Chromosome numbers in the tribe Anthemideae (Asteraceae) from north-east Anatolia

HUSEYIN INCEER* and SEMA HAYIRLIOGLU-AYAZ

Department of Biology, Faculty of Sciences and Arts, Karadeniz Technical University, 61080 Trabzon, Turkey

Received November 2005; accepted for publication July 2006

Twenty-two chromosome counts of 19 taxa in the tribe Anthemideae of the family Asteraceae are reported from north-east Anatolia, Turkey. The taxa belong to the subtribes Achilleinae (four Achillea taxa), Anthemidinae (five Anthemis taxa), Artemisiinae (two Artemisia species), Leucantheminae (one Lecanthemum species), Matricariinae (two Tripleurospermum species) and Tanacetinae (five Tanacetum taxa). Six counts are new reports, seven are not consistent with previous counts, and the remainder are confirmations of very limited previous data. Most of the populations of Anthemideae studied have the basic number \( x = 9 \), with ploidy levels ranging from \( 2x \) to \( 7x \) to \( 8x \), but dysploidy is also present, with one diploid species, Artemisia austriaca, having \( x = 8 \). © 2007 The Linnean Society of London, Botanical Journal of the Linnean Society, 2007, 153, 203–211.


INTRODUCTION

The Asteraceae is one of the largest plant families (Bremer, 1994). Its many genera and species, its worldwide distribution and the fact that it comprises many useful plants have made it the subject of large numbers of karyological studies (Watanabe, 2002 and references therein). The Anthemideae is a medium-sized tribe in the family, with about 1800 species (Valles et al., 2005). It is generally accepted as a relatively natural assemblage.

The tribe has a worldwide distribution but with main concentrations of taxa in Central Asia, the Mediterranean region and South Africa. Some members of the subtribes Ursiniinae, Artemisiinae, Chrysantheminae, Leucantheminae, Anthemidinae and Matricariinae are pernicious weeds, such as some of the Ursinia species introduced in Australia and New Zealand; species of Chrysanthemum, Anthemis, Artemisia, Achillea, Leucanthemum, Tripleurospermum and Matricaria are more widespread weeds in both the northern and southern hemispheres. However, most taxa have discrete ranges and very obvious areas of endemism (Bremer & Humphries, 1993).

The principal taxonomic problems within the tribe are almost entirely relationships between genera, but also circumscription of genera, especially within subtribal groups such as the Artemisiinae, Chrysantheminae and Tanacetinae (Bremer & Humphries, 1993). Although the Anthemideae tribe has been reviewed extensively (Bremer & Humphries, 1993), a complete convenient classification has not yet been achieved.

The relevance of karyological information to knowledge of the systematics and evolution of the tribe was noted long ago. In spite of the very large number of counts in the tribe, chromosomal data on Turkish populations are scarce, with chromosome numbers currently known for less than 14% of the taxa. Table 1 shows some previous counts made on Turkish material. The aim of the present study is to verify or to establish the chromosome numbers of several representatives of the Anthemideae from north-east Anatolian localities. Some of our data are new counts; others confirm unique or very scarce previous reports, or represent numbers which are different from those cited previously. We also discuss several systematic and
evolutionary aspects of the tribe in the light of the cytogenetic data.

MATERIAL AND METHODS

PLANT MATERIAL

The taxa studied were Achillea biebersteinii Afin., A. biserrata M. Bieb., A. millefolium L. ssp. millefolium, A. setacea Waldst. & Kit., Anthemis marschalliana Willd. ssp. pectinata (Boiss.) Grierson, A. melanoloma Trautv. ssp. trapezuntica Grierson, A. tinctoria L. var. pallida DC., A. tinctoria L. var. tinctoria, A. triumfetti (L.) All., Artemisia absinthium L., A. austriaca Jacq., Leucanthemum vulgare Lam., Tanacetum albipannosum Hub.-Mor. & Grierson, T. coccineum (Willd.) Grierson ssp. chamaemelifolium (Somm. & Lev.). Grierson, T. macrophyllum Sch. Bip., T. parthenium Sch. Bip., T. sorbifolium (Boiss.) Grierson, Tripleurospermum callosum (Boiss. & Meldr.) E. Hossain, and T. sevanense (Manden.) Pobed. These plants were collected in 2001–05 from the mountains of north-east Anatolia, Turkey. Vouchers are deposited in the herbarium at the Karadeniz Technical University, Department of Biology (KTUB).

