberry colour, a distinct but subtle fragrance, and the compact habit from the* sinense *parent. Good job, Kevin!*

My first thought upon seeing these photos from Pierre was that it looked very much like Cym. Auntie Mary Kovich (*sinense* X *insigne*) and that this could be viewed as evidence that *seidenfadenii* is indeed just a variety or subspecies of *insigne*. However, Sinseiden also bears strong resemblance to another *sinense* hybrid – Sweet Spring. A quick look through OrchidWiz shows that *sinense* often dominates in its progeny, producing starry flowers with fine spotting on the labellum. Given that *seidenfadenii* is clearly related to *insigne*, it is not surprising that the two primary hybrids are similar. Interestingly, Pierre confirmed that his Sinseiden is very compact (bulbs the same size as *sinense*), whereas the Auntie Mary Kovich I have seen were larger. That at least suggests that the smaller stature of *seidenfadenii* has influenced the plant size. Pierre's plant is a useful piece of information, but I do not think it can be used to argue for either combining with or separating *seidenfadenii* from *insigne*.

Unfortunately, very few hybrids have been registered using *Cym. seidenfadenii* and there are even fewer where the same partner has been used with both *insigne* and *seidenfadenii*, so it is difficult to compare its breeding behaviour. Excluding Cym. Durrell (the cross between *insigne* and *seidenfadenii*), the one *seidenfadenii* hybrid I have seen (Point Conception) shows the *seidenfadenii* influence in both the square shape of the blooms and its labellum (particularly the stripes). For the time being, I think it is worth keeping the two species separate until the genome of *seidenfadenii* has been sequenced – that will then provide a clear answer as to whether the two are genetically different enough to warrant separate species status.



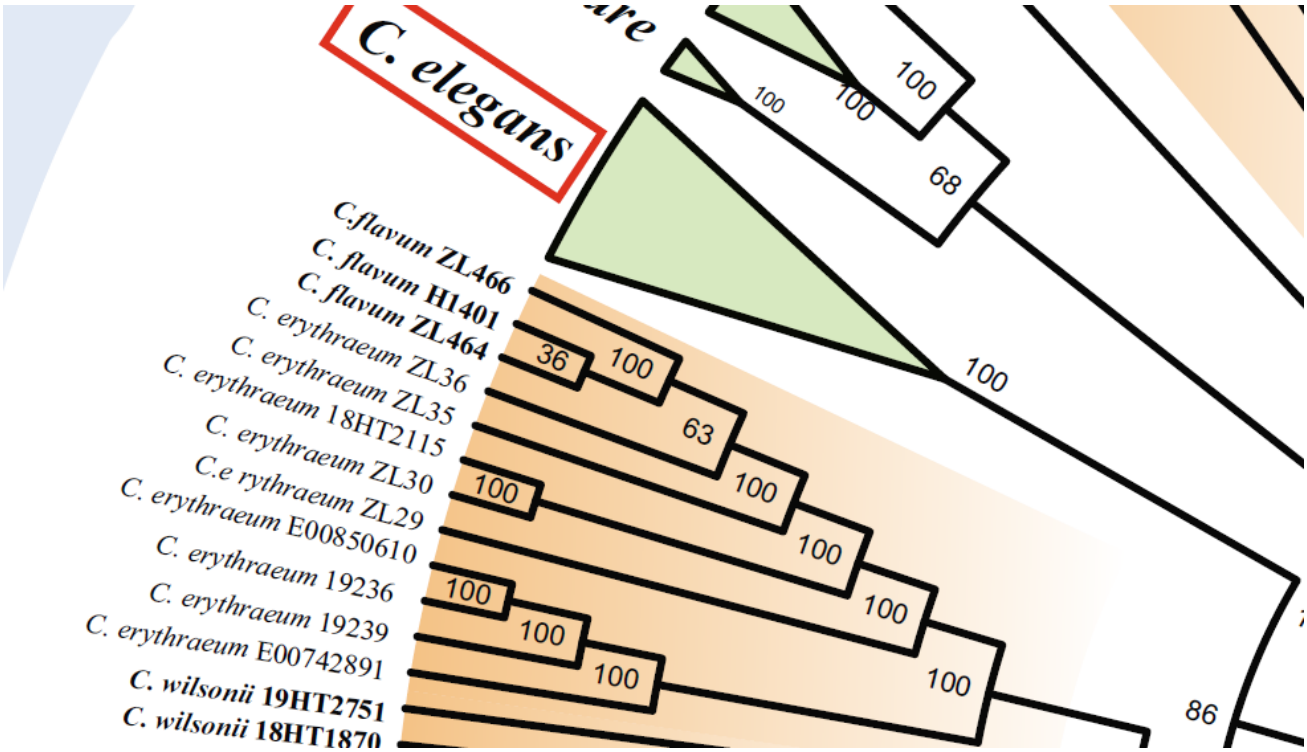
Cym. Sweet Spring in the editor's collection.

