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## A new family placement for Australian blue squill, *Chamaescilla*: Xanthorrhoeaceae (Hemerocallidoideae), not Asparagaceae

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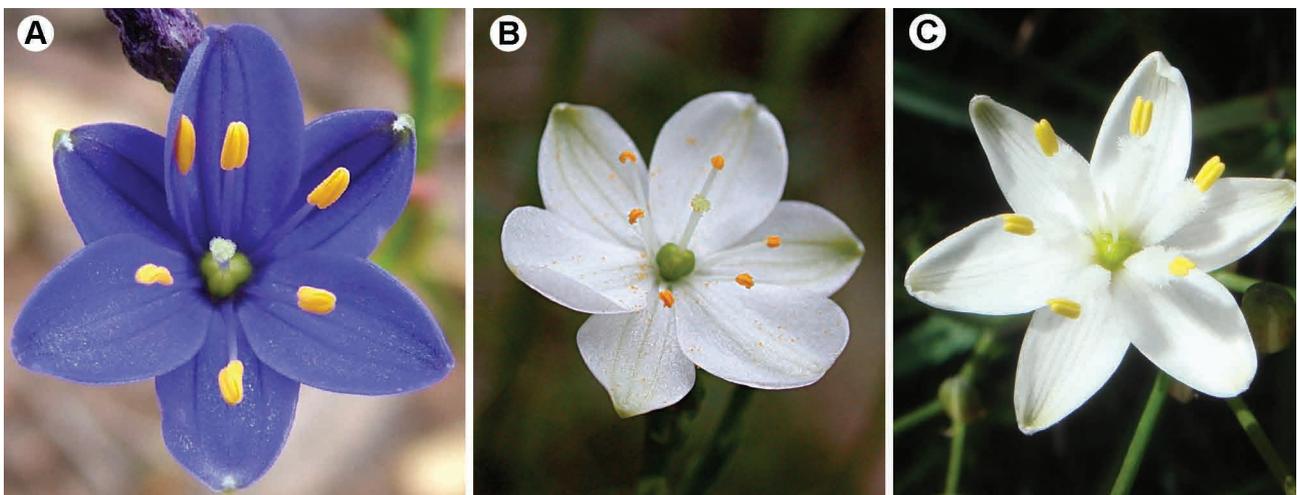
### Abstract

*Chamaescilla* is an endemic Australian genus, currently placed in the Asparagaceae, alongside other Australian endemic taxa in the tribe Lomandroideae. A recent molecular phylogeny indicated a relationship with another partly Australian family, the Xanthorrhoeaceae, but was not commented on by the authors. Here we added DNA sequence data for a single *Chamaescilla* specimen to an alignment representing all families in the Asparagales and performed parsimony and Bayesian phylogenetic analyses. *Chamaescilla* was strongly resolved as belonging to Xanthorrhoeaceae, subfamily Hemerocallidoideae, alongside two non-Australian members, *Simethis* and *Hemerocallis* in the hemerocallid clade. This position is corroborated by morphological characters, including pollen grain shape. We also produced an age-calibrated phylogeny and infer that the geographic distribution of the clade is the result of long distance dispersal between the Eocene and Miocene.

**Key words:** Asphodelaceae, biogeography, phylogeny, taxonomy

### Introduction

*Chamaescilla* Mueller ex Benth (1878: 48; commonly known as blue squill, blue stars or mudrut, Fig. 1) is a genus including four species that are endemic to Australia, found in the southwest and southeast of the country (Keighery 2001). All species are small, perennial tuberous herbs with annual leaves and flowers. Bisexual, typically blue flowers are borne on a scapose or corymbose inflorescence (Henderson 1987). *Chamaescilla corymbosa* (Brown 1810: 277) Mueller ex Benth in Mueller (1874: 90) subsp. *corymbosa* is widespread, occurring in both western and eastern parts of Australia, whereas the remaining taxa are restricted to the Western Australian biodiversity hotspot (Keighery 2001).



**FIGURE 1.** Flowers of: A, *Chamaescilla corymbosa* (Jane Duff Highway Park, western Victoria; photo M. Bayly) with typical blue/mauve flowers; B, *Chamaescilla corymbosa* (Brisbane Ranges, Victoria; photo, Chris Lindorff and Lauren Fraser) with unusual white flowers; C *Simethis mattiazii* (Ceuta, Spain; photo, Xemenendura).

Like many of the petaloid monocots, *Chamaescilla* has been placed in several different plant orders and families as monocot taxonomy has fluctuated. It was placed by Cronquist (1981) in the oversimplified Liliaceae, and treated as such for the *Flora of Australia* series (Henderson 1987), and then moved to Anthericaceae by Dahlgren (1989). Based on *rbcL* DNA sequences Chase *et al.* (1995, 1996) placed it sister to *Cordyline* Commerson ex Brown (1810: 280) in Lomandraceae (Asparagales), now subfamily Lomandroideae in Asparagaceae (Chase *et al.* 2009), along with several other former members of Australian Anthericaceae.

Asparagales is the largest monocot order, comprising approximately 50% of monocot species and 10–15% of all flowering plants (Chen *et al.* 2013), including the extremely diverse Orchidaceae, important food species such as *Asparagus* Linnaeus (1753a: 313) and *Allium* Linnaeus (1753a: 294), and garden plants like *Hemerocallis* Linnaeus (1753a: 324), which is also a potential new model organism (Rodriguez-Enriquez & Grant-Downton 2013). The order is united largely by molecular evidence and a black pigment in their seed coat, with few other morphological characters (Fay *et al.* 2000). Relationships between families in Asparagales are well understood with the sample-rich phylogeny of Chen *et al.* (2013) agreeing with the data-rich phylogeny of Steele *et al.* (2012), although Chen *et al.* (2013) note some level of reticulation at the base of the phylogeny. Nested clade analysis infers Australia as the centre of origin of the order, with many families such as Asparagaceae, Xanthorrhoeaceae, Orchidaceae and several early diverging lineages, such as the endemic Boryaceae and Doryanthaceae, occurring on that continent (Bremer & Janssen 2006). Chen *et al.* (2013) included a single sample of *Chamaescilla*, which their analysis placed in Hemerocallidoideae (Xanthorrhoeaceae) alongside two non-Australian members of the subfamily. This novel family placement was not commented on by Chen *et al.* (2013). An unpublished analysis of the Lomandroideae (Sirisenana 2010) also shows *Chamaescilla* sitting outside the rest of the subfamily, but that phylogenetic position might be an artefact of the enforced placement of *Asparagus* as an outgroup.

