Supporting Information for “Recent range expansion in Australian hummock grasses (Triodia) inferred using genotyping-by-sequencing”

BM Anderson, KR Thiele, PF Grierson, SL Krauss, PG Nevill, ID Small, X Zhong and MD Barrett

Table of Contents

Sampling........................................................................................................................................2
  Table S1. Samples used in the range expansion analyses..........................................................2
  Table S2. Chloroplast genomic samples used in divergence dating analyses..........................4
Range expansion results..............................................................................................................6
  Table S3.....................................................................................................................................6
Additional details for divergence dating analyses.....................................................................9
  MATERIALS AND METHODS.................................................................................................9
    Chloroplast genome sequencing and assembly.................................................................9
    Phylogenetic analysis.........................................................................................................11
    Divergence dating...............................................................................................................12
RESULTS......................................................................................................................................14
  Table S4....................................................................................................................................17
  Table S5....................................................................................................................................18
  Table S6....................................................................................................................................18
  Figure S1...............................................................................................................................19
  Figure S2...................................................................................................................................20
  Figure S3...................................................................................................................................21
  Figure S4...................................................................................................................................22
  Figure S5...................................................................................................................................23
SUPPORTING INFORMATION LITERATURE CITED....................................................................24
### Table S1. Samples used in the range expansion analyses.

| Sample ID       | Species                  | Voucher Collector   | Coll. #  | Locality          | Latitude      | Longitude      | Accession #     |
|-----------------|--------------------------|---------------------|----------|-------------------|---------------|---------------|-----------------|
| Base-bas-Andado05 | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 80   | Old Andado Hstd, NT | 25° 16' 29" S | 135° 24' 37" E | SAMN05942269    |
| Base-bas-Andado07 | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 80   | Old Andado Hstd, NT | 25° 16' 29" S | 135° 24' 37" E | SAMN05942270    |
| Base-bas-Andado12 | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 80   | Old Andado Hstd, NT | 25° 16' 29" S | 135° 24' 37" E | SAMN05942272    |
| Base-bas-Andado18 | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 80   | Old Andado Hstd, NT | 25° 16' 29" S | 135° 24' 37" E | SAMN05942273    |
| Base-bas-BMA53   | Triodia basedowii       | B.M. Anderson & K. Thiele | BMA 53   | Gunbarrel Hwy, WA  | 25° 42' 15.4" S | 125° 42' 47.6" E | SAMN05942274    |
| Base-bas-BMA77   | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 77   | Maryvale Stn, NT   | 24° 42' 16" S  | 134° 6' 7" E   | SAMN05942275    |
| Base-bas-Erl02   | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 81   | Eulalunda Jnctn, NT | 25° 11' 59" S | 133° 12' 0" E | SAMN05942276    |
| Base-bas-Ghan14  | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 79   | Finke, NT          | 25° 7' 37" S   | 134° 23' 40" E | SAMN05942277    |
| Base-bas-Huck03  | Triodia basedowii       | B.M. Anderson, M.D. Barrett & P. Jobson | BMA 72 | Huckitta Hstd, NT | 22° 54' 22" S | 135° 26' 43" E | SAMN05942278    |
| Base-bas-Lara06  | Triodia basedowii       | B.M. Anderson, M.D. Barrett & P. Jobson | BMA 75   | Larapinta Rd, NT   | 23° 52' 58" S  | 132° 26' 43" E | SAMN05942279    |
| Base-bas-Lara13  | Triodia basedowii       | B.M. Anderson, M.D. Barrett & P. Jobson | BMA 75   | Larapinta Rd, NT   | 23° 52' 58" S  | 132° 26' 43" E | SAMN05942280    |
| Base-bas-Lara19  | Triodia basedowii       | B.M. Anderson, M.D. Barrett & P. Jobson | BMA 75   | Larapinta Rd, NT   | 23° 52' 58" S  | 132° 26' 43" E | SAMN05942281    |
| Base-bas-Nart09  | Triodia basedowii       | B.M. Anderson, M.D. Barrett & P. Jobson | BMA 74   | Narwietooma Stn, NT | 23° 10' 53" S | 132° 39' 57" E | SAMN05942282    |
| Base-bas-Niftyb06 | Triodia basedowii       | B.M. Anderson       | BMA 37   | Nifty copper mine, WA | 21° 39' 49.7" S | 121° 33' 41.5" E | SAMN05942216    |
| Base-bas-Niftyb13 | Triodia basedowii       | B.M. Anderson       | BMA 37   | Nifty copper mine, WA | 21° 39' 49.7" S | 121° 33' 41.5" E | SAMN05942217    |
| Base-bas-Niftyb18 | Triodia basedowii       | B.M. Anderson       | BMA 37   | Nifty copper mine, WA | 21° 39' 49.7" S | 121° 33' 41.5" E | SAMN05942283    |
| Base-bas-NNew07  | Triodia basedowii       | B.M. Anderson & M.D. Barrett | BMA 31   | Roy Hill, WA       | 22° 47' 16.1" S | 120° 0' 14.3" E | SAMN05942284    |
| Base-bas-Olga13  | Triodia basedowii       | B.M. Anderson & P. Jobson | BMA 83   | W of the Olgas, NT | 25° 10' 0" S   | 130° 25' 8" E | SAMN05942285    |