CHROMOSOME ANALYSIS

Root tip meristems obtained directly from natural populations were used for chromosome analysis. The tips of roots were cleaned of soil particles, cut off and pretreated with 0.05% colchicine for 2–5 h (Inceer, Hayirlioglu-Ayaz & Beyazoglu, 2002). They were then fixed in ethanol-acetic acid (3:1) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 10 min and then rinsed in tap water for a minimum of 2–3 min. Staining was carried out in 1% aqueous aceto-orcein for 12–18 h at room temperature, and squashes made in 45% acetic acid. The best metaphase plates were photographed with ISO 50 Ilford film.

In order to assess the existence of published chromosome counts in the taxa studied we used the common indexes of plant chromosome numbers cited by Inceer & Beyazoglu (2004), as well as the on-line chromosome number databases, Index to Plant Chromosome Numbers (Missouri Botanical Garden, http://motot.mobot.org/W3T/Search/spcn.html) and Index to Chromosome Numbers in the Asteraceae (Watanabe, 2002; http://www-asteraceae.cla.kobe-u.ac.jp/index.html).

Table 1. Previous counts in some taxa of the tribe Anthemideae in Turkey

<table>
<thead>
<tr>
<th>Genus</th>
<th>Taxon</th>
<th>2n</th>
<th>Localities</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. spinulifolia</td>
<td>18</td>
<td>Nigde/Adana: Ak Dağ</td>
<td>Martin-Nouet (1969)</td>
</tr>
<tr>
<td>Anthemis</td>
<td>A. altissima</td>
<td>18</td>
<td>Tekirdağ: İneik</td>
<td>Strid (1980)</td>
</tr>
<tr>
<td></td>
<td>A. cretica ssp. lecanthemoides</td>
<td>36</td>
<td>Manisa: Manisa Dağı</td>
<td>Strid (1987)</td>
</tr>
<tr>
<td></td>
<td>A. cretica s. l.</td>
<td>18</td>
<td>Muğla: Çal Dağı</td>
<td>Strid (1987)</td>
</tr>
<tr>
<td></td>
<td>A. tinctoria</td>
<td>18</td>
<td>Tekirdağ: Kesan to Tekirdağ</td>
<td>Strid (1980)</td>
</tr>
<tr>
<td>Artemisia</td>
<td>A. abrotanum</td>
<td>18</td>
<td>Erzurum: Oltu-Göle</td>
<td>Johnson &amp; Brandham (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>Erzurum: Erzurum-Artvin</td>
<td>Johnson &amp; Brandham (1997)</td>
</tr>
<tr>
<td>Chrysanthemum</td>
<td>C. coronarium</td>
<td>18</td>
<td>İzmir: Bornova</td>
<td>Nemli (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>Rize: İkizdere</td>
<td>García et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36</td>
<td>Rize: İkizdere</td>
<td>García et al. (2005)</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The chromosome numbers \((2n)\), basic numbers and levels of ploidy of the 19 taxa studied are presented in Table 2. Except for the chromosome counts of *Achillea setacea* and *Anthemis tinctoria* var. *tinctoria*, the counts are new to the flora of Turkey. Six of these are also new to science.

The classification adopted here follows that of Bremer & Humphries, 1993).