The family Xanthorrhoeaceae *sensu lato* includes three subfamilies: Xanthorrhoeoideae, Asphodeloideae, and Hemerocallidoideae. These subfamilies were formerly treated as families, but Chase *et al.* (2009) created a larger Xanthorrhoeaceae in an effort to reduce the number of poorly known “Dahlgrenian” families based on molecular evidence (Chase *et al.* 2000, 2006). Xanthorrhoeaceae was used as the new name for this group as it was the only one of the three family names listed as conserved in Appendix IIB of the code (e.g. Wiersema *et al.* 2015), despite it being the smallest family and youngest name. This broader use of the name Xanthorrhoeaceae was not widely accepted after APG III (2009), leading Klopper *et al.* (2013) to suggest conservation of Asphodelaceae. This was approved by the Nomenclature Committee for Vascular Plants (Appelquist 2014) and ratification of that decision at the next Nomenclature Section of the International Botanical Congress (to be held in Shenzhen, China, July 2017) will restore the priority of Asphodelaceae over Xanthorrhoeaceae (APG IV 2016).

In order to test the phylogenetic position of *Chamaescilla* we added partial sequences of the four genes (*atpB*, *matK*, *ndhF*, *rbcL*) used by Chen *et al.* (2013) for a new collection of *C. corymbosa*, and an *rbcL* sequence for the unplaced genus *Hodgsoniola* Mueller (1861: 176). Sequences retrieved from GenBank were used to add the remaining genera of Hemerocallidoideae and Lomandroideae to confirm the new familial position of *Chamaescilla* and determine relationships between genera in those subfamilies.

## Materials and Methods

A total of 89 taxa was included in the dataset, including representatives of all genera from Hemerocallidoideae and Lomandroideae (Table 1). The taxon set included in the Nexus file from Chen *et al.* (2013) was reduced to represent core families and subfamilies of Asparagales. Additional sequences for eighteen taxa of Hemerocallidoideae and Lomandroideae were downloaded from GenBank. In some cases, sequences from different studies were used to represent a species, or sequences from different species were used to represent a genus (i.e. as a chimeric sequence).

DNA was extracted from ~20 mg dry tissue of both *Chamaescilla corymbosa* (MELU M113170a) and *Hodgsoniola junciformis* (Mueller 1860: 96) Mueller (1861: 185; PERTH 7488793) using a modified CTAB protocol (Shepherd & McLay 2011). The *atpB*, *rbcL*, *ndhF*, and *matK* regions were amplified using the primers of Chen *et al.* (2013; Table 2). PCRs were performed in 25  $\mu$ L reactions using 1 $\times$  MyTaq Reaction Buffer, 0.4 mM of each primer, 1.5 mM MgCl<sub>2</sub>, 1 $\times$  BSA and 1 unit of MyTaq DNA polymerase. PCR cycling conditions were 95°C for 1 min and 30 cycles of 95°C for 1 min, 50°C for 45 s, 65°C for 1 min and a final elongation at 65°C for 5 min. Various annealing temperatures and PCR additives were trialled, but generally produced poor amplification. This resulted in partial sequences of all four regions for *C. corymbosa* and only a partial *rbcL* sequence for *H. junciformis*. PCR products were purified, sent to the

Australian Genome Research Facility (AGRF, Melbourne), and sequenced by capillary separation using an AB3730 (Applied Biosystems, Foster City, CA, USA). Sequences were edited in Geneious R7 (Kearse *et al.* 2012).

**TABLE 1.** GenBank accession numbers for sequences used in dataset.

Taxa	Source	Family/subfamily	GenBank accession numbers for plastid markers: <i>atpB</i> , <i>matK</i> , <i>ndhF</i> , <i>rbcL</i>
<i>Acanthocarpus preisii</i> Lehm.	Chen <i>et al.</i> 2013	Lomandroideae	JX903820; JX903591; JX903403; JX903182
<i>Agapanthus africanus</i> Hoffmanns.	Chen <i>et al.</i> 2013	Amaryllidaceae	JX903728; HM640599; JX903309; HM640485
<i>Agave ghiesbreghtii</i> K.Koch.	Chen <i>et al.</i> 2013	Asparagaceae	JX903730; HM640592; JX903311; HM640478
<i>Agrostocrinum scabrum</i> (R.Br.) Baill.	GenBank	Hemerocallidoideae	–; –, AY225069; FN870757
<i>Allium thunbergii</i> G.Don.	Chen <i>et al.</i> 2013	Amaryllidaceae	AY147628; JX903547; JX903324; JX903136
<i>Aloe vera</i> (L.) Burm.f.	Chen <i>et al.</i> 2013	Asphodeloideae	AF168886; AJ511390; AY225054; AJ512309
<i>Amaryllis belladonna</i> L.	Chen <i>et al.</i> 2013	Amaryllidaceae	JX903750; JX903555; JX903333; JX903144
<i>Aphyllanthes monspeliensis</i> L.	Chen <i>et al.</i> 2013	Asparagaceae	JX903788; HM640614; JX903370; JF972914
<i>Apostasia wallichii</i> Wall.	Chen <i>et al.</i> 2013	Orchidaceae	JX903906; JX903642; JX903489; HM640552
<i>Arnocrinum preisii</i> Lehm.	GenBank	Hemerocallidoideae	–; JQ4355351; AY191172; FN870765
<i>Arthropodium cirratum</i> G.Benn.	Chen <i>et al.</i> 2013	Lomandroideae	JX903821; HM640634; JX903404; HM640516
<i>Asparagus densiflorus</i> (Kunth) Jessop	Chen <i>et al.</i> 2013	Asparagaceae	JX903790; JX903580; JX903372; JX903171
<i>Asphodeline lutea</i> Rchb.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903840; JX903600; JX903423; JX903192
<i>Asphodelus aestivus</i> Brot.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903841; HM640645; JX903424; HM640527
<i>Astelia alpina</i> R.Br.	Chen <i>et al.</i> 2013	Asteliaceae	JX903850; HM640648; JX903434; HM640530
<i>Astroloba foliolosa</i> (Haw.) Uitewaal	Chen <i>et al.</i> 2013	Asphodeloideae	JX903842; JX903601; JX903425; JX903193
<i>Blandfordia punicea</i> (Labill.) Sweet	Chen <i>et al.</i> 2013	Blandfordiaceae	JX903854; HM640650; JX903438; HM640532
<i>Borya septentrionalis</i> F.Muell.	Chen <i>et al.</i> 2013	Boryaceae	JX903855; HM640651; JX903439; HM640533
<i>Bowiea volubilis</i> Harv. ex Hook.f.	Chen <i>et al.</i> 2013	Asparagaceae	JX903800; HM640621; JX903382; HM640503
<i>Bulbine semibarbata</i> (R.Br.) Haw.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903843; HM640646; JX903426; HM640528
<i>Bulbinella cauda-felis</i> (L.f.) T.Durand & Schinz	Chen <i>et al.</i> 2013	Asphodeloideae	JX903844; JX903602; JX903427; JX903194
<i>Caesia contorta</i> T.Durand & Schinz	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903858; JX903610; JX903442; JX903201
<i>Chamaescilla corymbosa</i> (R.Br.) Benth.	This study	Hemerocallidoideae	KX421846; KX421845; KX421844; KX421843
<i>Chamaescilla</i> sp. F.Muell. ex Benth.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903822; JX903592; JX903405; JX903183
<i>Chamaexeros serra</i> (Endl.) Benth.	Chen <i>et al.</i> 2013	Lomandroideae	JX903593; JX903593; JX903406; JX903184
<i>Clivia nobilis</i> Lindl.	Chen <i>et al.</i> 2013	Amaryllidaceae	JX903753; HM640603; JX903336; JX903147
<i>Cordylone cannifolia</i> R.Br.	Chen <i>et al.</i> 2013	Lomandroideae	JX903824; JX903594; JX903407; JX903185
<i>Corynotheca micrantha</i> (Lindl.) Druce	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903859; JX903611; JX903443; JX903202
<i>Dasyllirion wheeleri</i> S.Watson.	Chen <i>et al.</i> 2013	Asparagaceae	JX903725; HM640588; JX903306; HM640474
<i>Dianella intermedia</i> 2 Endl.	GenBank	Hemerocallidoideae	FJ707485; –, FJ707515; FJ707495
<i>Dianella montana</i> 1 Blume	GenBank	Hemerocallidoideae	FJ707486; JQ435538; AY225071; FJ707496
<i>Dianella sandwicensis</i> 3 Hook. & Arn.	GenBank	Hemerocallidoideae	FJ707488; –, FJ707517; FJ707497
<i>Dianella tasmanica</i> 4 Hook.f.	GenBank	Hemerocallidoideae	FJ707489; –, FJ707518; FJ707498
<i>Dichopogon</i> sp. Kunth.	GenBank	Lomandroideae	–; –, –, FN870799
<i>Doryanthes palmeri</i> Benth.	Chen <i>et al.</i> 2013	Doryanthaceae	JX903857; HM640653; JX903441; HM640535
<i>Dracaena hookeriana</i> K.Koch.	Chen <i>et al.</i> 2013	Asparagaceae	JX903716; JX903540; JX903297; AM235113
<i>Eccremis coarctata</i> Baker	GenBank	Hemerocallidoideae	FJ707490; –, FJ707519; FJ707500
<i>Eremurus chinensis</i> O.Fedtsch.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903845; HM640644; JX903428; HM640526
<i>Eustrephus latifolius</i> R.Br.	GenBank	Lomandroideae	AF168912; JQ435554; AY191185; AY298831
<i>Gasteria rawlinsonii</i> Oberm.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903846; JX903603; JX903429; JX903195