| Base-bas-Roy1b03 | Triodia basedowii       | M.D. Barrett & W. Lewandrowski | MDB 3932 | E of Munjina, WA  | 22° 26' 35" S  | 118° 54' 31.1" E | SAMN05942286    |
| Base-bas-Roy1b08 | Triodia basedowii       | M.D. Barrett & W. Lewandrowski | MDB 3932 | E of Munjina, WA  | 22° 26' 35" S  | 118° 54' 31.1" E | SAMN05942288    |
| Base-bas-Roy1b12 | Triodia basedowii       | M.D. Barrett & W. Lewandrowski | MDB 3932 | E of Munjina, WA  | 22° 26' 35" S  | 118° 54' 31.1" E | SAMN05942289    |
| Base-bas-Roy1b15 | Triodia basedowii       | M.D. Barrett & W. Lewandrowski | MDB 3932 | E of Munjina, WA  | 22° 26' 35" S  | 118° 54' 31.1" E | SAMN05942290    |
| Base-bas-Roy1b20 | Triodia basedowii       | M.D. Barrett & W. Lewandrowski | MDB 3932 | E of Munjina, WA  | 22° 26' 35" S  | 118° 54' 31.1" E | SAMN05942291    |
| Base-bas-Stirling12 | Triodia basedowii     | M.D. Barrett, B.M. Anderson & P. Jobson | MDB 4520 | Stirling Hstd, NT | 21° 39' 38" S | 133° 44' 47" E | SAMN05942292    |
| Base-basswool-BMA41 | Triodia basedowii     | B.M. Anderson & K. Thiele | BMA 41   | E of Wiluna, WA     | 26° 39' 11.5" S | 121° 6' 57.7" E | SAMN05942299    |
| Base-basswool-Katb03 | Triodia basedowii     | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langlely & K. Brown | BMA 66   | Carnarvon Range, WA | 25° 2' 59" S | 120° 44' 5.3" E | SAMN05942300    |
| Base-basswool-Katb07 | Triodia basedowii     | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langlely & K. Brown | BMA 66   | Carnarvon Range, WA | 25° 2' 59" S | 120° 44' 5.3" E | SAMN05942301    |
| Base-basswool-Katb13 | Triodia basedowii     | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langlely & K. Brown | BMA 66   | Carnarvon Range, WA | 25° 2' 59" S | 120° 44' 5.3" E | SAMN05942302    |
| Sample ID         | Species               | Voucher Collector                                      | Coll. # | Locality             | Latitude       | Longitude       | Accession #    |
|------------------|-----------------------|--------------------------------------------------------|---------|----------------------|----------------|----------------|----------------|
| Base-basswool-Katb19 | Triodia basedowii     | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langley & K. Brown | BMA 66  | Carnarvon Range, WA  | 25° 2' 59" S  | 120° 44' 5.3" E | SAMN05942303  |
| Base-basswool-Leinster07 | Triodia basedowii     | B.M. Anderson, R. Davis & T. Hammer                       | BMA 4   | Leister, WA           | 28° 5' 16.6" S | 120° 53' 34" E | SAMN05942304  |
| Base-bas-TB1-02    | Triodia basedowii     | T.E. Erickson                                             | TEE 771 | Capricorn Roadhouse, WA | 23° 33' 13.9" S | 119° 45' 35.1" E | SAMN05942293  |
| Base-bas-TB1-09    | Triodia basedowii     | T.E. Erickson                                             | TEE 771 | Capricorn Roadhouse, WA | 23° 33' 13.9" S | 119° 45' 35.1" E | SAMN05942294  |
| Base-bas-TB1-13    | Triodia basedowii     | T.E. Erickson                                             | TEE 771 | Capricorn Roadhouse, WA | 23° 33' 13.9" S | 119° 45' 35.1" E | SAMN05942295  |
| Base-bas-TB1-17    | Triodia basedowii     | T.E. Erickson                                             | TEE 771 | Capricorn Roadhouse, WA | 23° 30' 2.1" S  | 119° 46' 56.5" E | SAMN05942296  |
| Base-bas-Tjuk14    | Triodia basedowii     | B.M. Anderson & K. Thiele                                 | BMA 58  | S of Tjukayirla, WA   | 27° 49' 28.3" S | 123° 56' 50.1" E | SAMN05942297  |
| Base-bas-Tjuk17    | Triodia basedowii     | B.M. Anderson & K. Thiele                                 | BMA 58  | S of Tjukayirla, WA   | 27° 49' 28.3" S | 123° 56' 50.1" E | SAMN05942298  |
| Base-isandy-Kat10  | Triodia birriliburu   | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langley & K. Brown | BMA 62  | Carnarvon Range, WA  | 25° 2' 47.8" S | 120° 44' 25.7" E | SAMN05942315  |
| Base-isandy-Kat15  | Triodia birriliburu   | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langley & K. Brown | BMA 62  | Carnarvon Range, WA  | 25° 2' 47.8" S | 120° 44' 25.7" E | SAMN05942316  |
| Base-isandy-Kat16  | Triodia birriliburu   | B.M. Anderson, S. van Leeuwen, N. Gibson, M. Langley & K. Brown | BMA 62  | Carnarvon Range, WA  | 25° 2' 47.8" S | 120° 44' 25.7" E | SAMN05942317  |
| Base-nana-Gibson02 | Triodia nana           | B.M. Anderson & K. Thiele                                 | BMA 49  | Gunbarrel Hwy, WA     | 25° 21' 13.7" S | 124° 26' 46.2" E | SAMN05942318  |
| Base-nana-Gibson05 | Triodia nana           | B.M. Anderson & K. Thiele                                 | BMA 49  | Gunbarrel Hwy, WA     | 25° 21' 13.7" S | 124° 26' 46.2" E | SAMN05942319  |
| Base-nana-Gibson15 | Triodia nana           | B.M. Anderson & K. Thiele                                 | BMA 49  | Gunbarrel Hwy, WA     | 25° 21' 13.7" S | 124° 26' 46.2" E | SAMN05942320  |
| Base-nana-Gibson19 | Triodia nana           | B.M. Anderson & K. Thiele                                 | BMA 49  | Gunbarrel Hwy, WA     | 25° 21' 13.7" S | 124° 26' 46.2" E | SAMN05942321  |
| Base-peed-Stew11   | Triodia glabra         | M.D. Barrett & W. Lewandrowski                            | MDB 3978 | W of Paraburdoo, WA   | 22° 25' 47.6" S | 115° 56' 35.5" E | SAMN05942242  |
| Base-peed-Stew13   | Triodia glabra         | M.D. Barrett & W. Lewandrowski                            | MDB 3978 | W of Paraburdoo, WA   | 22° 25' 47.6" S | 115° 56' 35.5" E | SAMN05942243  |
| Base-peed-Uaroo01   | Triodia glabra         | M.D. Barrett & B.M. Anderson                              | MDB 4120 | SW of Nanutarra, WA   | 22° 46' 11" S  | 115° 6' 37" E  | SAMN05942322  |
| Base-peed-Uaroo03   | Triodia glabra         | M.D. Barrett & B.M. Anderson                              | MDB 4120 | SW of Nanutarra, WA   | 22° 46' 11" S  | 115° 6' 37" E  | SAMN05942323  |
| Base-peed-Uaroo05   | Triodia glabra         | M.D. Barrett & B.M. Anderson                              | MDB 4120 | SW of Nanutarra, WA   | 22° 46' 11" S  | 115° 6' 37" E  | SAMN05942324  |
Table S2. Chloroplast genomic samples used in divergence dating analyses.