*Cym. Point Conception (*seidenfadenii *X Trigo Royale) at Santa Barbara Orchid Estate in 2019. Editor's photo.*

Investigating the Taxonomy of *Cym. erythraeum, Cym. flavum* and *Cym. daweishanense*

For some time now, I have been keeping an eye on the published research into the taxonomy of the Cymbidium genus. Over the past few years, work has been progressing to sequence the complete chloroplast genome for each of the species and use this genetic data to better assess the relationships between species in the genus (for those unfamiliar with the term, a chloroplast is the part of a cell – an organelle – that conducts photosynthesis). Much of this research has been carried out by the National Orchid Conservation Center of China or the Institute of Botany at the Chinese Academy of Sciences. Both groups have uploaded the sequenced genomes to the International Nucleotide Sequence Database Collaboration project, making them freely available to the public via online portals such as NIH's GenBank[1].

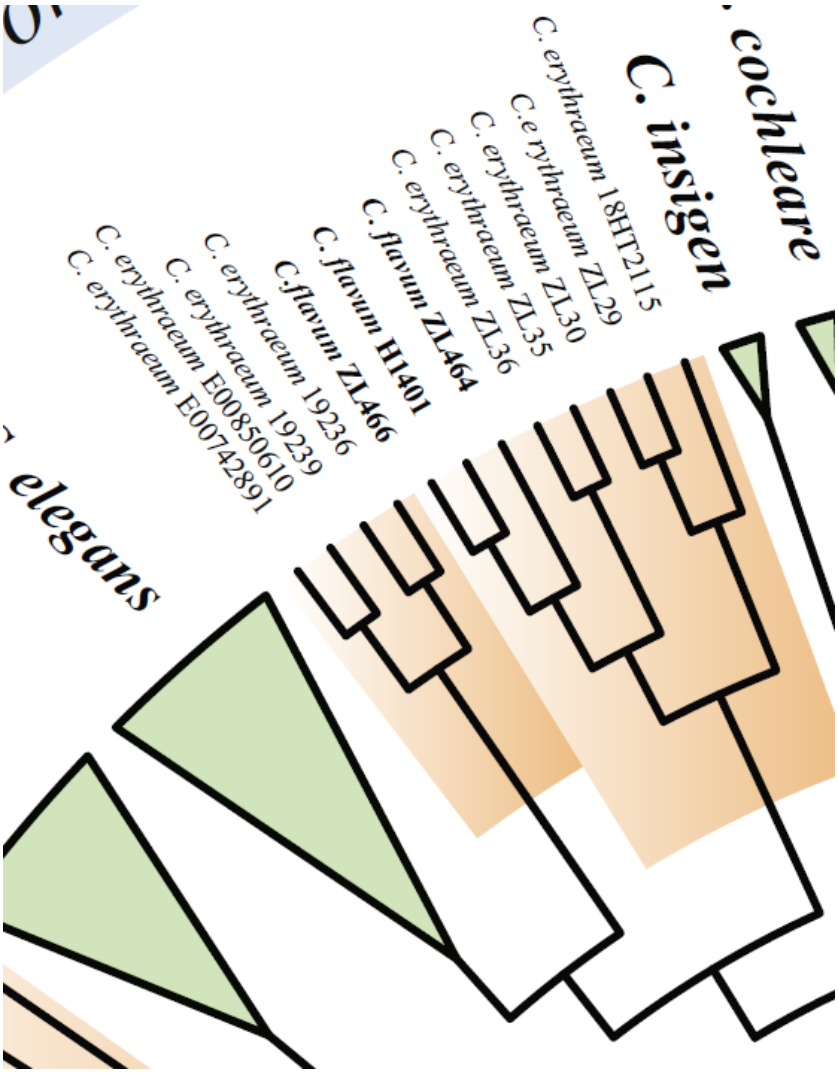
The publication of a large genetic analysis comprising over half the genus (Zhang, et al, 2023)[2] caught my attention earlier this year, as it had been the first to seriously consider the differences between *Cym. erythraeum* var. *erythraeum* (formerly *Cym. erythraeum*) and *Cym. erythraeum* var. *flavum* (formerly *Cym. flavum*). Unfortunately, the results were not as clear-cut as I had hoped, as both phylogenetic trees produced for the paper showed that *erythraeum* was not monophyletic (i.e., there was not a single point on the tree from which you could group all the *erythraeum* samples whilst excluding all other species). This did not bode well for my opinion (formed when I became familiar with these two taxa) that they should be treated as separate species.



