Phylogeny of the SE Australian clade of *Hibbertia* subg. *Hemistemma* (Dilleniaceae)

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Declaration

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This thesis is dedicated to my loving family and parents

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*The teacher who is indeed wise does not bid you to enter the house of his wisdom but rather leads you to the threshold of your mind--**Khalil Gibran***

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Prologue

The thesis is divided into 8 related chapters covering different aspects of the study. Chapter 1 is the introduction and literature review, placing the aims of the study into an historical context. Chapter 2 covers a morphological analysis of 87 taxa of the SE Australian clade of *Hibbertia* subg. *Hemistemma* recognised by Horn (2005, 2007) using 105 morphological characters to test the monophyly and relationships of the proposed 16 informal unpublished species groups of Toelken. Chapter 3 is the molecular analysis for the same taxa, using nuclear rDNA (ITS) and intergenic spacer regions of cpDNA (*trn*L–F) to test phylogenetic lineages and relationships. Chapter 4 combines the molecular and morphological data for the 87 taxa to explore their phylogenetic relationships, placement of intraspecific taxa and character evolution. Because of the complexity of the morphological data set, character exploration is divided into several sections. In chapter 5, 64 gross vegetative features and surface indumentum characters are plotted onto the combined analysis tree, to explore the significance of morphological characters in determining any distinct lineages as well as to investigate character evolution. In chapter 6, 41 floral characteristics of the studied taxa mapped onto the combined molecular and morphological analysis tree, to explore their relationship to the major lineages and character evolution in the subgenus. Chapter 7 examines the taxonomic importance of 43 leaf cuticular characters plotted onto the combined analysis tree in order to determine if leaf cuticular characters provide phylogenetic signal and/or taxonomic information in the species being studied. Finally, a general discussion synthesising these results and their implications for current and future research in *Hibbertia* is presented.

Abstract

Hibbertia Andrews (Dilleniaceae) has been studied for 200 years, but the systematics of species from SE Australia have not been addressed. Many of the taxa placed into 16 informal groups by Toelken are undescribed and their phylogenetic relationships are unknown. Therefore, a comprehensive morphological and molecular phylogenetic analysis of 87 SE Australian taxa within *Hibbertia* subg. *Hemistemma* was conducted.

Morphological studies were carried out including vegetative, indumentum, floral and leaf cuticular characters to assess intra-specific variation, determine any major lineages and taxon boundaries as well as to explore character evolution. Nuclear rDNA (ITS) and intergenic spacer regions of cpDNA (*trn*L-F) were amplified and the results compared and combined with morphological phylogenetic analysis.

The results, all the 16 informal species groups proposed by Toelken were placed instead into eight clades, with strong support for different species pairs and sister relationships. Most infrataxa within more variable species were not part of monophyletic lineages and none of the 16 informal species groups of Toelken were supported.

Vegetative, floral and leaf cuticular characters were important in understanding species of *Hibbertia* phylogenetic relationships, with general variation in numerous morphological characters such as hair type and density, androecium and gynoecium characteristics, stomata (shape and size), anticlinal and periclinal cell walls and trichome bases. The molecular data and combined data analysis enabled us to recognise eight clade, most with low support, with different groups of closely related taxa received strong support. Several infrataxa within more variable species did not form monophyletic lineages. Despite a high number of homoplasious, several morphological synapomorphies for Clade/taxon definition were discernible such as: lack of indumentum on stem, revolute leaf margins, acuminate inner sepal bases, >15 mm leaf blade width, 10.1–15 mm leaf blade width, absent of inner sepal, ≥10.1 mm inner sepal width, terminal pores anther dehiscence, ovate petal shape, spherical seed shape and 3.1–4 abaxial epidermal cell length to width ratio.

Contents

Chapter 1

General Introduction and Literature Review

Hibbertia (Dilleniaceae) is a large Australian genus of ~380 species (including c. 110 new and undescribed), mainly endemic to Australia (CHAH 2008). The main species diversity and endemism occurs in eastern Australia, Western Australia and the North Territory, but the genus extends to Fiji, Madagascar, New Caledonia and New Guinea (Tucker and Bernhardt 2000). The systematic delimitation of *Hibbertia* is poorly understood and species phylogenetic relationships are unknown. Phylogenetic research on this genus and in particular, the largely SE Australian clade of subgenus *Hemistemma* is required for a more accurate understanding of diversity and evolution in the group, as well as better taxon definition for conservation purposes.

Horn (2005, 2007) in a molecular phylogenetic study of Dilleniaceae produced a series of clades within *Hibbertia* subg. *Hemistemma*, one of which includes most of the taxa from eastern and southern Australia. This clade contains 93 species (many undescribed) and is currently divided into 16 informal species groups (H. Toelken pers. comm. 2009, see Table 1) based on morphological characters (Toelken 1998, 2000, 2010), As most of these taxa have not been investigated in detail, phylogenetic investigation of the 16 informal proposed groups within the SE Australian *Hemistemma* clade will form the basis of this current study.