| Subfamily        | Species                     | Source      | Data set 1 | Data set 2 | Genbank accession | Herb.     | Barcode | Voucher Collector | Coll. #      | Locality              | Latitude       | Longitude       |
|------------------|-----------------------------|-------------|------------|------------|-------------------|-----------|---------|-------------------|--------------|-----------------------|----------------|-------------------|
| Anomochloideae   | Anomochloa marantoidea      | Download    | yes        |            | NC_014062         |           |         |                   |              |                       |                 |                  |
| Aristidoideae    | Sardidia perrieri           | Download    | yes        |            | KJ819549         |           |         |                   |              |                       |                 |                  |
| Arundinoideae    | Monachather paradoxus       | Download    | yes        |            | KJ920235         |           |         |                   |              |                       |                 |                  |
| Arundinoideae    | Phragmites australis        | Download    | yes        |            | KJ825856         |           |         |                   |              |                       |                 |                  |
| Bambusoideae     | Arundinaria appalachiana    | Download    | yes        |            | KC817462         |           |         |                   |              |                       |                 |                  |
| Bambusoideae     | Bambusa oldhamii            | Download    | yes        |            | NC_012927        |           |         |                   |              |                       |                 |                  |
| Bambusoideae     | Olyra latifolia             | Download    | yes        |            | KF515509         |           |         |                   |              |                       |                 |                  |
| Chloridoideae    | Neyraudia reynaudiana       | Download    | yes        | yes        | KF356392         |           |         |                   |              |                       |                 |                  |
| Chloridoideae    | Sporobolus maritimus        | Download    | yes        | yes        | KP176438         |           |         |                   |              |                       |                 |                  |
| Chloridoideae    | Zoysia macrantha            | Download    | yay        |            | KT168390         |           |         |                   |              |                       |                 |                  |
| Danthonioideae   | Chionochloa macra           | Download    | yes        | yes        | KJ920227         |           |         |                   |              |                       |                 |                  |
| Danthonioideae   | Danthonia californica       | Download    | yes        | yes        | KJ920229         |           |         |                   |              |                       |                 |                  |
| Micrairoideae    | Isachne distichophylla      | Download    | yes        |            | KJ920233         |           |         |                   |              |                       |                 |                  |
| Micrairoideae    | Micraira sp. JLC-2014       | Download    | yes        |            | KJ920234         |           |         |                   |              |                       |                 |                  |
| Oryzoideae       | Chikusichloa aquatica       | Download    | yes        |            | NC_027184        |           |         |                   |              |                       |                 |                  |
| Oryzoideae       | Microlaena stipoides        | Download    | yes        |            | GU592211         |           |         |                   |              |                       |                 |                  |
| Oryzoideae       | Oryza sativa Japonica Group | Download    | yes        |            | NC_001320        |           |         |                   |              |                       |                 |                  |
| Oryzoideae       | Zizania latifolia           | Download    | yes        |            | KT161956         |           |         |                   |              |                       |                 |                  |
| Panicoideae      | Centotheca lappacea         | Download    | yes        |            | KJ920225         |           |         |                   |              |                       |                 |                  |
| Panicoideae      | Setaria viridis             | Download    | yes        |            | KT289405         |           |         |                   |              |                       |                 |                  |
| Panicoideae      | Sorghum bicolor             | Download    | yes        |            | NC_008602        |           |         |                   |              |                       |                 |                  |
| Pharoideae       | Pharus latifolius           | Download    | yes        |            | JN032131         |           |         |                   |              |                       |                 |                  |
| Pooideae         | Brachyelytrum aristosum     | Download    | yes        |            | KM974735         |           |         |                   |              |                       |                 |                  |
| Pooideae         | Poa palustris               | Download    | yes        |            | KM974749         |           |         |                   |              |                       |                 |                  |
| Pooideae         | Stipa hymenoides            | Download    | yes        |            | KM974729         |           |         |                   |              |                       |                 |                  |
| Puelioideae      | Puelia olyriformis          | Download    | yes        |            | KC534841         |           |         |                   |              |                       |                 |                  |
| Aristidoideae    | Aristida pruinosa           | New         | yes        |            | MK590083         | PERTH     | 8446334 | S. Dillon & A. Markey | CR 9260     | Cane River Conservation Park, WA | 21° 59' 59.3" S | 115° 51' 40.3" E |
| Micrairoideae    | Eriachne tenuiculmis        | New         | yes        |            | MK590078         | PERTH     | 8162107 | B. Cook            | C 27-04     | Coordinator Mining tenement, WA | 22° 58' 22" S  | 119° 36' 30" E   |
| Chloridoideae    | Aeluropus lagopoides        | New         | yes        |            | MK622397         | E         | E00358268 | M. S. Trudgen & P. Hoffmann | ESS PH 116  | NW of Tom Price, WA | 22° 17' 10.6" S | 117° 14' 39.9" E |
| Chloridoideae    | Astrebla lappacea           | New         | yes        |            | MK590086         | PERTH     | 8353697 | V. Goloskokov      | s.n.        | Kazakhstan            |                 |                  |
| Chloridoideae    | Cleistogenes sp.            | New         | yes        |            | MK622398         | E         | E00639685 | B. Vincent & N. Krawczyk | BV 141      | WNW of Paraburdo, WA | 22° 54' 50.6" S | 117° 7' 9.7" E   |
| Chloridoideae    | Dactyloctenium radulans     | New         | yes        |            | MK590085         | PERTH     | 8377332 |                         |             |                       |                 |                  |
| Subfamily     | Species             | Source          | Data set 1 | Data set 2 | Genbank accession | Herb.      | Barcode            | Voucher Collector        | Coll. # | Locality                      | Latitude | Longitude |
|---------------|---------------------|-----------------|------------|------------|-------------------|------------|-------------------|--------------------------|---------|-------------------------------|----------|-----------|
| Chloridoideae | Enneapogon caerulescens | New             | yes        | yes        | MK590084         | PERTH      | 8127085          | V. Long                   | VL 1494 39 | E of Burring Road, WA         | 20° 38' 18.3" S | 116° 46' 9" E |
| Chloridoideae | Enteropogon ramosus  | New             | yes        | yes        | MK590081         | PERTH      | 8446393          | S. Dillon & A. Markev     | CR 9267 | Cane River Conservation Park, WA | 22° 13' 11.7" S | 115° 27' 19.2" E |
| Chloridoideae | Eragrostis setifolia | New             | yes        | yes        | MK590079         | PERTH      | 6437788          | S. van Leeuwen            | 4683    | Coondewanna Flats, WA          | 23° 1' 15.2" S | 118° 45' 24.9" E |
| Chloridoideae | Eragrostis tenellula | New             | yes        | yes        | MK590080         | PERTH      | 8377391          | J. Atkinson                | JA 95    | WNW of Paraburadoo, WA         | 22° 25' 28.2" S | 116° 18' 41.5" E |
| Chloridoideae | Orinus kokononica   | New             | yes        | yes        | MK622399         | PERTH      | E0006427         | Sino-American-British Yushu Expedition (1996) | 2759 | China                          | 32° 17' N   | 96° 28' E |
| Chloridoideae | Tragus australianus | New             | yes        | yes        | MK590077         | PERTH      | 8446253          | S. Dillon & A. Markev     | CR 9282 | Cane River Conservation Park, WA | 22° 10' 11.8" S | 115° 33' 39.6" E |
| Chloridoideae | Triodia basedowii   | New             | yes        | yes        | MK622400         | PERTH      | 8618860          | M.D. Barrett & B.M. Anderson | MDB 4127 | Munjina, WA                   | 22° 20' 17.8" S | 118° 35' 46.5" E |
| Chloridoideae | Triodia chichesterensis | New             | yes        | yes        | MK622401         | PERTH      | 8618852          | M.D. Barrett & B.M. Anderson | MDB 4102 | Boodarie, WA                   | 20° 41' 15" S | 118° 41' 11.8" E |
| Chloridoideae | Triodia concinna    | New             | yes        | yes        | MK622402         | PERTH      | -                | B.M. Anderson & K. Thieke | BMA 45 | E of Carnegie Hstd, WA         | 25° 25' 38.4" S | 123° 46' 20.1" E |
| Chloridoideae | Triodia glabra      | New             | yes        | yes        | MK622403         | PERTH      | 8618968          | M.D. Barrett & W. Lewandowski | MDB 3978 | Mt Stuart, WA                   | 22° 25' 48.1" S | 115° 56' 19.1" E |
| Chloridoideae | Triodia lanigera    | New             | yes        | yes        | MK622413         | PERTH      | 8618895          | M.D. Barrett & B.M. Anderson | MDB 4099 | Marble Bar, WA                  | 20° 39' 54.1" S | 119° 13' 13" E |
| Chloridoideae | Triodia longicaps   | New             | yes        | yes        | MK622404         | PERTH      | 8618925          | M.D. Barrett & B.M. Anderson | MDB 4103 | Boodarie, WA                   | 20° 44' 24.9" S | 118° 40' 57.2" E |
| Chloridoideae | Triodia mallota     | New             | yes        | yes        | MK622405         | PERTH      | 8618909          | B.M. Anderson & M.D. Barrett | BMA 89 | Pannawonica, WA                | 21° 45' S   | 116° 18' E |
| Chloridoideae | Triodia plurinervata| New             | yes        | yes        | MK622406         | PERTH      | -                | C.E. Mayence              | s.n. | Shark Bay, WA                   | 26° 10' 14" S | 113° 23' 25.2" E |
| Chloridoideae | Triodia rigidissima | New             | yes        | yes        | MK622407         | PERTH      | 8957649          | M.D. Barrett & B.M. Anderson | MDB 3994 | Yellowine, WA                   | 31° 15' 28.5" S | 120° 2' 25.7" E |
| Chloridoideae | Triodia schinzii    | New             | yes        | yes        | MK622410         | PERTH      | 8618992          | M.D. Barrett & B.M. Anderson | MDB 4375 | Broome, WA                      | 17° 58' 30.4" S | 122° 10' 50.3" E |
| Chloridoideae | Triodia scintillans | New             | yes        | yes        | MK622411         | PERTH      | 8618887          | M.D. Barrett & W. Lewandowski | MDB 3944 | Kulbee Ck, WA                   | 22° 23' 13.5" S | 119° 58' 31.5" E |
| Chloridoideae | Triodia torrentosa  | New             | yes        | yes        | MK622408         | PERTH      | -                | M.D. Barrett & B.M. Anderson | MDB 3990 | Yellowine, WA                   | 31° 17' 35.1" S | 119° 39' 6.9" E |
| Chloridoideae | Triodia vanleeuwenii| New             | yes        | yes        | MK622409         | PERTH      | 8618879          | M.D. Barrett & W. Lewandowski | MDB 3969 | Karijini, WA                    | 22° 34' 28.5" S | 118° 12' 54.1" E |
| Chloridoideae | Triodia wiseana     | New             | yes        | yes        | MK622412         | PERTH      | 8618763          | M.D. Barrett & B.M. Anderson | MDB 4086 | Telfer Mine Road, WA           | 21° 18' 10" S | 121° 12' 1" E |
| Chloridoideae | Tripogon loliiformis| New             | yes        | yes        | MK590087         | PERTH      | 8415153          | K.R. Thiele                | 3969    | Pianto Road, WA                 | 29° 54' 6" S  | 121° 16' 37" E |
| Chloridoideae | Triraphis mollis    | New             | yes        | yes        | MK590082         | PERTH      | 7851472          | R. Orifici                 | 89     | SSW of Mt Brockman, WA          | 22° 33' 46.5" S | 117° 14' 24.7" E |
Range expansion results