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**TABLE 1.** (Continued)

Taxa	Source	Family/subfamily	GenBank accession numbers for plastid markers: <i>atpB</i> , <i>matK</i> , <i>ndhF</i> , <i>rbcL</i>
<i>Geitonoplesium cymosum</i> R.Br.	GenBank	Hemerocallidoideae	–; –; AY191174; AY298833
<i>Gladiolus illyricus</i> Sturm	Chen <i>et al.</i> 2013	Iridaceae	JX903884; JX903627; JX903467; HM640542
<i>Haworthia coarctata</i> Haw.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903847; JX903604; JX903430; JX903196
<i>Hemerocallis dumortierii</i> 3 C.Morren	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903861; JF972938; JX903445; JF972904
<i>Hemerocallis fulva</i> 4 L.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903862; JF972939; JX903446; JF972905
<i>Hemerocallis hongdoensis</i> 2 M.G.Chung & S.S.Kang	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903863; JX903612; JX903447; AY149364
<i>Hemerocallis minor</i> 1 Mill.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903864; HM640655; JX903448; HM640537
<i>Hemiphylacus latifolius</i> S.Watson	Chen <i>et al.</i> 2013	Asparagaceae	JX903793; HM640617; JX903375; HM640499
<i>Hensmania</i> (chimera of two species) W.Fitzg	GenBank	Hemerocallidoideae	FJ707492; JQ435540; AY191175; FJ707501
<i>Herpolirion novae-zelandiae</i> Hook.f.	GenBank	Hemerocallidoideae	–; –; –; Z77303
<i>Hodgsoniola junciformis</i> F.Muell.	This study	Hemerocallidoideae	–; –; –; KX421842
<i>Iris odaesanensis</i> Y.N.Lee	Chen <i>et al.</i> 2013	Iridaceae	JX903888; JF972933; JX903471; JF972899
<i>Iris sanguinea</i> Donn	Chen <i>et al.</i> 2013	Iridaceae	JX903891; JF972936; JX903474; JF972902
<i>Ixiolirion tataricum</i> Schult.f.	Chen <i>et al.</i> 2013	Ixioliriaceae	JX903904; HM640660; JX903487; HM640543
<i>Johnsonia pubescens</i> Lindl.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903865; JX903613; JX903449; JX903203
<i>Kniphofia</i> sp. Moench	Chen <i>et al.</i> 2013	Asphodeloideae	AJ417572; JX903605; JX903431; Z73689
<i>Laxmannia squarrosa</i> Lindl.	Chen <i>et al.</i> 2013	Lomandroideae	JX903826; HM640636; JX903409; JF972915
<i>Leucocrinum montanum</i> Nutt. ex A.Gray	Chen <i>et al.</i> 2013	Asparagaceae	JX903787; HM640595; JX903369; HM640481
<i>Leucojum roseum</i> F.Martin	Chen <i>et al.</i> 2013	Amaryllidaceae	JX903765; JX903568; JX903348; JX903158
<i>Lomandra hastilis</i> (R.Br.) Ewart	Chen <i>et al.</i> 2013	Lomandroideae	JX903827; HM640635; JX903410; HM640517
<i>Lomandra ordii</i> (F.Muell.) Schltr.	Chen <i>et al.</i> 2013	Lomandroideae	JX903829; JX903596; JX903412; JX903188
<i>Milla biflora</i> Cav.	Chen <i>et al.</i> 2013	Asparagaceae	JX903837; HM640641; JX903420; HM640523
<i>Murchisonia fragrans</i> Brittan	GenBank	Lomandroideae	–; –; –; FN870878
<i>Narcissus tazetta</i> var <i>chinensis</i> L.	Chen <i>et al.</i> 2013	Amaryllidaceae	JX903768; HM640601; JX903351; HM640487
<i>Nolina bigelovii</i> S.Watson	Chen <i>et al.</i> 2013	Asparagaceae	JX903726; JX903543; JX903307; JX903132
<i>Pasithea caerulea</i> (Ruiz & Pav.) D.Don	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903866; JX903614; JX903450; JX903204
<i>Phoenix dactylifera</i> L.	Chen <i>et al.</i> 2013	Arecaceae	JX903945; JX903671; JX903525; JX903254
<i>Phormium tenax</i> J.R.Forst & G.Forst	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903867; JX903615; JX903451; JX903205
<i>Poellnitzia rubiflora</i> Uitewaal	Chen <i>et al.</i> 2013	Asphodeloideae	JX903848; JX903606; JX903432; JX903197
<i>Rhodohypoxis milloides</i> (Baker) Hilliard & B.L.Burtt	Chen <i>et al.</i> 2013	Hypoxidaceae	AJ235582; AY368377; AY225062; Z77280
<i>Rhuacophila javanica</i> Blume	GenBank	Hemerocallidoideae	–; JQ435543; AY225073; –
<i>Romnaldia ophiopogonoides</i> Conran, P.I.Forst. & Donnon	GenBank	Lomandroideae	–; –; –; KF496520
<i>Simethis mattiazii</i> (Vand.) Sacc.	GenBank	Hemerocallidoideae	–; JQ435544; AY225074; Z69231
<i>Sowerbaea juncea</i> Andrews	Chen <i>et al.</i> 2013	Lomandroideae	JX903830; JX903597; JX903413; JX903189
<i>Stawellia dimorphantha</i> F.Muell.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903868; JX903616; FJ707520; Z77306
<i>Stypandra glauca</i> R.Br.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903869; JX903617; JX903452; JX903206
<i>Tecophilaea cyanocrocus</i> Leyb.	Chen <i>et al.</i> 2013	Tecophilaceae	JX903919; HM640661; JX903500; HM640544
<i>Thelionema</i> (chimera of two species) R.J.F.Hend	GenBank	Hemerocallidoideae	–; JQ435546; AY225076; FN870752
<i>Thysanotus</i> sp. R.Br.	Chen <i>et al.</i> 2013	Lomandroideae	JX903831; JX903598; JX903414; JX903190
<i>Trachyandra esterhuysenae</i> Oberm.	Chen <i>et al.</i> 2013	Asphodeloideae	JX903849; JX903607; JX903433; JX903198
<i>Trichopetalum plumosum</i> J.F.Macbr.	Chen <i>et al.</i> 2013	Lomandroideae	JX903832; JX903599; JX903415; JX903191