Excerpt of the maximum-likelihood phylogenetic tree from Zhang, et al (2023). The bootstrap rates are shown at the nodes and indicate the level of confidence in the placement of that node.

[1] https://www.ncbi.nlm.nih.gov/genbank/

[2] Zhang, et al (2023). *DNA barcoding of Cymbidium by genome skimming: Call for next-generation nuclear barcodes*. https://www.researchgate.net/publication/364374193_DNA_barcoding_of_Cymbidium_by_genome_skimming_call_for_next-generation_nuclear_barcodes



Excerpt of the Skmer tree from Zhang, et al (2023).

However, two pieces of information stood out to me in the paper – first, that the maximum-likelihood bootstrap values did not indicate a good fit for the *flavum* group (the *flavum* parent node has a value of 63%, which is usually consider poor in this type of analysis – values of 90% or higher are preferred); and secondly, that four of the *erythraeum* samples were associated with *elegans* in the Skmer tree (shown left).

I had already been doing some of my own phylogenetic analyses using the data available from GenBank, so I decided to investigate this further. Several of the sequences used by the 2023 paper were available on GenBank, along with that of a related species – *Cym. daweishanense.* Classified in 2018[3], this relatively new species is clearly related to *Cym. flavum*, bearing a strong resemblance to it and some of its hybrids. I decided to include its genome sequence in my analysis to see just how closely related it was to *flavum*. I also selected several other species that were identified as being close relatives, both by my previous analysis and the phylogenetic trees produced by Zhang, et al (2022).

Left: Cym. flavum*. Unfortunately, I was unable to get permission to reproduce a photo of* Cym. daweishanense.

After some initial analysis, it became apparent that some of the samples were duplicates and existed in GenBank under multiple accession numbers. There were also two samples that I chose to reject from the analysis as outliers (with the reason noted below). The final list of species and the samples I used for my analysis are in the table below.