Table S3.
Results from running the range expansion analyses for variations in filtering SNPs and samples. SNP states were polarized using different outgroups: *Triodia birriliburu, T. nana,* and *T. glabra*. Loci sets were (1) assembled, (2) unassembled, or (3) a combination of the two. SNPs were chosen (i) randomly or (ii) with a bias toward SNPs with multiple copies of the rare allele. Sampling subsets are I: one per population (random), II: one per population (alternative choice), III: one per population excluding tetraploids, IV: all samples (populations downsampled), and V: all samples excluding tetraploids (populations downsampled). The number of polarized SNPs indicates how many remain after the scripts remove those that have an ambiguous ancestral state. Geographic sample subsets are "all", "west", "eastern" and "intermediate", and the signal for expansion was deemed significant if P < 0.01. NT is the Northern Territory, SA is South Australia, and QLD is Queensland.

| Outgroup | SNP set | SNP choice | Sampling | Samples | polarized SNPs | Sig Reg | P-value  | Location   | Eastern origin? |
|----------|---------|------------|----------|---------|----------------|---------|----------|------------|-----------------|
| *T. birriliburu* | 1       | i          | I        | 18      | 2353           | all     | 6.53E-09 | SE NT      | Y               |
|          |         |            | II       | 18      | 2395           |         |          |            |                 |
|          |         |            | III      | 15      | 2101           | all     | 7.64E-12 | S NT       | Y               |
|          |         |            | IV       | 36      | 3409           | all     | 7.99E-17 | S NT       | Y               |
|          |         |            | V        | 27      | 2913           | all     | 1.18E-19 | NE SA      | Y               |
|          | ii      | I          | I        | 18      | 2397           | all     | 8.74E-03 | N NT       | Y               |
|          |         |            | II       | 18      | 2393           | all     | 1.06E-06 | S NT       | Y               |
|          |         |            | III      | 15      | 2136           |         |          |            |                 |
|          |         |            | IV       | 36      | 3387           | all     | 7.04E-17 | S NT       | Y               |
|          |         |            | V        | 27      | 2872           | all     | 7.83E-19 | SE NT      | Y               |
|          | 2       | i          | I        | 18      | 2902           | all     | 1.14E-05 | NW QLD     | Y               |
|          |         |            | II       | 18      | 2958           |         |          |            |                 |
|          |         |            | III      | 15      | 2570           | all     | 1.59E-06 | NE NT      | Y               |
|          |         |            | IV       | 36      | 4193           | all     | 1.16E-11 | NE SA      | Y               |
|          |         |            | V        | 27      | 3460           | all     | 7.87E-10 | W QLD      | Y               |
|          | ii      | I          | I        | 18      | 2978           | all     | 1.08E-04 | NW QLD     | Y               |
|          |         |            | II       | 18      | 2991           | all     | 8.07E-07 | NW QLD     | Y               |
|          |         |            | III      | 15      | 2631           | all     | 5.02E-05 | NW QLD     | Y               |
|          |         |            | IV       | 36      | 4201           | all     | 7.89E-14 | E NT       | Y               |
|          |         |            | V        | 27      | 3515           | all     | 2.30E-13 | W QLD      | Y               |
|          | 3       | i          | I        | 18      | 5255           | all     | 3.58E-08 | W QLD      | Y               |
|          |         |            | II       | 18      | 5353           | all     | 9.57E-03 | NW QLD     | Y               |
|          |         |            | III      | 15      | 4671           | all     | 3.92E-11 | SE NT      | Y               |
| Outgroup | SNP set | SNP choice | Sampling | Samples | polarized SNPs | Sig Reg | P-value | Location | Eastern origin? |
|----------|---------|------------|----------|---------|---------------|---------|---------|-----------|----------------|
| IV       | 36      | 7602       | all      | 4.06E-15 | S NT          | Y       |
| V        | 27      | 6373       | all      | 9.91E-16 | NE SA         | Y       |
|          | ii      | I          | 18       | 5375    | 4.35E-06      | NE NT   | Y       |
|          |         | II         | 18       | 5384    | 1.01E-08      | NW QLD  | Y       |
|          | III     | 15         | 4767     | 3.04E-06 | NW QLD        | Y       |
|          | IV      | 36         | 7588     | 3.62E-17 | S NT          | Y       |
|          | V       | 27         | 6387     | 4.75E-18 | W QLD         | Y       |
| T. nana  | 1       | i          | I        | 18      | 2326          | all     | 2.50E-10 | W QLD     | Y       |
|          |         | II         | 18       | 2332    | 2.75E-09      | NW QLD  | Y       |
|          |         | III        | 15       | 2075    | 1.89E-12      | NW QLD  | Y       |
|          |         | IV         | 36       | 3251    | 1.97E-19      | S NT    | Y       |
|          | V       | 27         | 2737     | 3.13E-20 | W QLD         | Y       |
|          | 1       | ii         | I        | 18      | 2323          | -       |         |           |         |
|          |         | II         | 18       | 2337    | 2.15E-07      | S NT    | Y       |
|          |         | III        | 15       | 2055    | 1.15E-05      | E NT    | Y       |
|          |         | IV         | 36       | 3282    | 1.64E-13      | E NT    | Y       |
|          | V       | 27         | 2758     | 7.46E-20 | W QLD         | Y       |
|          | 2       | i          | I        | 18      | 2830          | -       |         |           |         |
|          |         | II         | 18       | 2848    | 2.27E-03      | NE NT   | Y       |
|          |         | III        | 15       | 2494    | -             |         |         |           |         |
|          |         | IV         | 36       | 3917    | 6.89E-12      | NW QLD  | Y       |
|          |         | V          | 27       | 3229    | 1.59E-12      | NW QLD  | Y       |
|          | 2       | ii         | I        | 18      | 2761          | eastern | 1.97E-04 | S NT      | N       |
|          |         | II         | 18       | 2802    | -             |         |         |           |         |
|          |         | III        | 15       | 2396    | -             |         |         |           |         |
|          |         | IV         | 36       | 3876    | 1.55E-16      | E NT    | Y       |
|          |         | V          | 27       | 3198    | 3.04E-21      | W QLD   | Y       |
|          | 3       | i          | I        | 18      | 5156          | all     | 1.64E-08 | NW QLD    | Y       |
|          |         | II         | 18       | 5180    | 2.07E-09      | NW QLD  | Y       |
|          |         | III        | 15       | 4569    | 3.25E-11      | NE NT   | Y       |
|          |         | IV         | 36       | 7168    | 5.30E-18      | E NT    | Y       |
|          |         | V          | 27       | 5966    | 8.63E-20      | W QLD   | Y       |
|          | 3       | ii         | I        | 18      | 5084          | -       |         |           |         |
|          |         | II         | 18       | 5139    | 4.14E-08      | NW QLD  | Y       |
|          |         | III        | 15       | 4451    | 2.81E-05      | NW QLD  | Y       |
|          |         | IV         | 36       | 7158    | 2.16E-16      | E NT    | Y       |
|          |         | V          | 27       | 5956    | 2.62E-24      | W QLD   | Y       |
| Outgroup | SNP set | SNP choice | Sampling | Samples | polarized SNPs | Sig Reg | P-value | Location | Eastern origin? |
|----------|---------|------------|----------|---------|---------------|---------|---------|----------|----------------|
| T. glabra | 1       | i          | I        | 18      | 2099          | all     | 2.27E-03 | S NT     | Y              |
|           | II      |            | 18       | 2145    | all           | 9.58E-04 | S NT     | Y        |                |
|           | III     | 15         | 1883     | all     | 8.38E-04      | S NT     | Y        |          |                |
|           | IV      | 36         | 2973     | all     | 3.72E-11      | S NT     | Y        |          |                |
|           | V       | 27         | 2553     | all     | 1.83E-11      | S NT     | Y        |          |                |
| 1        | ii      | I          | 18       | 2144    | -             |          |          |          |                |
|           | II      | 18         | 2170     | -       |               |          |          |          |                |
|           | III     | 15         | 1928     | -       |               |          |          |          |                |
|           | IV      | 36         | 2966     | all     | 3.59E-13      | S NT     | Y        |          |                |
|           | V       | 27         | 2553     | all     | 6.50E-13      | SE NT    | Y        |          |                |
| 2        | i       | I          | 18       | 2727    | -             |          |          |          |                |
|           | II      | 18         | 2686     | -       |               |          |          |          |                |
|           | III     | 15         | 2424     | all     | 9.38E-07      | central NT | Y        |          |                |
|           | IV      | 36         | 3714     | all     | 4.94E-09      | E NT     | Y        |          |                |
|           | V       | 27         | 3136     | all     | 8.64E-12      | NW QLD   | Y        |          |                |
| 2        | ii      | I          | 18       | 2759    | -             |          |          |          |                |
|           | II      | 18         | 2767     | -       |               |          |          |          |                |
|           | III     | 15         | 2464     | all     | 1.32E-03      | S NT     | Y        |          |                |
|           | IV      | 36         | 3706     | all     | 1.48E-06      | S NT     | Y        |          |                |
|           | V       | 27         | 3149     | all     | 9.37E-09      | W QLD    | Y        |          |                |
| 3        | i       | I          | 18       | 4826    | all           | 5.31E-04 | NW NT    | Y        |                |
|           | II      | 18         | 4831     | all     | 1.27E-02      | NW QLD   | Y        |          |                |
|           | III     | 15         | 4307     | all     | 2.60E-08      | central NT | Y        |          |                |
|           | IV      | 36         | 6687     | all     | 5.70E-12      | S NT     | Y        |          |                |
|           | V       | 27         | 5689     | all     | 3.54E-15      | W QLD    | Y        |          |                |
| 3        | ii      | I          | 18       | 4903    | -             |          |          |          |                |
|           | II      | 18         | 4937     | -       |               |          |          |          |                |
|           | III     | 15         | 4392     | all     | 4.15E-04      | S NT     | Y        |          |                |
|           | IV      | 36         | 6672     | all     | 1.53E-11      | S NT     | Y        |          |                |
|           | V       | 27         | 5702     | all     | 1.15E-13      | NE SA    | Y        |          |                |
Additional details for divergence dating analyses