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**TABLE 1.** (Continued)

Taxa	Source	Family/subfamily	GenBank accession numbers for plastid markers: <i>atpB</i> , <i>matK</i> , <i>ndhF</i> , <i>rbcL</i>
<i>Tricoryne elatior</i> R.Br.	Chen <i>et al.</i> 2013	Hemerocallidoideae	JX903870; JX903618; JX903453; JX903207
<i>Walleria gracilis</i> (Salisb.) S.Carter	Chen <i>et al.</i> 2013	Tecophiliaceae	JX903920; JX903648; JX903501; JX903232
<i>Xanthorrhoea media</i> 2 R.Br.	Chen <i>et al.</i> 2013	Xanthorrhoeaceae	JX903922; JX903650; JX903503; JX903234
<i>Xanthorrhoea resinosa</i> 1 Pers.	Chen <i>et al.</i> 2013	Xanthorrhoeaceae	JX903923; HM640663; JX903504; HM640546
<i>Xerolirion divaricata</i> A.S.George	GenBank	Lomandroideae	–; –; –; Z77299
<i>Xeronema callistemon</i> W.R.B.Oliv	Chen <i>et al.</i> 2013	Xeronemataceae	JX903924; HM640664; JX903505; HM640547
<i>Zephyra elegans</i> D.Don	Chen <i>et al.</i> 2013	Tecophiliaceae	JX903921; JX903649; JX903502; JX903233

**TABLE 2.** Primer sequences for the four plastid markers used in this study.

Primer	Sequence 5'-3'	Source
<i>atpB</i> -2F	TATGAGAATCAATCCTACTACTTCT	Savolainen <i>et al.</i> 2000
<i>atpB</i> -1494R	TCAGTACACAAAGATTTAAGGTCAT	Savolainen <i>et al.</i> 2000
<i>rbcL</i> -1F	ATGTCACCACAAACAGAAAC	Savolainen <i>et al.</i> 2000
<i>rbcL</i> -1460R	TCCTTTTAGTAAAAGATTGGGCCGAG	Savolainen <i>et al.</i> 2000
<i>ndhF</i> -32F	TACCTTTTCTTCCACTTCCAGTT	Terry <i>et al.</i> 1997
<i>ndhF</i> -1318R	GAACATATAAAAATGCGGTTAATCC	Terry <i>et al.</i> 1997
<i>ndhF</i> -745F	TGGTTACCTGATGCTATGGAAGG	Terry <i>et al.</i> 1997
<i>ndhF</i> -2110R	CCCCCTATATATTTGATACCTTCTCC	Terry <i>et al.</i> 1997
MO <i>matK</i> -480F	CATWTGGAAATCTTGGTTC	Hilu <i>et al.</i> 2003
<i>trnK</i> -2R	AACTAGTCGGATGGAGTAG	Johnson & Soltis 1994

Sequences for each locus were aligned in Geneious R7 (Kearse *et al.* 2012) using the inbuilt aligner with default settings to determine locus boundaries. The alignments for each locus were then concatenated and the alignment was refined using MUSCLE (Edgar 2004) and by eye. The final alignment of the four regions was 6530 bp long and consisted of 89 terminal taxa.

A parsimony analysis was performed in PAUP\* 4.0 b10 (Swofford 2002) using a heuristic search with stepwise addition closest, TBR, and MaxTrees set at 20,000. Bootstrap analysis was based on 1000 full heuristic replicates. Bayesian analysis was implemented in MrBayes on the CIPRES portal (Miller *et al.* 2010) using 15,000,000 generations, with 30% burn in and a sampling frequency of 1000. The data were partitioned into the four gene regions and these were unlinked to reduce the effect of different evolutionary histories of each gene conflating the results. The most appropriate model for each region was selected using MrModeltest v2 (Nylander 2004) using the Akaike Information Criterion. After burn-in, the remaining trees were used to produce a 50% majority-rule consensus tree showing the posterior probabilities of all branches. Both analyses used *Phoenix dactylifera* Linnaeus (1753b: 1188) as an outgroup.