Table 2. Chromosome numbers \((2n)\), basic numbers and levels of ploidy of the Anthemideae taxa studied

<table>
<thead>
<tr>
<th>Genus</th>
<th>Taxon</th>
<th>Chromosome number ((2n))</th>
<th>Basic number ((x))</th>
<th>Ploidy level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achillea</td>
<td><em>A. biebersteinii</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. biserrata</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. millefolium</em> ssp. millefolium</td>
<td>67**</td>
<td>9</td>
<td>7x-8x</td>
</tr>
<tr>
<td></td>
<td><em>A. setacea</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td>Anthemis</td>
<td><em>A. marschalliana</em> ssp. pectinata</td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. melanoloma</em> ssp. trapezuntica</td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. tinctoria</em> var. pallida</td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. tinctoria</em> var. tinctoria</td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. triumfetti</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td>Artemisia</td>
<td><em>A. absinthium</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>A. austriaca</em></td>
<td>16</td>
<td>8</td>
<td>2x</td>
</tr>
<tr>
<td>Leucanthemum</td>
<td><em>L. vulgare</em></td>
<td>36</td>
<td>9</td>
<td>4x</td>
</tr>
<tr>
<td>Tanacetum</td>
<td><em>T. albipannosum</em></td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>T. coccineum</em> ssp. chamaemelifolium</td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>T. macrophyllum</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>T. parthenium</em></td>
<td>18</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td><em>T. sorbilfolium</em></td>
<td>18*</td>
<td>9</td>
<td>2x</td>
</tr>
<tr>
<td>Tripleurospermum</td>
<td><em>T. callasum</em></td>
<td>36</td>
<td>9</td>
<td>4x</td>
</tr>
<tr>
<td></td>
<td><em>T. sevanense</em></td>
<td>36</td>
<td>9</td>
<td>4x</td>
</tr>
</tbody>
</table>

*New record; **new record for the genus.

SUBTRIBE ACHELLEINAE BREMER & HUMPHRIES

*Achillea biebersteinii* Afan.

A7: Gümüşhane: near Köse Dağı pass. Grassy stream banks, margins of *Pinus sylvestris* forest, 1800 m a.s.l., 23.vii.2001, *Inceer* 142. \(2n = 18\) (Fig. 1).

Our count on Turkish material confirms the existence of two ploidy levels in this species. Our diploid report agrees with three reports from different territories (Watanabe, 2002 and references therein), although we found only one previous record of the tetraploid level, occurring in plants from Poland (Dabrowska, 1989).

*A. biserrata* Bieb.


A7: Trabzon: Zigana Dağı. Open slopes, roadsides, 1700 m a.s.l., 28.vi.2005, *Inceer* 188. \(2n = 18\) (Fig. 2).

Our two counts agree with one report of somatic number (Daniela, 1997) and one of gametic number (Fedorov, 1969) in plants from Russia.

*A. millefolium* L. ssp. *millefolium*

A7: Gümüşhane: Torul. Open slopes, roadsides, 1300 m a.s.l., 28.vi.2005, *Inceer* 199. \(2n = 67\) (Fig. 3).

Our chromosome number of this taxon was uniform in the five specimens that we studied from one population. It was aneuploid, between 7\(x\) and 8\(x\), representing simply a loss of normal chromosomes, as is to be expected of products of the irregular meiosis that occurs in high autopolyploids. The plants’ survival results from the presence in them of adequate numbers of complete chromosome sets. This result shows that the aneuploidy in this population is stable. Sexual products of aneuploids at this high ploidy level would be expected to display variation in chromosome number between individuals, so our observations demonstrate that the studied population was a single clone spreading by vegetative or possibly apomictic means.
Figures 1–12. Somatic metaphases. Fig. 1. *Achillea biebersteinii* (2n = 18). Fig. 2. *A. biserrata* (A7 Trabzon, 2n = 18). Fig. 3. *A. millefolium* ssp. *millefolium* (2n = 67). Fig. 4. *A. setacea* (2n = 18). Fig. 5. *Anthemis marschalliana* ssp. *pectinata* (2n = 18). Fig. 6. *A. melanoloma* ssp. *trapezuntica* (2n = 18). Fig. 7. *A. tinctoria* var. *pallida* (2n = 18). Fig. 8. *A. tinctoria* var. *tinctoria* (2n = 18). Fig. 9. *A. triumfetti* (2n = 18). Fig. 10. *Artemisia absinthium* (2n = 18). Fig. 11. *A. austriaca* (2n = 16). Fig. 12. *Leucanthemum vulgare* (Zigana Daği, 2n = 36). Scale bar = 10 µm.
A. setacea Waldst. & Kt.
A7: Trabzon: Zigana Dağı pass. Damp alpine meadows, 2000 m a.s.l., 10.vii.2001, Inceer 131. 2n = 18 (Fig. 4).