MATERIALS AND METHODS

Chloroplast genome sequencing and assembly

For divergence time estimation across Poaceae, we obtained chloroplast genomes for members of all subfamilies, 26 downloaded from GenBank and 13 newly sequenced and assembled (Table S2). We aimed to include two or three genomes from each subfamily, four for Oryzoideae and 14 for Chloridoideae, but were only able to obtain one representative from each of Anomochloideae, Pharoideae and Puelioideae. Sample classification and subfamilial taxonomy follows Soreng et al. (2015). For a second analysis with denser sampling of Chloridoideae and Triodia, we included an additional ten Triodia and five other Chloridoideae genomes newly sequenced and assembled.

Silica-dried leaf material was ground in liquid nitrogen and genomic DNA extracted using a CTAB method (Doyle and Dickson 1987) as described in Anderson et al. (2016) but with RNAse added prior to heating. DNA was also extracted from herbarium samples using the commercial DNeasy Plant Mini Kit (Qiagen, USA) following manufacturer’s instructions, with DNA eluted in 100 μl of AE buffer. DNA concentration was quantified on a NanoDrop ND-1000 spectrophotometer and samples with concentrations of 3–20 ng/μl sent to the Australian Genome Research Facility node in Melbourne, Victoria. 200 ng of DNA was sheared in a volume of 50 μl using a Coavris E220 Focused ultrasonicator. Following shearing, sequencing libraries were prepared using Illumina’s TruSeq Nano DNA Library preparation kit (350 bp median insert) following the manufacturer’s protocol. Libraries were assessed by gel electrophoresis (Agilent D1000 ScreenTape Assay) and quantified by qPCR (KAPA Library Quantification Kits for Illumina). Sequencing was performed on the Illumina
HiSeq 2500 system with 2×125 bp paired-end reads using the HiSeq PE Cluster Kit v. 5 and HiSeq SBS Kit v. 4 (250 cycles).

For 19 genomes, adapter sequences (“AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC” and “AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT”) in raw paired-end reads were removed using the software CUTADAPT v. 1.9.1 (Martin 2011). Sequencing read errors were corrected with SPAdes v. 3.6.1 (Bankevich et al. 2012). The filtered reads were normalised for sequencing depth based on k-mer counts and merged into single reads using BBNorm and BBMerge, respectively, from the BBMap package v. 35.82 (https://sourceforge.net/projects/bbmap/ ; visited June 2016). The processed reads were assembled into contigs using Velvet v. 1.2.09 (Zerbino and Birney 2008) with k-mer values 51, 71, 91 and 111, and coverage cut-off values 7, 10, 15 and 20. Assembled contigs were aligned to the chloroplast genome of *Spinacia oleracea* (GenBank accession NC_002202) using MUMmer v. 3.1 (Kurtz et al. 2004). Based on the alignments, assembled chloroplast contigs were identified, ordered and then merged into a single circular draft genome for each specimen using a custom script. Reads were mapped back to the assembly using BWA v. 0.7.5a-r405 (Li and Durbin 2009) and the assembly further refined with Pilon v. 1.16 (Walker et al. 2014). The *Setaria viridis* genome (GenBank accession NC_028075) was used as a reference for gene annotation.

One chloroplast genome (*T. vanleeuwenii*) was sequenced using 454 shotgun sequencing by M. Gardner, using the method outlined in Gardner et al. (2011), and assembled by M. Duvall, S. Burke and W. Wysocki, (Northern Illinois University) using plastome assembly and verification methods outlined in Wysocki et al. (2014) and Duvall et al. (2016).

The remaining eight genomes were assembled using a beta-test version of the de novo assembler NOVOPlasty (available at: https://github.com/ndierckx/NOVOPlasty, verified...
August 2017) (Dierckxsens et al. 2017), using various conserved seed sequences where necessary. Genomes were validated by mapping raw reads onto the assembly in Geneious v. 6.1.8 (http://www.geneious.com, Kearse et al. 2012) to check for ambiguously aligned regions. The NOVOPlasty algorithm was applied to three of the genomes assembled using the first method above, to demonstrate its utility in Triodia. The three genomes assembled using the two methods differed in, at most, a few heterozygous/homozygous calls and/or the number of units in repeat regions.

**Phylogenetic analysis**

We created two datasets for phylogenetic and divergence dating analyses: (1) "all grasses" (39 samples from across Poaceae with focused sampling in Chloridoideae), and (2) "chloridoids" (29 samples of Chloridoideae with focused sampling in Triodia plus two samples of Danthonioideae as an outgroup). Dataset 1 comprised only chloroplast coding regions, while dataset 2 included coding regions as well as introns and intergenic spacers. Whole chloroplast genomes were aligned in Geneious v. 6.1.8 (http://www.geneious.com, Kearse et al. 2012) using the MAFFT (Katoh and Standley 2013) plugin with gap creation 2.0 and gap extension 0.12. Target regions were manually extracted from the alignment and checked by eye for alignment ambiguities, which were removed or adjusted. A custom Python v. 2.7.2 (Python Software Foundation 2016) script was used to remove positions in the alignment with more than one missing or 'N' state.