Time calibrated phylogenies of Xanthorrhoeaceae were inferred using a reduced alignment of 33 taxa and BEAST v1.7 (Drummond *et al.* 2012). As outlined below, the calibration points were within Xanthorrhoeaceae, therefore, in order to reduce the effect of different substitution rates throughout the rest of Asparagales on age estimates within Xanthorrhoeaceae, a representative sample of each genus (two samples for *Hemerocallis*) within the rest of the family was used and *Xeronema callistemon* Oliver (1926: 1) was used as an outgroup. An uncorrelated lognormal (UCLN) clock model was used, with a general time reversible substitution model (GTR+I+G) and a Yule speciation model of tree branching. Two calibration points were used as tree priors following Crisp *et al.* (2014): 1) a *Dianella*-like fossil from the Eocene was placed at the crown of *Phormium* Forster & Forster (1776: 47, t. 24) and *Dianella* Lamark ex Jussieu (1789: 41), excluding *Pasithea* Don (1832: 236), which lacks isobifacial leaves, using a lognormal prior (offset = 45.0, SD = 1.0); 2) a normally distributed secondary calibration of the Xanthorrhoeaceae crown (71.2, SD = 1.0). The MCMC chain was run for 15 million generations to ensure convergence between four replicate runs. Trees from each run were combined using LogCombiner and annotated in TreeAnnotator (Drummond *et al.* 2012); Tracer v1.6 (Drummond *et al.* 2012) was used to check stationarity and establish that effective sample sizes were greater than 200 for the combined logs after 30% burn-in.

Scanning electron microscopy was used to determine the pollen type of *Chamaescilla*. Pollen was removed from the pressed sample of *C. corymbosa* onto a stub and coated with gold to a thickness of 10 nm. Pollen morphology was observed on a Philips XL30 Field Emission Scanning Microscope.

## Results

**Phylogenetic analyses:**—Summary statistics from parsimony analyses and MrModelTest are shown in Table 3. Parsimony and Bayesian analyses were very similar so posterior probability (PP) and parsimony bootstrap (BS) support were mapped onto a Bayesian 50% majority rule tree (Fig. 2). Relationships between Asparagales families were identical to those resolved in previous analyses (Chen *et al.* 2013; Seberg & Petersen 2012; Steele *et al.* 2012). The newly sequenced *Chamaescilla corymbosa* specimen was resolved as sister to the sample from Chen *et al.* (2013), strongly within Hemerocallidoideae, sister to *Simethis* Kunth (1843: 618) and *Hemerocallis* within the hemerocallid clade. *Hodgsoniola* was placed in the johnsonioid clade, along with other genera with phylloclades rather than leaves (Clifford & Conran 1998).

Within the phormioid clade the South American genus *Pasithea* was placed sister to the rest of the tribe with strong support (PP = 1/BS = 82). The relationships of some phormioid genera are still unclear, with a polytomy between the sister pairs of *Dianella–Eccremis* Willdenow ex Baker (1876: 319), *Rhuacophila* Blume (1827: 15)—*Stypandra* Brown (1810: 278), and *Thelionema* Henderson (1985: 109)—*Herpolirion* Hooker (1853: 258) in the phylogeny based on Bayesian inference. The family Xanthorrhoeaceae was well supported (PP = 1.0/BS=95) and relationships between its subfamilies were well resolved, with Asphodeloideae strongly supported (1.0/100) as sister to Xanthorrhoeoideae (1.0/100) and Hemerocallidoideae (1.0/88). The *Asphodeline* Reichenbach (1830: 116) + *Asphodelus* Linnaeus (1753a: 309) clade was strongly supported as sister to a clade including the remaining Asphodeloideae (PP=1/BS=100). The monophyly of Lomandroideae is also strongly supported using Bayesian inference, and moderately supported by parsimony (PP = 1.0, BS = 76).

**Divergence time estimates in the Hemerocallidoideae:**—The maximum clade credibility tree produced by BEAST analysis of Xanthorrhoeaceae is shown in Fig. 3, and ages of major clades are given in Table 4. The median age of the divergence of the most recent common ancestor (MRCA) of the hemerocallid clade and the johnsonioid clade was 52.51 mya (million years ago) (95% Highest Posterior Density, HPD = 45.58–59.42). Estimated divergence between *Chamaescilla* and *Hemerocallis–Simethis* was 29.11 mya, though this date is associated with high uncertainty (95% HPD = 17.34–41.48).

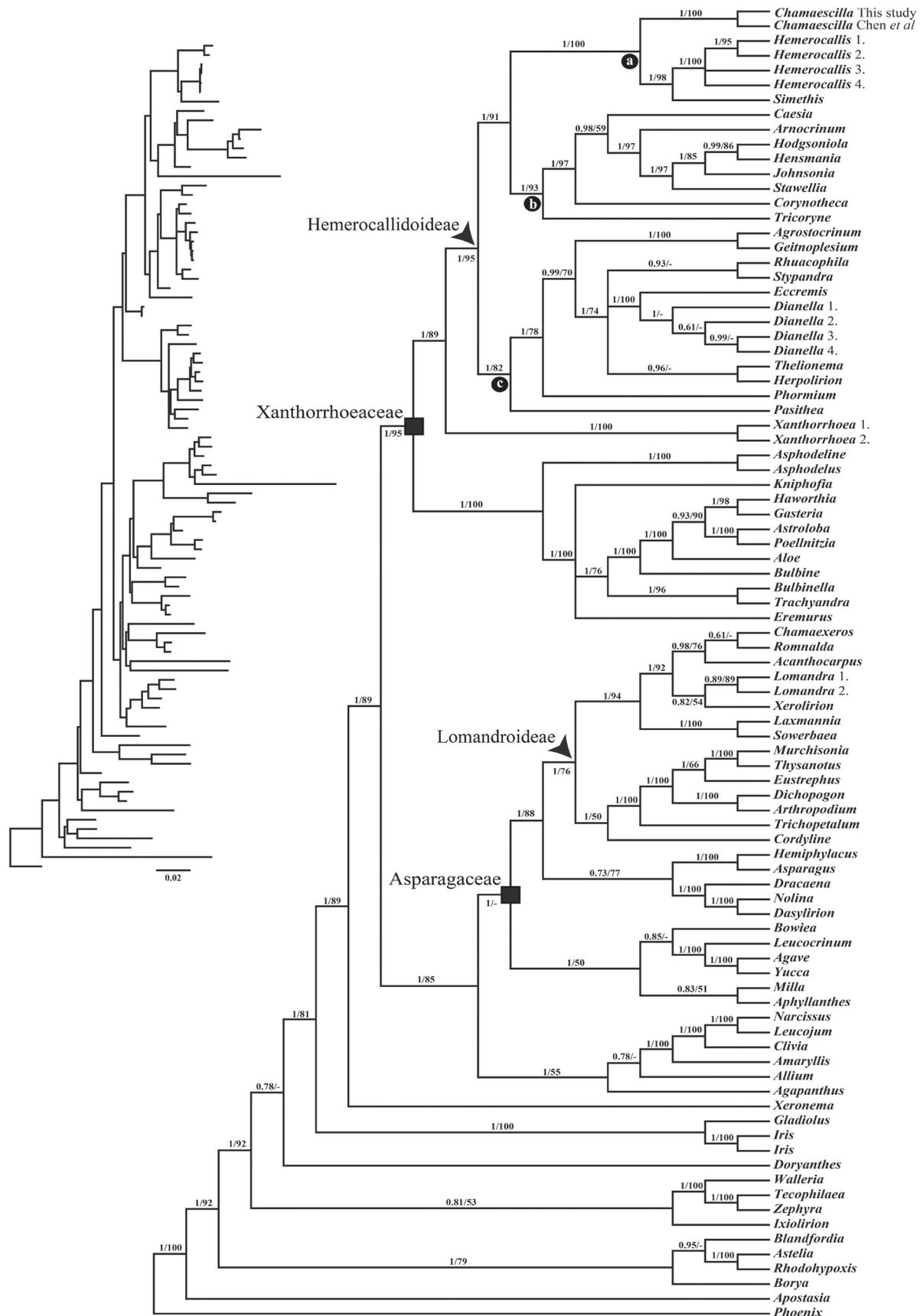
**Pollen type of *Chamaescilla*:**—*Chamaescilla* pollen is ovoid, approximately 60 µm long and 20 µm wide (Fig. 4). The aperture is monosulcate and the pollen surface is reticulate.