Our diploid count agrees with one report from Turkey and many others from different countries (Ehrendorfer, 1959; Gadella & Kliphuis, 1970; Kuzmanov, Georgieva & Nikolava, 1986; Kuzmanov, Zdravkova & Georgieva, 1989; Khaniki, 1995). We found only one previous record of the tetraploid level, in Russian plants (Androshchuk & Kostinenko, 1981).

SUBTRIBE ANTHEMIDINAE DUMORF. EMEND. BREMER & HUMPHRIES

Anthemis marschalliana Willd. ssp. pectinata (Boiss.) Grierson
A7: Gümüşhane: near Limonsuyu. Roadsides and distributed steppe, 2300 m a.s.l., 24.vii.2005, Inceer 205. 2n = 18 (Fig. 5).

According to our data, this is the first report of the chromosome number of this subspecies. It agrees with the number recorded for the species in Russian plants (Watanabe, 2002).

A. melanoloma Trautv. ssp. trapezuntica Grierson
A7: Gümüşhane: near Limonsuyu. Limestone slopes and scree, distributed steppe, 2300 m a.s.l., 24.vii.2005, Inceer 204. 2n = 18 (Fig. 6).

To our knowledge, this is the first report for this subspecies. It agrees with the number recorded for the species in Russian plants (Magulaev, 1992).

A. tinctoria L. var. pallida DC.
A7: Trabzon: Arakh above village of Ayvadere. Roadsides, margins of fields, boggy ground, 300 m a.s.l., 2.vii.2005, Inceer 202. 2n = 18 (Fig. 7).

According to our data, this is the first report of the chromosome number of this variety. It agrees with the number recorded for the species (Watanabe, 2002 and references therein).

A. tinctoria L. var. tinctoria
A7: Gümüşhane: Pınus forests near Torul. Roadsides, streamsides, 1400 m a.s.l., 28.vi.2005, Inceer 194. 2n = 18 (Fig. 8).

Our diploid count agrees with one report from Turkish material and many others from different territories (Watanabe, 2002 and references therein). This number is shown to be very stable, because it is also the same in many other reports based on plants from very different regions.

A. triumfetti (L.) All.
A7: Gümüşhane: Pınus forests near Köse Dağı pass. Rocky slopes and meadows, 1800 m a.s.l., 23.vii.2001, Inceer 141. 2n = 18 (Fig. 9).

Our count agrees with the only three previous ones, on Spanish and Italian plants (Watanabe, 2002 and references therein).

SUBTRIBE ARTEMISINAE LESS. EMEND. BREMER & HUMPHRIES

Artemisia absinthium L.
A7: Gümüşhane: Pınus forests near Köse Dağı pass. Slopes, streamside, 1800 m a.s.l., 23.vii.2001, Inceer 139. 2n = 18 (Fig. 10).

Our count agrees with many others from different territories (Watanabe, 2002 and references therein). This number is shown to be very stable, because it is also the same in many other reports based on plants from different regions.

A. austriaca Jacq.
A7: Gümüşhane: Pınus forests near Köse Dağı pass. Slopes, streamside, roadsides, 23.vii.2001, Inceer 140. 2n = 16 (Fig. 11).