To determine the optimum partitioning and model scheme, the loci for each dataset were run through PartitionFinder v. 2.0.0-pre13 (Lanfear et al. 2012), using models available for MrBayes, the Bayesian information criterion to select the optimal model, and a greedy search. The optimal partitioning scheme (see Table S4) was used in phylogenetic inference with RAxML v. 8.1.21 (Stamatakis 2014), which implements the GTRGAMMA model for all
partitions. RAxML was run with a 100 replicate rapid bootstrap (Stamatakis et al. 2008) followed by a search for the maximum likelihood tree (‘-f a’ option). We also ran the partitions and best models under Bayesian inference in MrBayes v. 3.2.6 (Ronquist et al. 2012). MrBayes was executed for three runs, each using four chains and two million generations, sampling every 500, with 10% of samples discarded as burn-in. Trees from each run were combined into a single file with a custom script, and a maximum clade credibility tree constructed using TreeAnnotator v. 2.4.0 (Bouckaert et al. 2014) and visualized in FigTree v. 1.4.3 (Rambaut 2016).

**Divergence dating**

We included five fossils and a root prior (Table S5) in Bayesian analyses of dataset 1 (all grasses) in BEAST v. 2.4.6 (Bouckaert et al. 2014). The placement of the Prasad et al. (2005, 2011) phytoliths is somewhat controversial (see Christin et al. 2014), but has been set at crown Oryzoideae in a recent dating analysis of grasses (Burke et al. 2016), consistent with recently discovered fossils of a spikelet from c. 100 Ma (Poinar et al. 2015). We report our results with the phytoliths at stem Oryzeae (common ancestor of *Oryza* and *Microlaena* in our tree), but we also ran analyses with them placed conservatively at stem BOP+PACMAD (Bambusoideae, Oryzoideae, Pooideae + Panicoideae, Aristidoideae, Chloridoideae, Micrairoideae, Arundinoideae, and Danthonioideae). In setting the prior probability distributions, maximum ages were arbitrarily chosen as 1.5 times the minimum age except for stem Chloridoideae, which was extended (making a less informative prior) to account for the evidence of C₄ grasses from the Oligocene (Urban et al. 2010). Internal node priors were implemented using log-normal distributions with a mean of 1, an offset to the minimum age, and standard deviations set such that 95% of the probability distribution was contained within the chosen intervals. The prior on the root of the tree was set as a uniform distribution from 0 to a hard maximum age of 125 Ma, under the assumption that lineages of Poaceae were not
present prior to the earliest eudicot fossil (Doyle 1992). *Anomochloa* was fixed as the outgroup (see Bouchenak-Khelladi *et al.* 2008).

For dataset 1, BEAST was run for 120 million generations, sampling every 10,000, using the Yule model for branching under a relaxed uncorrelated log-normal clock (UCLN; Drummond *et al.* 2006), and using the optimal partitioning scheme from PartitionFinder. BEAST had difficulty starting the analysis from a random tree because of the prior for placement of the 65 Ma phytoliths at stem Oryzeae, so a tree in Newick format was provided with appropriate branch lengths for that node to initialize the analysis. Rather than fixing models for the optimum partitions, we allowed the substitution models to be sampled as part of the analysis using the bModelTest v. 1.0.4 package (Bouckaert and Drummond 2017). Log files were examined in Tracer v. 1.6.0 (Rambaut *et al.* 2014) to check that the run had reached stationarity and that effective sample sizes (ESS) were large. A nominal 10% burn-in was used, after observing that the chain had reached stationarity by that point, and a maximum clade credibility tree was created using TreeAnnotator v. 2.4.6 (Bouckaert *et al.* 2014) and visualized in FigTree v. 1.4.3 (Rambaut 2016).

For dataset 2, we calibrated the node age for crown Chloridoideae using the 95% highest posterior density (HPD) interval from the analysis of dataset 1. The prior distribution was set (see Table S4) with a gamma distribution and an offset, with the shape and scale set iteratively to closely approximate the 95% HPD from the first analysis. A recent molecular dating analysis of *Triodia* (Toon *et al.* 2015) found that a random local clocks model (RLC; Drummond and Suchard 2010) provided a better fit to account for rate changes in *Triodia* than the UCLN clock (see also Crisp *et al.* 2014), which produced younger ages in *Triodia*. We ran BEAST with the same settings as in the analysis of dataset 1, except using an RLC clock model, again using the optimal partition scheme obtained from PartitionFinder. Examining log
files in Tracer showed some instability in the MCMC chain for some parameters, with stationarity reached around 60 million generations. To obtain higher ESS of parameters, we ran a second analysis with the same settings then combined the two runs and constructed a maximum clade credibility tree after discarding appropriate burn-ins (50% and 25%, respectively).

We ran two additional analyses to assess the impact of clock model and alignment size. First, we ran the analysis of dataset 2 again with the same settings, but this time using a UCLN clock model. Examining the log file in Tracer showed stationarity after approximately 30% burn-in, so this was used to construct a maximum clade credibility tree. Second, we assessed whether differences in the dates we obtained compared to those of Toon et al. (2015) might be due to the length of our alignment. We extracted the matK region from our alignment of dataset 2 and ran an analysis with just that locus, using the same settings as in the original analysis of dataset 2 (with an RLC clock model). Examining the run in Tracer showed wide variation in the prior and posterior (with low ESS) as well as a few anomalous samples well outside the range of the chain. We combined the results with those of a second run, after discarding burn-in and removing anomalous samples with a custom script, but still had low ESS for the posterior and prior, possibly reflecting low information content in the matK locus. Results from the matK analyses should therefore be interpreted cautiously.

RESULTS
The 28 newly-assembled grass chloroplast genomes were on average c. 135,000 bp (132,643–137,308 bp) in length. There were no major discrepancies in gene content or order. After filtering the aligned genomic regions, dataset 1 comprised 79 loci (coding regions) with a combined length of 62,005 bp, while dataset 2 comprised 163 loci (coding regions, introns or spacers) with a combined length of 101,510 bp. Phylogenetic inference for both datasets
resulted in highly resolved trees (Figs. S1–S4), with high (100%/1.00 posterior probability) support for all grass subfamilies except Pharoideae and Anomochloideae (single samples), and high support for *Triodia* as a genus and for the *T. basedowii* species complex. Phylogenetic relationships of grass subfamilies are in agreement with the current understanding of evolution in Poaceae (Grass Phylogeny Working Group II 2012; Soreng *et al.* 2015). Topologies are identical between RAxML and MrBayes trees, aside from the lack of resolution at the base of the trees for dataset 1.

Divergence dates for important nodes across the grasses (Fig. S5, Table 1) are similar to previous studies with comparable placement of the controversial phytoliths (Prasad *et al.* 2011; Christin *et al.* 2014; Burke *et al.* 2016). Our estimate for the node age of crown Chloridoideae (41.7 Ma; used for a secondary calibration in analysis of dataset 2) is slightly older than previous estimates but is within the 95% highest posterior density (HPD) interval of those studies (Table 1). Alternative placement of the controversial phytoliths at stem BOP+PACMAD led to estimates for crown Chloridoideae c. 10 Ma younger (32.1 Ma; Table S6).