1.1 Classification

Hibbertia (Dilleniales; Dilleniaceae) from the order Dilleniales currently includes 11 genera: *Acrotrema* Jac, *Curatella* Loefl., *Davilla* Vand., *Didesmandra* Stapf., *Dillenia* L., *Doliocarpus* Rol., *Hibbertia* Andrews, *Pachynema* R.Br. ex DC., *Pinzona* Mart.& Zucc., *Schumacheria* Vahl, and *Tetracera* (Stevens 2001; Horn 2005, 2007). There are four subgenera recognised within *Hibbertia* (Horn 2007), based on a molecular phylogenetic study by Horn (2005):

1. *Hibbertia* subg. *Hibbertia*
2. *Hibbertia* subg. *Adrastea* (DC.) J.W.Horn

3. *Hibbertia* subg. *Hemistemma* (Thouars) J.W.Horn
4. *Hibbertia* subg. *Pac*hynema (R.Br. ex DC.) J.W.Horn

Recent phylogenetic analyses of the class Magnoliopsida placed Dilleniaceae to the order Dilleniales (Thorne and Reveal 2007; APG III 2009) and a sister to either Caryophyllales (Chase *et al.* 1993; Soltis *et al.* 2000) or Vitaceae (Bernhardt 1996; Savolainen *et al.* 2000; Hilu *et al.* 2003).

1.2 Geographical distribution

According to APNI (2007) most of the 93 species of *Hibbertia* of the SE Australian *Hemistemma* clade in 1) Eastern Australia particularly in the coastal side of Great Dividing Range in Queensland; 2) New South Wales; 3) Tasmania; 4) Victoria; and 5) Coastal New South Wales.

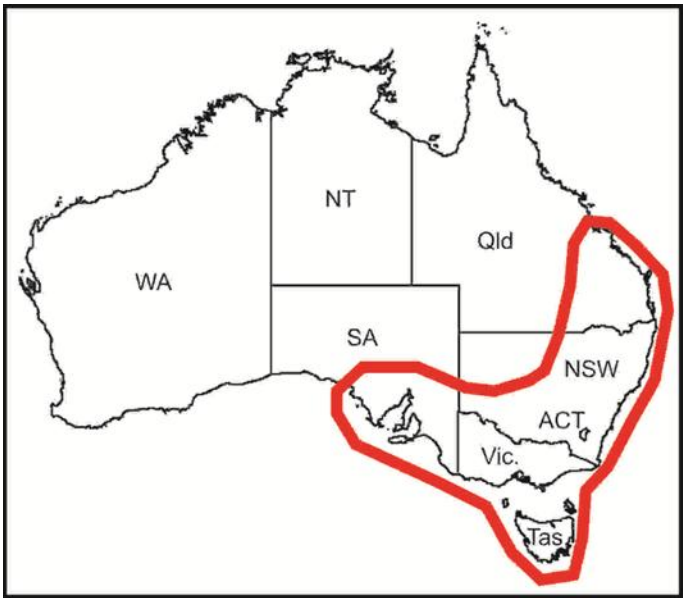

Figure 1. The distribution of south-eastern Australian clade of *Hibbertia* subgenus *Hemistemma.*

1.3 Biology

Toelken (1998) reported that *Hibbertia* hybrids were very uncommon, agreeing with Stebbins and Hoogland (1976). Bernhardt (1984) mentioned that *Hibbertia stricta* pollen are orange or mustard colour and social bees are the main pollinations. In addition, in *H. fasciculata* pollen is collected by bees from the anther clusters using thoracic vibration and their abdomen is placed parallel to the stigma so that it can receive the pollen (Bernhardt 1986).

Dickison (1982) described the pollen grains of Dilleniaceae and Actinidiaceae, but concluded that pollen morphology may not be useful to describe species relationships, with only one difference noticed in Dilleniaceae: tectum incomplete punctuate to reticulate.

1.4 History

Hibbertia was first described by de Candolle (1817) who included 19 Australian species: *H. grossulariaefolia* from Western Australia and from eastern Australia: *H. volubilis, H. dentata, H. saligna, H. procumbens, H. virgata, H. fasciculata, H. linearis, H. obtusifolia*, *H. diffusa*, *H. monogyna*, *H. pedunculata*, *H. serpyllifolia*, *H. aspera*, *H. hermaniifolia*, *H. oblongata*, *H. cistifolia*, *H. tomentosa* and *H. lepidota.* He also recognized *H. sericea*, based on Robert Brown's specimens collected near present-day Melbourne. Bentham (1863) described 41 species of *Hibbertia* within section *Pleurandra* from Australia, providing illustrations of some morphological characters, along with a description of their distribution within Australia. Two species from Madagascar were also affiliated with *H.* sect. *Hemistemma,* despite possessing opposite leaves. Inflorescence characteristics of subgenus *Hemistemma* were observed by de Candolle (1824), but some of his subgeneric characteristics overlap, making the distinction of subgenus *Hemistemma* unclear.