**TABLE 3.** Summary information for the four plastid markers used in this study, including aligned length, parsimony informative characters (PIC), parsimony consistency index (CI), retention index (RI) and rescaled consistency index (RC), as well as the model of evolution used for Bayesian analysis.

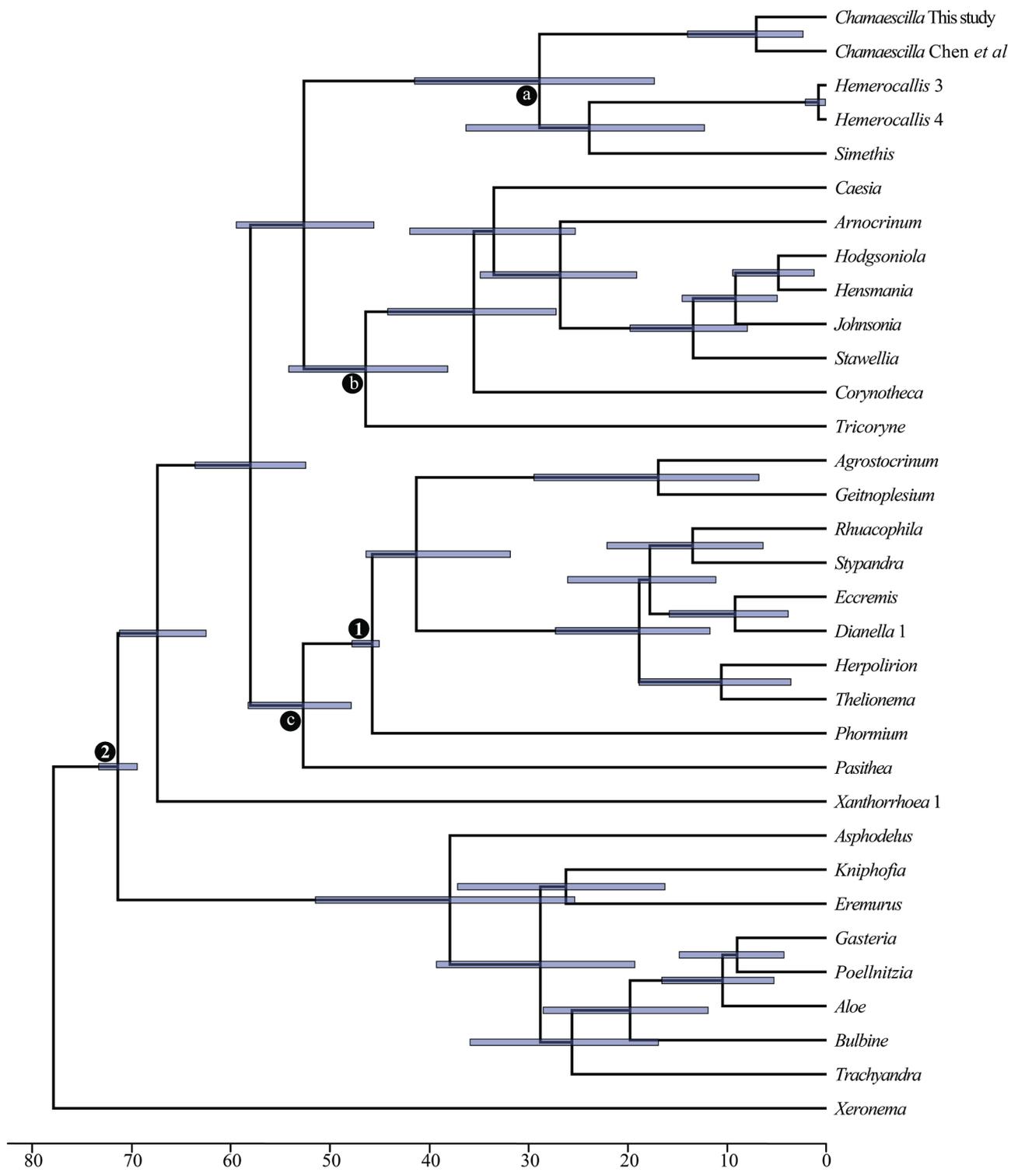
	<i>atpB</i>	<i>matK</i>	<i>ndhF</i>	<i>rbcL</i>	Combined
# of taxa	77	77	83	88	89
Aligned length (bp)	1463	1653	2077	1335	6528
Total variable/PIC	464/301	1046/713	1044/738	406/278	2959/2030
CI	0.464	0.447	0.412	0.400	0.432
RI	0.639	0.646	0.656	0.669	0.653
RC	0.297	0.289	0.272	0.268	0.282
MrModeltest	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G	

**TABLE 4.** Divergence time estimates for the Xanthorrhoeaceae and clades of subfamily Hemerocallidoideae estimated using BEAST.