The analysis focusing on Chloridoideae and *Triodia* chloroplast genomes allowed incorporation of non-coding regions into the alignment and produced a well-supported (posterior probabilities >0.99 for most nodes) chronogram for evolution in the group (Fig. 3). Estimated node ages (Table 2, rounded to two significant figures) indicate that *Triodia* began to diversify in the late Miocene (7.9 Ma; 7.0–8.8 Ma 95% HPD), and that the *T. basedowii* complex began to radiate in the Pleistocene (2.3 Ma; 1.9–2.7 Ma 95% HPD). Using a secondary calibration from the analysis with the alternate placement of the phytoliths at stem BOP+PACMAD had a small effect on the estimate for the crown age of the complex (1.7 Ma; 1.4–2.0 Ma 95% HPD; Table S6), but still indicated a radiation beginning in the Pleistocene.
As with a previous study on diversification timing for *Triodia* (Toon *et al.* 2015), the UCLN clock model produced younger estimates for node ages compared to the preferred RLC model (see Table 2). Restricting the alignment to *matK* resulted in older crown age estimates (Table 2) for *Triodia* (10 Ma) and the *T. basedowii* complex (3.8 Ma) compared to the full alignment; however, the estimates were still younger than those in Toon *et al.* (2015).

Our estimate for the timing of diversification should be interpreted cautiously, given our limited sampling of *Triodia* and the lack of a fossil calibration for our ingroup. Indeed, our estimate for the age of crown *Triodia* (7.0–8.8 Ma) is significantly younger than the 11.4–18.3 Ma estimate obtained in a recent study (Toon *et al.* 2015), although our estimate for stem *Triodia* is similar (see Table 2). This is surprising, especially given Toon *et al.* (2015) used a younger secondary calibration (c. 32 Ma) for crown Chloridoideae. There are several potential reasons for our dates being younger. First, we used a larger dataset (chloroplast genomic alignment vs. ITS + *matK*), which we expect to be more informative for inferring rates of evolution. When we used the *matK* region alone, we obtained an older estimate for crown *Triodia* (5.5–17 Ma), although still not as old as that of Toon *et al.* (2015). Second, our sampling of *Triodia* and outgroup Chloridoideae is sparser, which may have produced a node density effect (see Heath *et al.* 2008; Simon Ho, pers. comm.), limiting the number of inferred substitutions. While undersampling across a tree has been shown to result in younger ages, undersampling of a specific clade was not observed to have the same effect on the age of the subtending node (Linder *et al.* 2005), suggesting that undersampling in *Triodia* may not fully explain the discrepancy. Third, our sampling of *Triodia* lacks species from northern tropical regions, which were included in Toon *et al.* (2015) and which may have higher rates of evolution and could bias lower nodes to be older (see Beaulieu *et al.* 2015). Broader sampling of *Triodia* chloroplast genomes in the future may help to resolve whether our younger dates are primarily a result of limited sampling or a better estimate based on greater sequence data.
**Table S4.**

Partitions and models for datasets 1 and 2. The number of loci refers to genes, introns or intergenic spacers and is the number of possible partitions that PartitionFinder assigned to that partition.

| Dataset | Partition | Length (bp) | # loci | Model   |
|---------|-----------|-------------|--------|---------|
| 1       | 1         | 8103        | 10     | GTR+I+G |
|         | 2         | 7146        | 13     | GTR+I+G |
|         | 3         | 9049        | 9      | GTR+I+G |
|         | 4         | 8235        | 14     | GTR+I+G |
|         | 5         | 6600        | 12     | GTR+I+G |
|         | 6         | 1543        | 1      | GTR+G   |
|         | 7         | 4780        | 3      | GTR+I+G |
|         | 8         | 5390        | 6      | GTR+I+G |
|         | 9         | 5924        | 6      | GTR+I+G |
|         | 10        | 4605        | 4      | HKY+I   |
|         | 11        | 630         | 1      | K80+G   |
| 2       | 1         | 13659       | 24     | GTR+G   |
|         | 2         | 21270       | 26     | GTR+I+G |
|         | 3         | 13859       | 39     | GTR+I+G |
|         | 4         | 7625        | 8      | GTR+I+G |
|         | 5         | 6643        | 6      | HKY+I+G |
|         | 6         | 1558        | 5      | HKY+I+G |
|         | 7         | 7847        | 14     | GTR+G   |
|         | 8         | 7570        | 15     | HKY+G   |
|         | 9         | 3433        | 6      | F81+I   |
|         | 10        | 1021        | 1      | HKY     |
|         | 11        | 6563        | 8      | GTR+G   |
|         | 12        | 5849        | 6      | HKY+I+G |
|         | 13        | 2888        | 1      | HKY+I   |
|         | 14        | 1725        | 4      | K80+I   |
Table S5.

Molecular dating calibrations and their parameterization in BEAST. In the case of the secondary calibration, the age range is the 95% highest posterior density (HPD) interval, with the gamma implementation that closely approximates that HPD. The secondary calibration (S) is obtained from the analysis of dataset 1.

| Calibration | Type     | Tree placement                              | Age (Ma) (95% quantile) | Distrib.   | offset | mean | std dev |
|-------------|----------|---------------------------------------------|--------------------------|------------|--------|------|---------|
| 1           | fossil   | stem Oryzeae                                | 65 (–97.5)               | log-normal | 65     | 1    | 1.508   |
| 2           | fossil   | stem Pooidae                                | 40 (–60)                 | log-normal | 40     | 1    | 1.212   |
| 3           | fossil   | common ancestor of Stipa and Poa            | 34 (–51)                 | log-normal | 34     | 1    | 1.115   |
| 4           | fossil   | crown Bambusoideae†                          | 35 (–52.5)               | log-normal | 35     | 1    | 1.131   |
| 5           | fossil   | stem Chloridoideae                          | 19 (–50)                 | log-normal | 19     | 1    | 1.48    |
| 6           | root     | bounding root (crown Poaceae)               | 0 (–125)                 | uniform    | 0      | -    | -       |
| S           | secondary| crown Chloridoideae                         | 41.7 (38.1–45.7)         | gamma (41.7; 38.4–45.6) | 36.1 | 7 (shape) | 0.8 (scale) |

Footnotes: References for fossils are: (1) (Prasad et al. 2005, 2011), (2) (Zucol et al. 2010), (3) (MacGinitie 1953; Manchester 2001), and (4) & (5) (Strömberg 2005). †(Piperno and Pearsall 1998).

Table S6.

Node ages (Ma) from analyses of datasets 1 (clear) and 2 (greyed) with the phytoliths placed alternatively at stem BOP+PACMAD or at stem Oryzeae (preferred). HPD is the highest posterior density interval.

| Analysis | Phytoliths at stem BOP+PACMAD | Phytoliths at stem Oryzeae |
|----------|-------------------------------|---------------------------|
| Node     | Age  | 95% HPD         | Age  | 95% HPD         |
| crown Poaceae | 110  | 96.6–124       | 123  | 119–125         |
| crown BOP+PACMAD | 62.5 | 59.6–66.5       | 82.4 | 78.5–86.5       |
| crown Oryzoideae | 44.4 | 39.8–49.2       | 65.6 | 65–66.6         |
| crown Bambusoideae | 38.6 | 35.1–43.3       | 50   | 40.9–59         |
| crown Pooidae   | 47.2 | 44.1–50.4       | 60.1 | 55.1–65.2       |
| crown Chloridoideae | 32.1 | 29–35.1        | 41.7 | 38.1–45.7       |
| stem Triodia    | 15.2 | 13.6–16.8       | 20.2 | 18.4–22.2       |
| crown Triodia   | 5.92 | 5.19–6.65       | 7.89 | 6.98–8.82       |
| crown T. basedowii complex | 1.73 | 1.44–2.02 | 2.29 | 1.91–2.70 |
Figure S1.