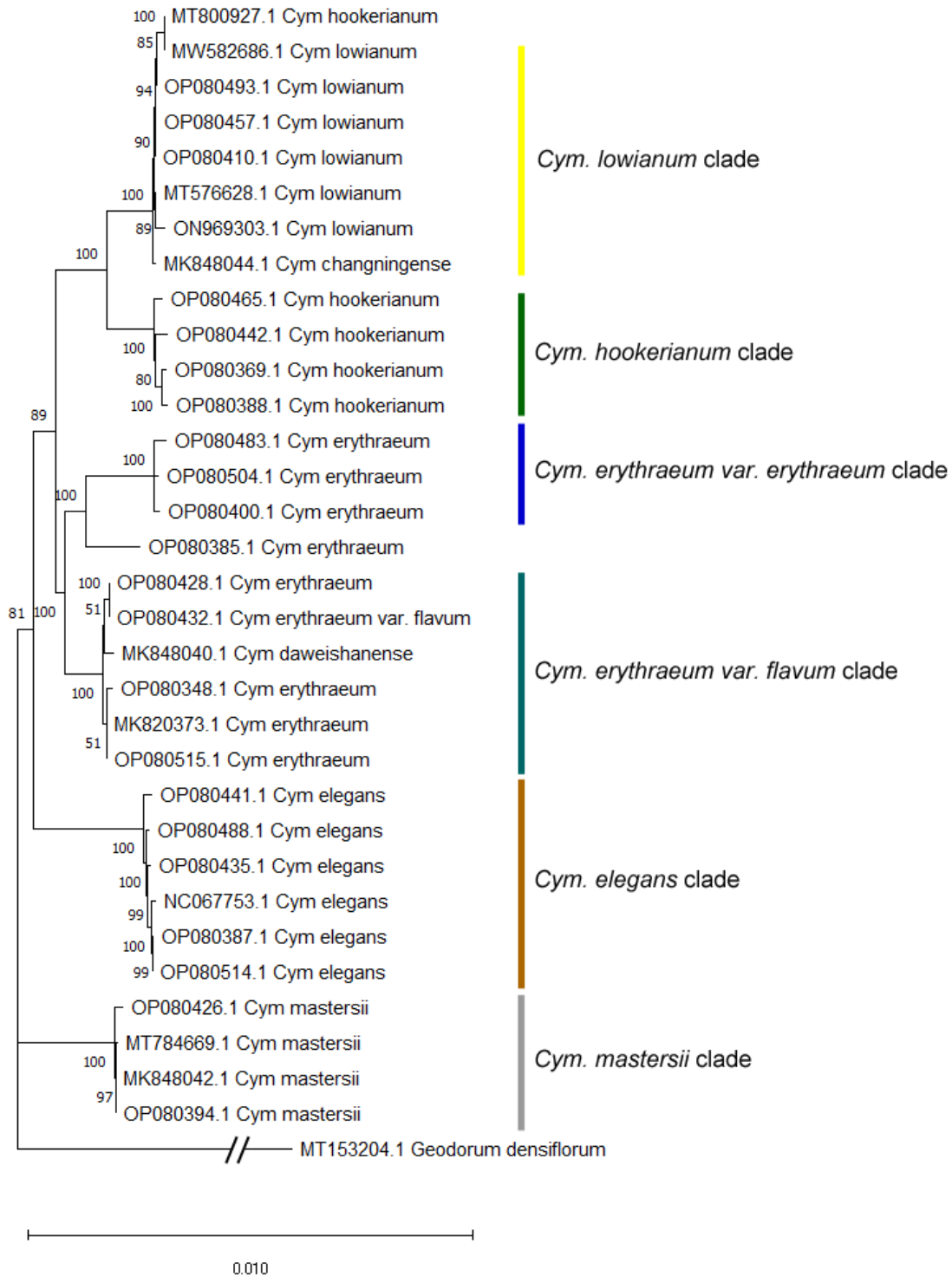
[3] *Cymbidium daweishanense, a new species from China: evidence from morphological and molecular analyses.* https://www.researchgate.net/publication/328782716_Cymbidium_daweishanense_Orchidaceae_Epidendroideae_a_new_species_from_China_evidence_from_morphological_and_molecular_analyses in Phytotaxa 374(3) pp249-256.

Species	Samples Accepted	Samples Rejected
Cym. changningense	MK848044.1	
Cym. daweishanense	MK848040.1	
Cym. elegans	OP080387.1 OP080435.1 OP080441.1 OP080488.1 OP080514.1	
***Cym. erythraeum* (no variety specified*)**	MK820373.1 OP080348.1 OP080385.1 OP080400.1 OP080428.1 OP080483.1 OP080504.1 OP080515.1	
Cym. flavum	OP080432.1	
Cym. lowianum	MT576628.1 MW582686.1 ON969303.1 OP080410.1 OP080457.1 OP080493.1	
Cym. hookerianum	OP080369.1 OP080388.1 OP080442.1 OP080465.1	MT800927.1 – this sample showed strong affinity with *lowianum* rather than the other four *hookerianum* samples. I suspect it is mislabelled.
Cym. mastersii	MK848042.1 MT784669.1 OP080394.1 OP080426.1	MT576627.1 – this was an outlier compared to the other four *mastersii* samples. It showed some affinity with *eburneum* and *maguanense*, so my suspicion is that this is misidentified.

* The *erythraeum* & *flavum* samples posed a slight problem, as only one sample was labelled with the variety (the Indian var. *erythraeum* or the Chinese var. *flavum*). I proceeded on the assumption that if *erythraeum* and *flavum* were genetically distinct enough to warrant separate species status, two clades (a type of taxonomical group) would appear in the results – one associated with the known *flavum* sample and one not.