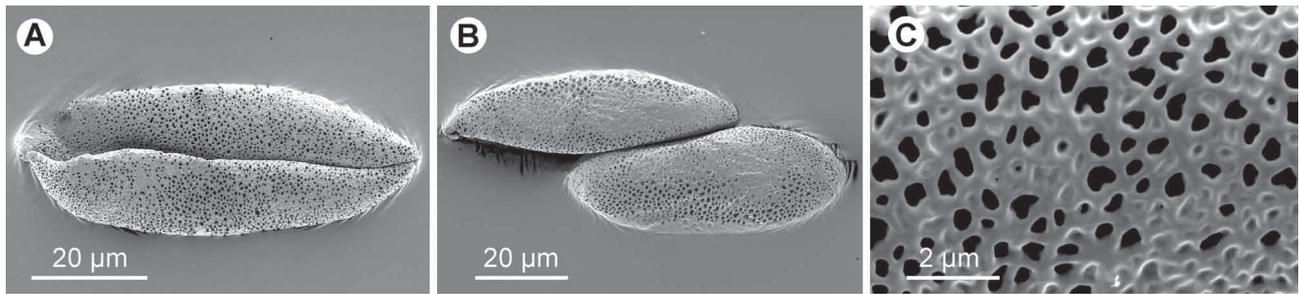
Taxon	Median (crown age)	95% HPD
Xanthorrhoeaceae	71.35	69.4–73.28
Hemerocallidoideae stem	67.09	62.46–71.18
Hemerocallidoideae crown	58	52.43–63.57
Phormioid clade crown	52.94	47.86–58.23
Johnsonoid-hemerocallid crown	52.51	45.58–59.42
Johnsonioid clade crown	46.3	38.15–54.14
Hemerocallid clade	29.11	17.34–41.48



**FIGURE 2.** Cladogram based on combined plastid dataset showing Bayesian topology and posterior probability (PP)/parsimony bootstrap support (BS). A Bayesian phylogram showing mean branch lengths is shown as an inset on the left. Relevant families and subfamilies are shown. Letters correspond to the three Hemerocallidaceae clades: a) hemerocallid, b) johnsonioid, c) phormioid.



**FIGURE 3.** Maximum clade credibility tree of Xanthorrhoeaceae with divergence times estimated. The 95% highest posterior density estimates for each clade are represented by bars. Calibration points are indicated by numbers at nodes. Numbers correspond to calibration points explained in text. Letters correspond to the three Hemerocallidaceae clades: a) hemerocallid, b) johnsonioid, c) phormioid.



**FIGURE 4.** Scanning electron micrographs of *Chamaescilla* pollen (voucher MELU M113170a). A, distal polar view; B, proximal polar view; C, surface details.

## Discussion

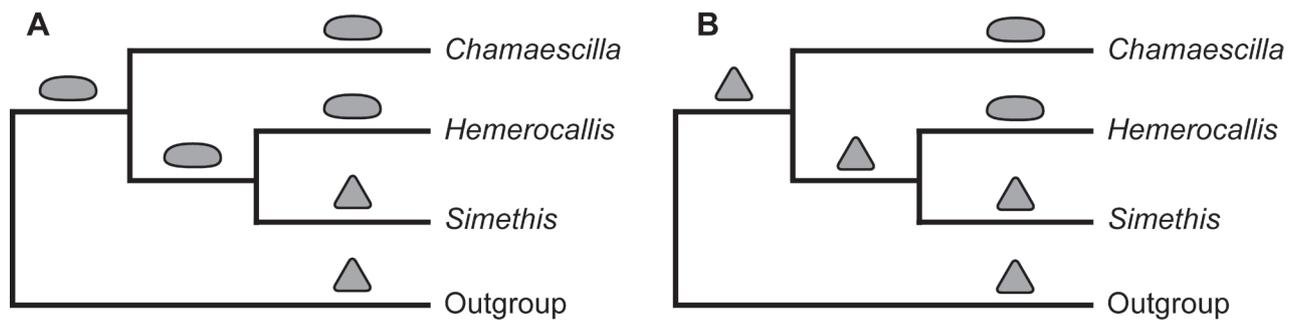
### A new familial placement of *Chamaescilla* and resolution of relationships of the genera within Hemerocallidoideae and Lomandroideae:—

As found by Chen *et al.* (2013), *Chamaescilla* is placed with strong support in Hemerocallidoideae, sister to a group formed by the western European/northern African genus *Simethis* and the Asian genus *Hemerocallis*. The three genera share a similar cymose paniculate inflorescence structure. *Chamaescilla* and *Simethis* have very similar floral morphology (Fig. 1), with undifferentiated, free tepals, characteristic of much of Asparagales. This differs to species in Lomandroideae, which tend to have more elaborate tepals that are basally united and show differentiation between whorls (Conran 1998). *Hemerocallis* has slightly zygomorphic, larger flowers than *Chamaescilla* and *Simethis*. While the petals of *Chamaescilla* are predominantly blue, white petal morphs can occasionally be found (Henderson 1987; Fig. 1B), matching the petal colour of *Simethis* (Clifford *et al.* 1998).

The *rbcL* sequence from Chase *et al.* (1995) is not available on GenBank, so it is unclear whether the position resolved for *Chamaescilla* in that study, in Lomandraceae *sensu lato* alongside *Cordyline*, was due to sample misidentification, phylogenetic inaccuracy, or contamination of DNA. Morphological studies conducted concurrently with that molecular work did not include *Chamaescilla* in comparative leaf analyses (Rudall & Chase 1996) and Chase *et al.* (1996) concluded that *Chamaescilla* differed slightly to *Cordyline* in pollen microsporogenesis. The work on microsporogenesis was based on microscope slides previously prepared by Prof. U. Hamann and his students (University of Bochum), i.e. it was likely not from the same source as the material used in the molecular study of Chase *et al.* (1995).

To test the position of *Chamaescilla*, the 14 genera of Lomandroideae were included here and this is the first time that a phylogeny of the whole group is published. The relationships within Lomandroideae are similar to those shown in the thesis of Sirisena (2010), but with the addition here of *Dichopogon* Kunth (1843: 622) as sister to *Arthropodium* Brown (1810: 276). Relationships between several Lomandroideae genera were weakly supported in our study; *Chamaexeros* Benthams (1878: 110), *Romnaldia* Stevens (1978: 148), *Acanthocarpus* Lehmann (1848: 274), *Lomandra* Labillardiere (1805: 92) and *Xerolirion* George (1986: 98, 229) form a clade, but relationships among the genera are poorly resolved. Conran (1998) suggested these taxa represent a *Lomandra* complex and all taxa may form a broader *Lomandra*. Donnon (2009) also resolved this pattern using two markers, *trnL-trnF* plastid DNA and ITS2 nuclear ribosomal DNA, from 113 samples. This complex is morphologically diverse and contains rainforest, sclerophyll (heathland) and arid species widespread in Australia and Pacific Islands (Conran 1998, Donnon 2009). Phylogenetic study of this subfamily, including the poorly resolved *Lomandra* complex, is warranted.