RAxML tree for analysis of dataset 1. Alignment of 62,005 bp of chloroplast coding regions across Poaceae. Branch support from 100 rapid bootstrap replicates shown for values > 80. Grass subfamilies are indicated. Scale bar units are branch lengths from RAxML (inferred substitutions per site).
**Figure S2.**

MrBayes tree for analysis of dataset 1. Alignment of 62,005 bp of chloroplast coding regions across the Poaceae. Branch support values are posterior probabilities and are shown for values > 0.95. Grass subfamilies are indicated. Scale bar units are branch lengths from MrBayes (inferred substitutions per site).
Figure S3.
RAxML tree for analysis of dataset 2. Alignment of 101,510 bp of chloroplast coding regions, spacers and introns for Chloridoideae with two outgroup Danthonioideae. Branch support from 100 rapid bootstrap replicates shown for values > 80. The *Triodia basedowii* complex is indicated. Scale bar units are branch lengths from RAxML (inferred substitutions per site).
Figure S4.
MrBayes tree for analysis of dataset 2. Alignment of 101,510 bp of chloroplast coding regions, spacers and introns for Chloridoideae with two outgroup Danthonioideae. Branch support values are posterior probabilities and are shown for values > 0.95. The *Triodia basedowii* complex is indicated. Scale bar units are branch lengths from MrBayes (inferred substitutions per site).
Figure S5.

Chronogram from the BEAST analysis of dataset 1, comprising chloroplast alignments from members of all grass subfamilies. Fossil calibration points are shown with black triangles and numbered as in Table S5. Node bars for selected nodes of interest are 95% highest posterior density intervals. Node support values are posterior probabilities and are shown for values < 1.00. Grass subfamilies in BOP+PACMAD are indicated at right; O: Oryzoideae, Po: Pooidae, B: Bambusoideae, Ar: Aristidoideae, P: Panicoideae, A: Arundinoideae, M: Micrairoideae, D: Danthonioideae, C: Chloridoideae.
SUPPORTING INFORMATION LITERATURE CITED

Anderson BM, Barrett MD, Krauss SL, Thiele K. 2016. Untangling a species complex of arid zone grasses (Triodia) reveals patterns congruent with co-occurring animals. *Molecular Phylogenetics and Evolution* **101**: 142–162.

Bankevich A, Nurk S, Antipov D, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* **19**: 455–477.

Beaulieu JM, O’Meara BC, Crane P, Donoghue MJ. 2015. Heterogeneous rates of molecular evolution and diversification could explain the Triassic age estimate for angiosperms. *Systematic Biology* **64**: 869–878.

Bouchenak-Khelladi Y, Salamin N, Savolainen V, et al. 2008. Large multi-gene phylogenetic trees of the grasses (Poaceae): progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution* **47**: 488–505.

Bouckaert RR, Drummond AJ. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* **17**: 42.

Bouckaert R, Heled J, Kühnert D, et al. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537–e1003537.

Burke SV, Lin C-S, Wysocki WP, Clark LG, Duvall MR. 2016. Phylogenomics and plastome evolution of tropical forest grasses (Leptaspis, Streptochaeta: Poaceae). *Frontiers in Plant Science* **7**: 1993.

Christin P-A, Spriggs E, Osborne CP, Strömberg CAE, Salamin N, Edwards EJ. 2014. Molecular dating, evolutionary rates, and the age of the grasses. *Systematic Biology* **63**: 153–165.

Crisp MD, Hardy NB, Cook LG. 2014. Clock model makes a large difference to age estimates of long-stemmed clades with no internal calibration: a test using Australian grasstrees. *BMC*
Evolutionary Biology 14: 263.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Research 45: e18.

Doyle JA. 1992. Revised palynological correlations of the lower Potomac group (USA) and the cocobeach sequence of Gabon (Barremian-Aptian). Cretaceous Research 13: 337–349.

Doyle JJ, Dickson EE. 1987. Preservation of Plant Samples for DNA Restriction Endonuclease Analysis. Taxon 36: 715–722.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4: e88.

Drummond AJ, Suchard MA. 2010. Bayesian random local clocks, or one rate to rule them all. BMC Biology 8: 114.

Duvall MR, Fisher AE, Travis Columbus J, et al. 2016. Phylogenomics and plastome evolution of the chloridoid grasses (Chloridoideae: Poaceae). International Journal of Plant Sciences 177: 235–246.

Gardner MG, Fitch AJ, Bertozzi T, Lowe AJ. 2011. Rise of the machines--recommendations for ecologists when using next generation sequencing for microsatellite development. Molecular Ecology Resources 11: 1093–1101.

Grass Phylogeny Working Group II. 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C₄ origins. The New Phytologist 193: 304–312.

Heath TA, Hedtke SM, Hillis DM. 2008. Taxon sampling and the accuracy of phylogenetic analyses. Journal of Systematics and Evolution 46: 239–257.

Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30: 772–780.
Kearse M, Moir R, Wilson A, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kurtz S, Phillippy A, Delcher AL, et al. 2004. Versatile and open software for comparing large genomes. *Genome Biology* 5: R12–R12.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754–1760.

Linder HP, Hardy CR, Rutschmann F. 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution* 35: 569–582.

MacGinitie HD. 1953. Fossil plants of the Florissant beds, Colorado. *Publications of the Carnegie Institution of Washington* 599.

Manchester SR. 2001. Update on the megafossil flora of Florissant, Colorado. *Proceedings of the Denver Museum of Nature & Science* 4: 137–161.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17: 10–12.

Piperno DR, Pearsall DM. 1998. The silica bodies of tropical American grasses: morphology, taxonomy, and implications for grass systematics and fossil phytolith identification. *Smithsonian Contributions to Botany* 85: 1–40.

Poinar G, Alderman S, Wunderlich J. 2015. One hundred million year old ergot: psychotropic compounds in the Cretaceous? *Paleodiversity* 8: 13–19.

Prasad V, Strömberg CAE, Alimohammadian H, Sahni A. 2005. Dinosaur coprolites and the early
evolution of grasses and grazers. *Science* **310**: 1177–1180.

**Prasad V, Strömberg CAE, Leaché AD, et al. 2011.** Late Cretaceous origin of the rice tribe provides evidence for early diversification in Poaceae. *Nature Communications* **2**: 480.

**Python Software Foundation. 2016.** Python Language Reference, version 2.7.

**Rambaut A. 2016.** *FigTree* [http://tree.bio.ed.ac.uk/software/figtree/].

**Rambaut A, Suchard M, Xie D, Drummond A. 2014.** *Tracer* [http://tree.bio.ed.ac.uk/software/tracer/].

**Ronquist F, Teslenko M, van der Mark P, et al. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

**Soreng RJ, Peterson PM, Romaschenko K, et al. 2015.** A worldwide phylogenetic classification of the Poaceae (Gramineae). *Journal of Systematics and Evolution* **53**: 117–137.

**Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

**Stamatakis A, Hoover P, Rougemont J. 2008.** A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.

**Strömberg CAE. 2005.** Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 11980–11984.

**Toon A, Crisp MD, Gamage H, et al. 2015.** Key innovation or adaptive change? A test of leaf traits using Triodiinae in Australia. *Scientific Reports* **5**: 12398.

**Urban MA, Nelson DM, Jiménez- Moreno G, Châteauneuf J-J, Pearson A, Hu FS 2010.** Isotopic evidence of C₄ grasses in southwestern Europe during the Early Oligocene–Middle Miocene. *Geology*
Walker BJ, Abeel T, Shea T, et al. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* 9: e112963.

Wysocki WP, Clark LG, Kelchner SA, et al. 2014. A multi-step comparison of short-read full plastome sequence assembly methods in grasses. *Taxon* 63: 899–910.

Zerbino DR, Birney E. 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research* 18: 821–829.

Zucol AF, Brea M, Bellosi ES. 2010. Phytolith studies in Gran Barranca (central Patagonia, Argentina): the middle-late Eocene In: Madden RH, Carlini AA, Vucetich MG, Kay RF, eds. *The Paleontology of Gran Barranca: Evolution and Environmental Change through the Middle Cenozoic of Patagonia*. New York: Cambridge University Press, 317–340.