Different chromosome numbers and ploidy levels have been assigned to this species; 2n = 16 in populations from Poland (Skalinska, Czapic & Piotrowicz, 1959) and Bulgaria (Kuzmanov et al., 1986; Kuzmanov, 1993): 2n = 32 in a Russian population (Lavrenko & Serditov, 1991); 2n = 36 in a Slovak population (Kawatani & Ohno, 1964); 2n = 48 in a Slovak population (Majovsky & Murin, 1987); one Hungarian and one Austrian population (Feráková, 1997) and one Armenian and one Iranian population (Torrel et al., 2001) and 2n = 54 in an Iranian population (Tavassoli & Derakhshandeh-Peikar, 1993) and in a Slovak population (Feráková, 1997).

Our diploid count is based on x = 8. This basic number is frequent in section Absinthium; moreover, some members of section Artemisia with x = 8 appear in the Absinthium clade in the ITS phylogeny (Torrel et al., 1999). For A. austriaca, Feráková (1997) concluded that the diploid populations belong to var. advena Rech. f., but did not cite the tetraploid and the hexaploid cytotypes based on x = 9.

SUBTRIBE LECANTHEMINAE BREMER & HUMPHRIES

Leucanthemum vulgare Lam.
A7: Trabzon: Zigana Dağı. Grassy slopes, stony pastures, meadows, 1700 m a.s.l., 30.vi.2005, Inceer 200. 2n = 36 (Fig. 12).

Our counts agree with many other reports on material from other areas (Watanabe, 2002 and references therein). They confirm that the tetraploid level predominates in this species, although diploid, hexaploid and octoploid levels have also been reported in Austrian, Bulgarian and German plants (2n = 18;
Subtribe Matricarinae Bremer & Humphries

*Tripleurospermum callosum* (Boiss. & Meldr.) E. Hossain

A9: Artvin: Savsat, above Sahara National Park. Damp subalpine pastures, roadsides and grassy slopes, 1900 m a.s.l., 8.vi.2002, *Inceer* 149. 2n = 36 (Fig. 13).

The present count agrees with the previous ones carried on this Turkish endemic species in north-east Anatolian populations (Inceer & Beyazoglu, 2004; Garcia et al., 2005).

*T. sevanense* (Manden.) Pobed.

A8: Rize: Ikizdere, between Ikizdere and Anzer. Streamsides and roadsides, 1400 m a.s.l., 12.vii.2001, *Inceer* 138. 2n = 36 (Fig. 14).

Our count confirms the existence of the tetraploid cytotype of this species, reported by Inceer & Beyazoğlu (2004) and Garcia et al. (2005) on Turkish material. Apart from this, one count on Armenian plants has reported the diploid level, 2n = 18 (Avetisian & Oganesian, 1995).

Subtribe Tanacetinae Bremer & Humphries

*Tanacetum albipannosum* Hub.-Mor. & Grierson

A7: Trabzon: Zığına Dağı. Stony pastures on limestone and roadsides, 1600 m a.s.l., 28.vi.2005, *Inceer* 191. 2n = 18 (Fig. 15).

To our knowledge, this is the first report of the chromosome number of this species, which is endemic to Turkey.

*T. coccineum* (Willd.) Grierson ssp. *chamaemelifolium* (Somm. & Lev.). Grierson


A9: Artvin: Savsat, above Sahara National Park. Damp subalpine pastures on limestone and grassy slopes, 15.vi.2002, *Inceer* 153. 2n = 18 (Fig. 16).

According to our data, this is the first report of the chromosome number of subspecies. Our counts, the same in both populations studied, agree with the num-

13 14 15 16 17 18 19

Figures 13–19. Somatic metaphases. Fig. 13. *Tripleurospermum callosum* (2n = 36). Fig. 14. *T. sevanense* (2n = 36). Fig. 15. *Tanacetum albipannosum* (2n = 18). Fig. 16. *T. coccineum* ssp. *chamaemelifolium* (Artvin, 2n = 18). Fig. 17. *T. macrophyllum* (2n = 18). Fig. 18. *T. parthenium* (2n = 18). Fig. 19. *T. sorbifolium* (2n = 18). Scale bar = 10 μm.
ber recorded for the species (Watanabe, 2002 and references therein).