**Pollen shape and pollination syndrome:—***Chamaescilla* pollen is similar to *Hemerocallis* pollen, but varies slightly in that the tectum lacks both a granulated muri and pollen kit globules (Furness *et al.* 2014). Pollen within Hemerocallidoideae, including *Simethis*, is predominantly trichotomosulcate and triangular in shape in polar view, but both *Hemerocallis* and *Chamaescilla* (Figs. 4, 5) have monosulcate pollen that is oval in polar view. The monosulcate condition is widespread in Asparagales (Luo *et al.* 2015) and the trichotomosulcate condition is considered to be a synapomorphy of the Hemerocallidoideae (Furness *et al.* 2014). The topology of the phylogeny suggests either two independent changes to monosulcate pollen in *Hemerocallis* and *Chamaescilla*, or a change to monosulcate pollen some time since the divergence of the hemerocallid clade from the johnsonioid clade, with a reversion to trichomosulcate pollen in *Simethis*, as illustrated in Fig. 5.



**FIGURE 5.** Four-taxon tree showing alternative pathways of pollen evolution in the hemerocallid clade. Triangular and elliptical shapes represent trichotomosulcate and monosulcate pollen grains, respectively.

Buzz pollination is thought to be common in the Hemerocallidoideae and has been observed in several genera (Furness *et al.* 2014). Typical flower characteristics for buzz pollination include a *Solanum*-like flower shape, densely hairy filaments to enhance vibration amplitude, and a small pollen size to aid pollen release through the anther pore (Buchmann 1983; De Luca & Vallejo-Marín 2013). These features are found in most of the johnsonioid and phormioid genera, except for *Phormium*, which has large tubular flowers for bird pollination (Furness *et al.* 2014). In the hemerocallid clade, *Hemerocallis* has large pollen and slightly zygomorphic flower symmetry for pollination by butterflies or hawkmoths (Hirota *et al.* 2012), while *Simethis* retains the buzz pollination features found in the rest of the family. *Chamaescilla* lacks hairy filaments and has large pollen indicating that buzz pollination may have been lost, but blue flower colour is considered to be a feature of bee pollination (Kevan 1983). A pollination study of the orchid *Thelymitra epipactoides* Mueller (1866: 174) found multiple *Chamaescilla* pollen grains on the orchid stigma, which was pollinated by several native bees (Cropper & Calder 1990), indicating that bees serve as a vector for dispersal of *Chamaescilla* pollen. The loss of specific buzz pollination traits in *Chamaescilla* may indicate a change to a more general pollination syndrome.

**Biogeography of hemerocallid clade:**—The new phylogenetic position of *Chamaescilla* presents an interesting and disjunct distribution pattern. *Simethis* and *Hemerocallis* are the only two extant members of the Hemerocallidoideae that have a distribution outside former Gondwana. *Simethis* occurs in Western Europe and Northern Africa (Gianguzzi *et al.* 2012) and *Hemerocallis* is found from Central Europe to China and Japan (Clifford *et al.* 1998). The estimated age for the hemerocallid clade (17.34–41.48 mya) suggests a dispersal rather than vicariance as a reason for this distribution. Dispersal out of Australia is one explanation for this pattern, given the nested placement of the hemerocallid clade within related lineages concentrated in Australia (i.e. the johnsonioid clade is nearly entirely Australian, and the phormioid clade occurs in Australia, New Zealand, the Pacific Islands and parts of South America). However, alternative explanations could include a European or Asian origin for the clade.

Disjunctions between extra-tropical Australia and mainland Asia or Europe are uncommon, but not unique, e.g. distribution patterns in *Scleranthus* Linnaeus (1753a: 406; also in New Zealand, Smissen *et al.* 2003), *Austrobryonia* Schaefer in Schaefer *et al.* (2008: 126), *Bryonia* Linnaeus (1753b: 1012), *Ecballium* Richard in De Saint-Vincent (1824: 19) (Schaefer *et al.* 2008), and *Pleurosorus* Fee (1852: 179, t. 16.C; also in New Zealand and South America, Ohlsen *et al.* 2015). Australia-Asia floristic relationships tend to be between Southeast Asia and northern Australia in tropical groups. These dispersal events go in both directions and greatly increased in frequency when the Sunda and Sahul continental plates began to collide during the Miocene 25 mya (Crayn *et al.* 2015). Anti-tropical relationships (where the same or sister taxa are absent from tropical regions, but are found to the north and south) are less common, but some examples occur between temperate Australia and mainland Asia-Japan. Among these taxa, older dispersal events are inferred in Sapindaceae (30–60 mya; Buerki *et al.* 2011) and Apiaceae (46–71 mya; Calviño *et al.* 2016); more recent dispersal events are inferred in *Cucumis* Linnaeus (1753b: 1011) in Cucurbitaceae (2–10 mya; Sebastian *et al.* 2010), *Solenogyne* Cassini in Cuvier (1828: 174) in Asteraceae (0.9–4 mya; Nakamura *et al.* 2012) and within Australian *Poa* sect. *Brizoides* Pilger ex Potztl (1969: 472) (1.4–6 mya; Birch *et al.* 2014). While the lower bounds of the 95% HPD estimates for the divergence age of *Chamaescilla* and *Hemerocallis* + *Simethis* overlap with the timing of the collision of the Sahul and Sunda plates, which could be associated with a recent anti-tropical dispersal, the majority of the confidence interval surrounding the divergence suggests an older dispersal event.

The lack of well-resolved phylogenies for *Hemerocallis* and *Chamaescilla* limits interpretation of their biogeographic histories. Kim *et al.* (2012) used five plastid DNA markers to resolve relationships between Korean

Asparagales, including five *Hemerocallis* taxa, and identified *Hemerocallis minor* Miller (1768: 359) as the first diverging lineage. This species is widespread throughout Asia, from Siberia through China, Japan and Korea (eFloras 2008) and thus an ancestral area for the genus is hard to determine. A phylogeny of the four *Chamaescilla* species would not affect interpretation of the overall pattern of dispersal from Australia to Asia. However, understanding whether the earliest diverging species are from western or eastern Australia may help in identifying taxa with similar distributions and dispersal patterns.

Long distance dispersal is inferred in other Hemerocallidoideae, with *Eccremis* found in South America (as well as *Pasithea*, but this distribution could be the result of Gondwanan vicariance). Similarly *Dianella*, *Rhuacophila* and *Geitonoplesium* Cunningham ex Brown in Hooker (1832: t. 3131) are found throughout Australia and the South Pacific (Clifford *et al.* 1998) where the fleshy fruit associated with these genera could enable bird dispersal. *Chamaescilla*, *Simethis* and *Hemerocallis* have membranous capsular fruits and are less likely to be bird dispersed, making the mechanism of dispersal less obvious.

## Conclusion

Our molecular analyses support the transfer of *Chamaescilla* from Asparagaceae to Xanthorrhoeaceae subfamily Hemerocallidoideae. The transfer is from one group of largely Australian taxa to another, but the position within the hemerocallid clade facilitates new interpretations of evolutionary patterns in floral morphology and pollination biology. Furthermore, it identifies a biogeographically interesting distribution pattern between temperate Australia and mainland Asia and Europe that is worthy of further investigation.

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