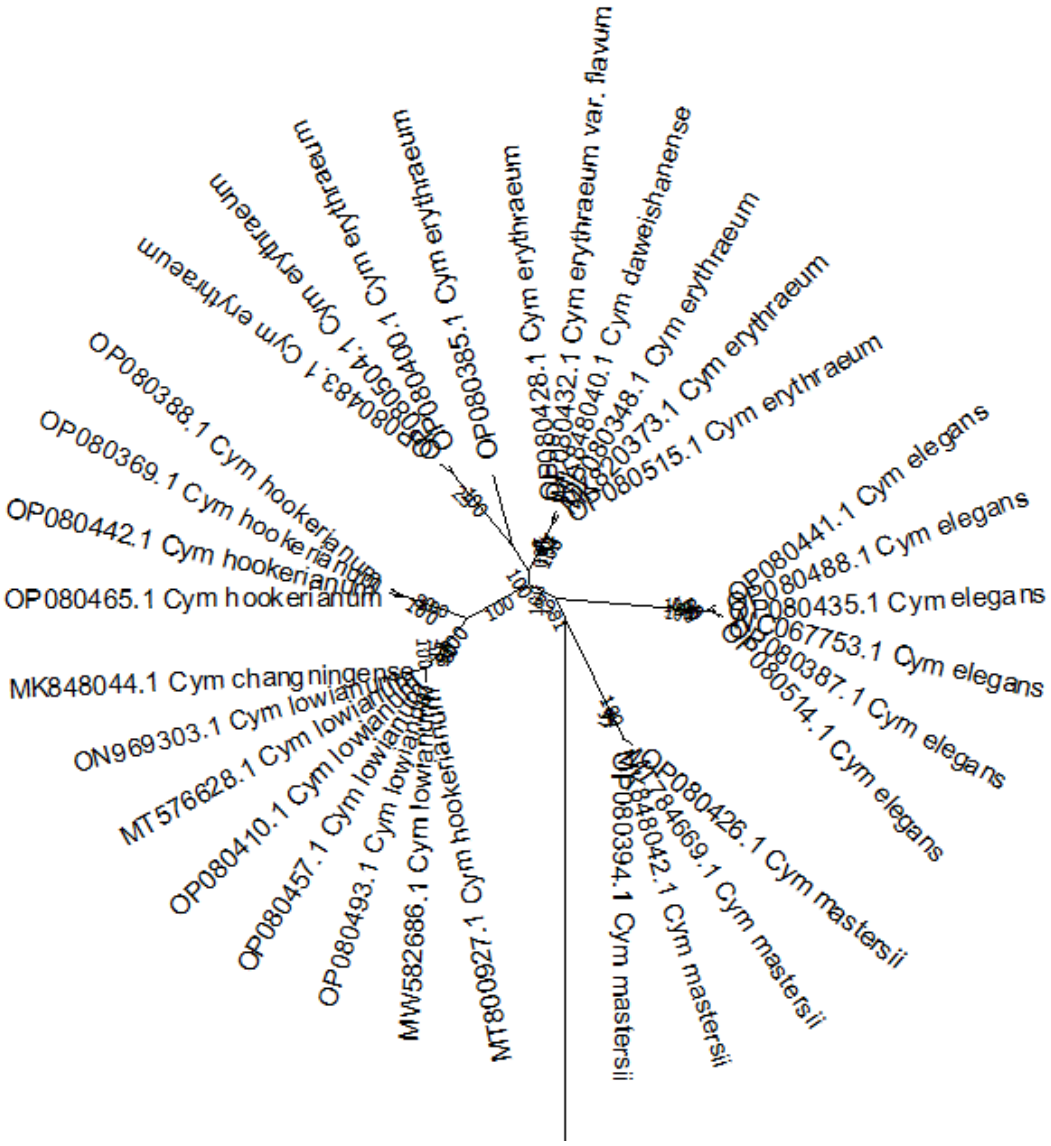
Once I had collated the dataset (GenBank allows for sequences to be exported in several formats, including the popular FASTA format), I used a program called MAFFT[4] to align the sequences. This was required since genes don't always have the same length across a genus (nor even within the same species!) and so an educated guess must be made as to how the genetic sequences from different species match up. Once the sequences were aligned, further analysis was carried out using MEGA[5], a genetic analysis software tool freely available for research purposes.

[4] MAFFT: https://mafft.cbrc.jp/alignment/software/
[5] MEGA: https://www.megasoftware.net/



Rectangular maximum-likelihood phylogenetic tree with the distinct clades colour-coded. Two of the flavum *clade confidence values are poor, indicating that the relative position of these samples within the clade is questionable.*

I ran a maximum-likelihood (ML) phylogenetic analysis in MEGA X, using *Geodorum densiflorum* as the outgroup. The ML tree was then plotted two different ways: 1) as a traditional rectangular tree with the branch length proportional to genetic divergence; and 2) as a radial tree, which more clearly highlighted the distinct clades.



A radial version of the same phylogenetic tree.