*T. macrophyllum* Sch. Bip.

A7: Trabzon: Araklı, between Dağbasi and Pazarlık. Roadside and streamside, 1300 m a.s.l., 24.vii.2005, *Inceer* 203. 2*n* = 18 (Fig. 17).

The count confirms four previous reports on material from other areas (Watanabe, 2002 and references therein), although we found only one previous record of the tetraploid level (2*n* = 36), in Hungarian plants (Sz.-Borsos, 1970). In addition, Kuzmanov, Thin & Georgieva. (1981) reported 2*n* = 14 in Bulgarian material.

*T. parthenium* Sch. Bip.

A7: Gümüshane: *Pinus* forests near Torul. Roadsides, streamside, stony pastures, 1400 m a.s.l., 28.vi.2005, *Inceer* 193. 2*n* = 18 (Fig. 18).

Our count agrees with previous reports (Watanabe, 2002 and references therein), although we found only one previous record of the tetraploid level in German plants (cult.) (Abd. El-Twab & Kordo, 2001). In addition, Vilmorin & Chopinet (1954) reported 2*n* = 16 in material from France (cult.).

*T. sorbilfolium* (Boiss.) Grierson

A8: Trabzon: Caykara, above the village of Akdoğan. Stony pastures and roadsides, 950 m a.s.l., 24.vi.2001, *Inceer* 129. 2*n* = 18 (Fig. 19).

To our knowledge, this is the first report of the chromosome number of this species.

### CONCLUSIONS

Most of the Anthemideae studied have the basic number *x* = 9, which is predominant in this tribe and in the entire family Asteraceae (Solbrig, 1977; Schweizer & Ehrendorfer, 1983; Oliva & Valles, 1994; Valles & Siljak-Yakovlev, 1997; Valles *et al.*, 2001; Inceer & Beyazoglu, 2004; Valles *et al.*, 2005). Among the considered taxa, only *Artemisia austriaca* has the less common basic number *x* = 8; this is evidence for the descending dysploidy that occurs in the genus (Valles & Siljak-Yakovlev, 1997; Torrel *et al.*, 2001). This phenomenon is common in many genera of the family Asteraceae (Fernandez Casas & Susanna, 1986; Siljak-Yakovlev, 1996; Torrel *et al.*, 2001; Valles *et al.*, 2001, 2005).

Polploidy is another relevant evolutionary mechanism in plants (Bretagnolle *et al.*, 1998 and references therein) and in some groups of the Anthemideae in particular (Solbrig, 1977; Valles *et al.*, 2001, 2005; Inceer & Beyazoglu, 2004; Guo *et al.*, 2005). This statement is supported by the present study, because five populations belonging to different Anthemideae genera studied (*Achillea*, *Leucanthemum*, *Tripleurospermum*) are polyploid, four being tetraploid and one heptaploid or octoploid.

Subtribal classifications of the Anthemideae were elaborated cladistically by Bremer & Humphries (1993). In that classification, except for the subtribe Tanacetinae, which is at best paraphyletic, other subtribes (*Achilleinae*, *Anthemidinae*, *Artemisiinae*, *Leucantheminae*, *Matricariinae*) are monophyletic. However, molecular studies in the Anthemideae (Francisco-Ortega *et al.*, 1997; Oberprieler & Vogt, 2000; Watson, Evans & Boluarte, 2000) revealed that these subtribes are not monophyletic. Chromosome morphometries of the studied genera show that the subtribes of the Anthemideae may be heterogenous groups. A combination of detailed karyotype analysis with molecular studies is needed in order to achieve a convenient classification of this pool of plants.

### ACKNOWLEDGEMENTS

We thank Miss Melahat Ozcan for her help in collecting some of the plant material. We also thank Dr P. E. Brandham (Royal Botanic Gardens, Kew) for his suggestions that improved the manuscript.

### REFERENCES