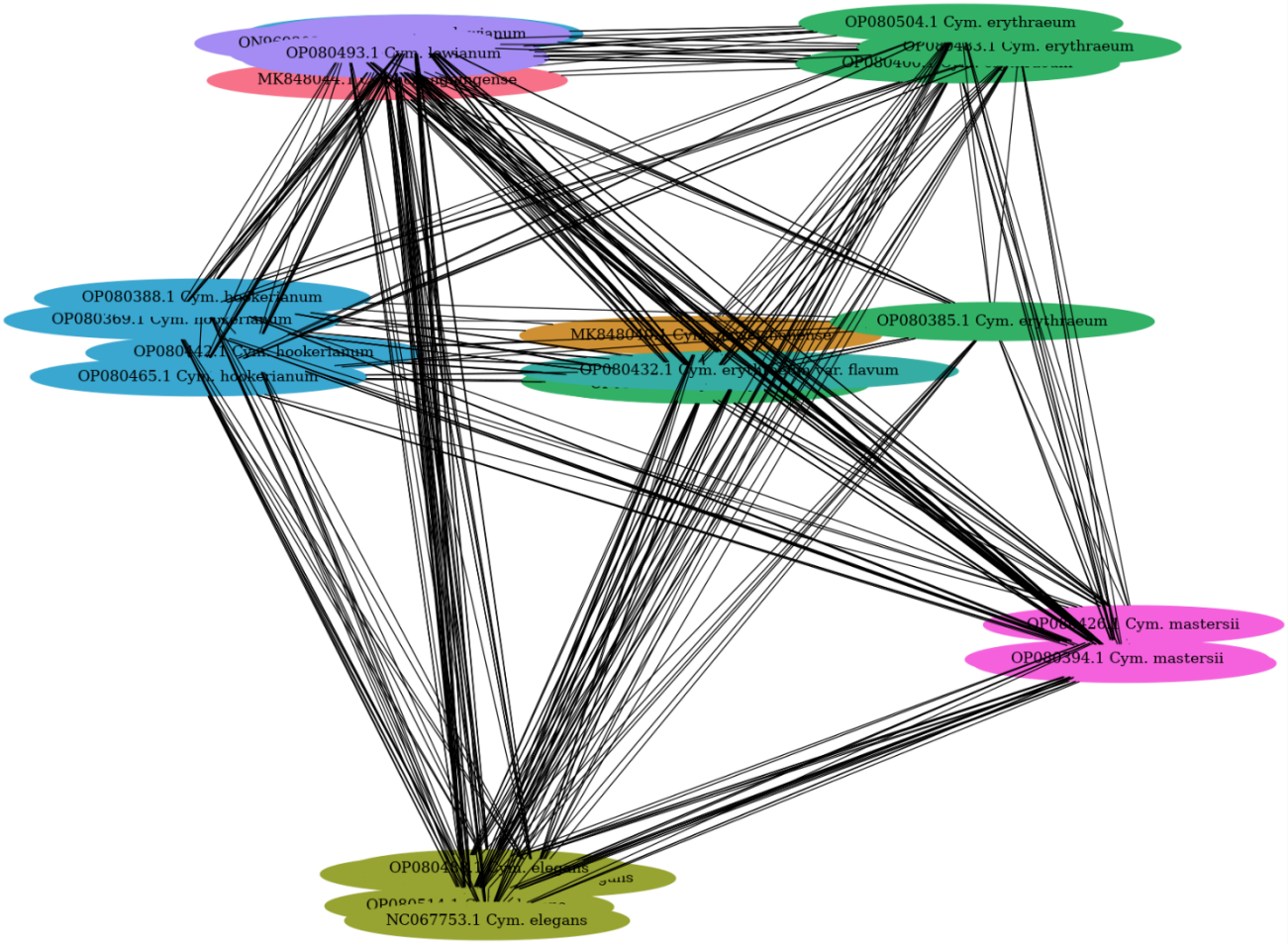
I followed the phylogenetic tree analysis with a genetic distance analysis (this computes the number of differences between each sample and produces a matrix of values to represent how closely related the samples are to each other). The resulting matrix was then plotted as a network graph using Graphviz[6] in order to visualise any clustering of samples.

In both the phylogenetic trees and the distance network graph, it was clear that the *Cym. erythraeum, flavum* and *daweishanense* samples fell into two distinct clades. Both clades had high bootstrap confidence values, supporting the hypothesis that there are two distinct species involved:

- The Indian *Cym. erythraeum*
- The Chinese *Cym. flavum*, with *Cym. daweishanense* as a subspecies or variety

Intriguingly, sample OP080385.1 did not neatly fit into either the *erythraeum* or *flavum* clades. In fact, it showed significant divergence from both, suggesting that it may be instead from a hybrid (perhaps it originated from the region where the two taxa overlap in nature?). It would be very

[6] Graphviz neato model: https://graphviz.org/docs/layouts/neato/



A network graph created using Graphviz and the neato model to represent the genetic distance between samples. The flavum *clade is near the centre of the graph, whilst the* erythraeum *clade is in the top right.*

interesting (as well as helpful) to have additional background information on the *erythraeum* and *flavum* samples provided to GenBank to further clarify these results.

Another interesting observation was the close association of *changningense* with the *lowianum* clade. This suggested that this taxon may be more appropriately described as a subspecies or variety of *lowianum*, rather than a species in its own right – but that is a discussion for another time.

It is my hope that as further genetic analyses are carried out, we will see the various taxonomical issues within the Cymbidium genus resolved and a clearer understanding of species relationships will be developed. Apart from the *erythraeum-flavum-daweishanense* group, other relationships that need clarifying include: *lowianum* and *i'ansonii*, *tracyanum* and *gaoligongense*, *insigne* and *seidenfadenii*, the whole *parishii-eburneum-sanderae* group (including the unique 'Sanderae'/'Emma Menninger' clones), not to mention almost the entire Jensoa section (based on the findings of Zhang, et al, 2022)!

In the meantime, I will continue my desktop studies using the data available to me and report any interesting findings.

Deflasking Tips from Justin Priddy

Justin recently explained to me how he deflasks his seedlings and was happy for me to share his experience in Cymbidium Chatter. He has kindly provided photos as well to demonstrate the process.

I thought I would share how I remove my plants from flask. I can't take credit for this, as Scott Barrie told me this is what he does after I had a conversation with him about how much of a nightmare some flasks are to get the plants out of without damage when they have extensive root systems. It's a very simple process and speeds up deflasking dramatically when you have a lot of bottles to do.

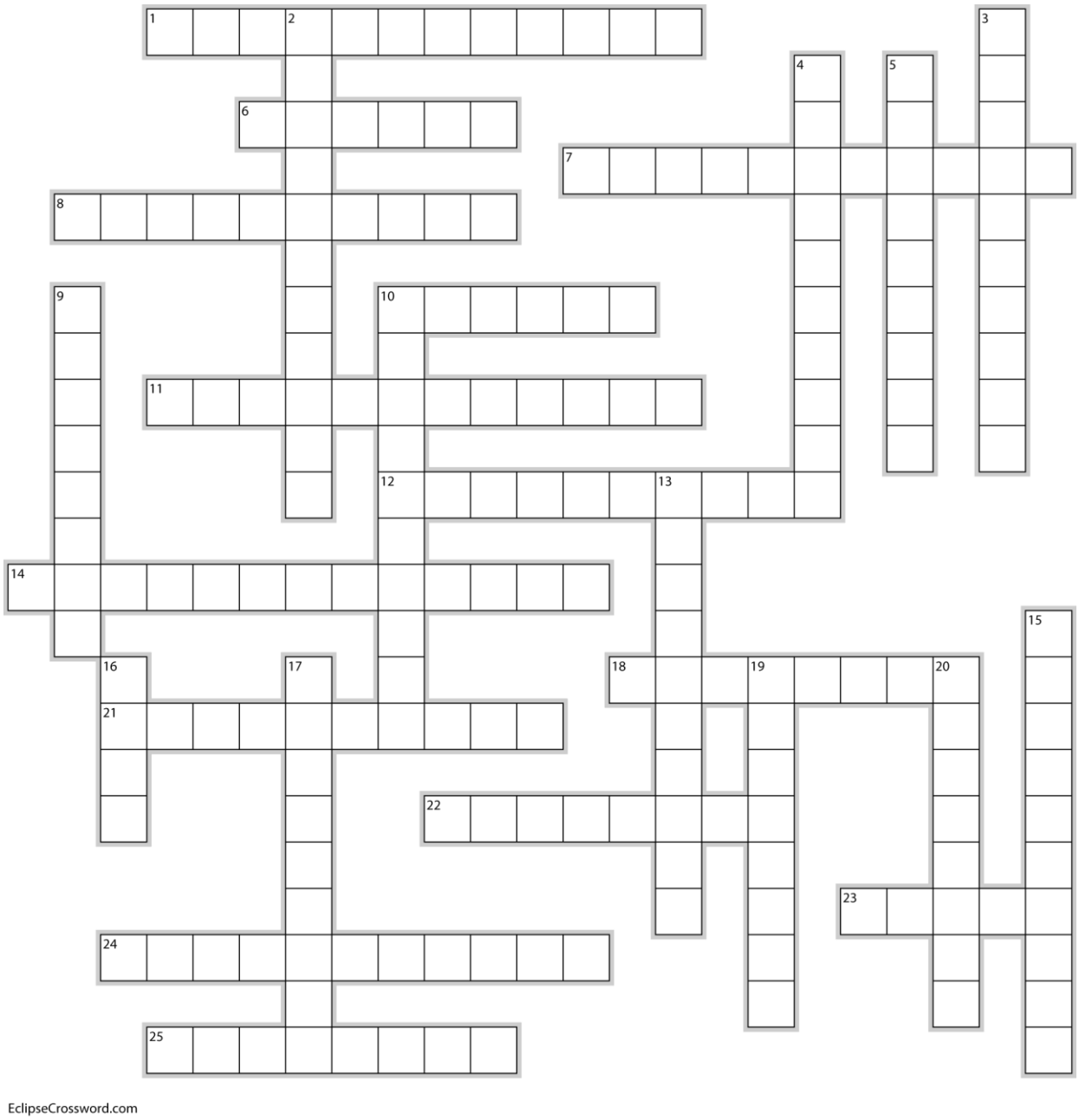
Get yourself a glass bottle cutter. They are easily available on eBay and Amazon. Don't get one where the cutting head swivels and moves, as it's hard to hold to cutter stable while you score the bottle and the score lines don't line up very well. Instead, use one that has a fixed cutting head.

The process is as follows:

1. Place bottle on the cutter.
2. Rotate the bottle to make a score line all the way around the bottle.
3. Hold bottle over a box or bin and gently tap the bottle on the bottom side of the score line and the top of the bottle will just fall off.
4. The plants then come out easily with no damage.

It produces a lot less glass shards this way and is a lot quicker than breaking the flask manually. I deflasked 22 bottles and washed and prepared all the plants ready to pot in 1.5hrs this way.

Cymbidium Crossword #1



Hopefully readers will enjoy this little puzzle, which I created in Eclipse Crossword. The solution will be in the next issue. If there is enough interest, I may create other crosswords or puzzles in future.

Across

1. Popular orange Cym bred from Electric Ladyland (2)
6. Family who produced the precursor to the RHS Orchid Register
7. Largest green-flowered species
8. Name of the grex that received the first Australian FCC
10. Black Magic
11. The process of creating clones
12. Type species for the Cymbidium genus
14. Australian native species that prefers a high pH
18. Name for a pair of pollen globules

21. Famous grex that produced an awarded chance tetraploid
22. A plant that grows on other plants but is not a parasite
23. The flower stem
24. A type of plant that grows in or on the ground
25. The grex from which most "Khan" progeny originates

Down

2. Famous selection of Cym. Dolly
3. Part of the plant that stores nutrients and moisture
4. 'Picture' was a selection of this grex that won at Dural (2)
5. Popular Australian novelty grex created by Kevin Hipkins (2)
9. Australian nursery that produced Wallamurra
10. A plant with four sets of chromosomes
13. A type of plant that grows on rocks
15. Name of the Australian hybridiser known for his *suave* hybrids (2)
16. A common component of orchid media
17. Popular floribundum hybrid in Australia, usually white or pale green/yellow (2)
19. A modified petal
20. A lack of anthocyanins

Errata

In my article on albinism in Issue 35, I incorrectly explained tetraploid breeding behaviour due to my misunderstanding of the terminology involved at the time (allotetraploids, autotetraploids and amphidiploids). Due to the extent of the changes required, I am planning to update the article (rather than produce an addendum) and send out a revised version of Issue 35 to the mailing list once it's ready. I will also replace the version available on the COSV website at the same time.

Acknowledgements and Contributions

I hope you have enjoyed this issue. If you have any feedback or would like to contribute (whether it be just one or two photos, an idea for an article, or to volunteer for an interview), please get in touch! I can be reached at jwhite88@gmail.com.

Previous issues are available at https://www.cosv.com.au/publications-and-resources. All material is copyright © the original owners and used with permission. Thanks to all those who have contributed to this issue, including Michael Coker, Kobsukh Kaenratana, Justin Priddy and Pierre Pujol.

The next issue is planned for September 2023.