Bentham (1863) listed 67 Australian species of *Hibbertia*, providing descriptions of their morphological characteristics and geographical ranges. Significant taxonomic studies on *Hibbertia* species were also made in Western Australia by Wheeler (Wheeler 2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b), who recognised 19 new endemic species and new six varieties, providing a key to 126 species and varieties of *Hibbertia*, based on morphology. Similarly, Toelken and Wheeler (2002) made taxonomic notes on three *Hibbertia* species from Tasmania, based on surface indumentum, floral morphology and ecological distribution. Toelken also informally arranged 16 species and subspecies into one group (*Aspera* group) and 17 species and subspecies in another (*Sericea* group) (see Ch. 2: Tab. 1).

1.5 Circumscription and phylogenetic position of *Hibbertia* Andrews within Dilleniaceae

The family circumscription of *Hibbertia* has changed throughout the years as illustrated in table 1.

Table 1. Comparison between different classifications of Dilleniaceae.

Pre 1960	Post 1960
1- de Candolle (1824), Dilleniaceae tribes: a) Delimeae b) Dillenieae	1- Eames (1961): a close relationship between the orders Dilleniales and Ranales
2- Bentham (1863), Dilleniaceae genera: a) *Adrastaea* b) *Candollea* c) *Hibbertia* d) *Pachynema* e) *Wormia*	2- Hutchinson (1964), merged Gilg Werdermann (1925) tribes: a) Acrotremeae + Dillenieae b) Hibbertieae c) Tetracereae
3- Gilg and Werdermann (1924), Dilleniaceae tribes: a) Acrotremeae b) Dillenieae c) Hibbertieae d) Tetracereae	3- Horn (2005, 2007), Dilleniaceae subfamilies: a) Delimoideae b) Doliocarpoideae c) Hibbertioideae d) Dillenioideae
4- Hoagland (1952), elevated the above tribes to subfamilies	

The first classification system was based on de Candolle (1824) who classified Dilleniaceae into two tribes: a) Delimeae; b) Dillenieae. Bentham (1863) subsequently described five genera:

a) *Adrastaea*: Staminodia absent.

b) *Candollea*: Stamens are united in three clusters.

c) *Hibbertia*: Stamens free, more than 10 (or less), grouped one side of the pistil or surrounding the pistil.

d) *Pachynema*: Androecium bicyclic, outer (2–10) fertile stamens, two in the inner or staminodes.

e) *Wormia*: Trees with large leaves.

A new classification system was developed by Gilg and Werdermann (1925), who reported four tribes within the Dilleniaceae depending on the carpel number (1–20). The tribes were as follows:

a) Acrotremeae
b) Dillenieae
c) Hibbertieae
d) Tetracereae

In contrast, Hoagland's (1952) classification elevated four tribes mentioned above to the rank subfamily based on characters of the cymose inflorescences.

Eames (1961) suggested a close relationship between the orders Dilleniales and Ranales, based on the presence of fasciculate stamens and arillate seeds. Conversely, there are numerous characters not supporting this classification, and the characters tricolpate pollen and the transformation of stamens into nectaries are absent from the Dilleniales. Hutchinson (1964) made significant changes to the four tribes of Dilleniaceae created by Gilg and Werdermann (1925) merging the Acrotremeae into the Dillenieae.

Tucker and Bernhardt (2000) observed two types of floral development in Australian species of *Hibbertia*, symmetrical and zygomorphic flower. In the first, the floral parts (calyx and corolla) arise in quincuncial helices, while in the second arises in unidirectionally from one side of the flower. The stamens number increases over the time in organogeny period for androecium in the meristematic activity time; meanwhile, variations appear among population in different areas.

Horn (2005) proposed a new classification based on combined molecular and morphological data sets which was different from the previous classifications, characterised by:

a) Leaf architecture and the venation patterns (parallel or non-parallel).
b) Anther dehiscence (longitudinal slits or apical pores).
c) The carpel fusion (apocarpous or syncarpous).

Horn (2007) also divided the family into 4 subfamilies Delimoideae, Doliocarpoideae, Hibbertioideae and Dillenioideae (Fig. 2). Delimoideae was sister to the rest of the family and included only one genus: (*Tetracera*).

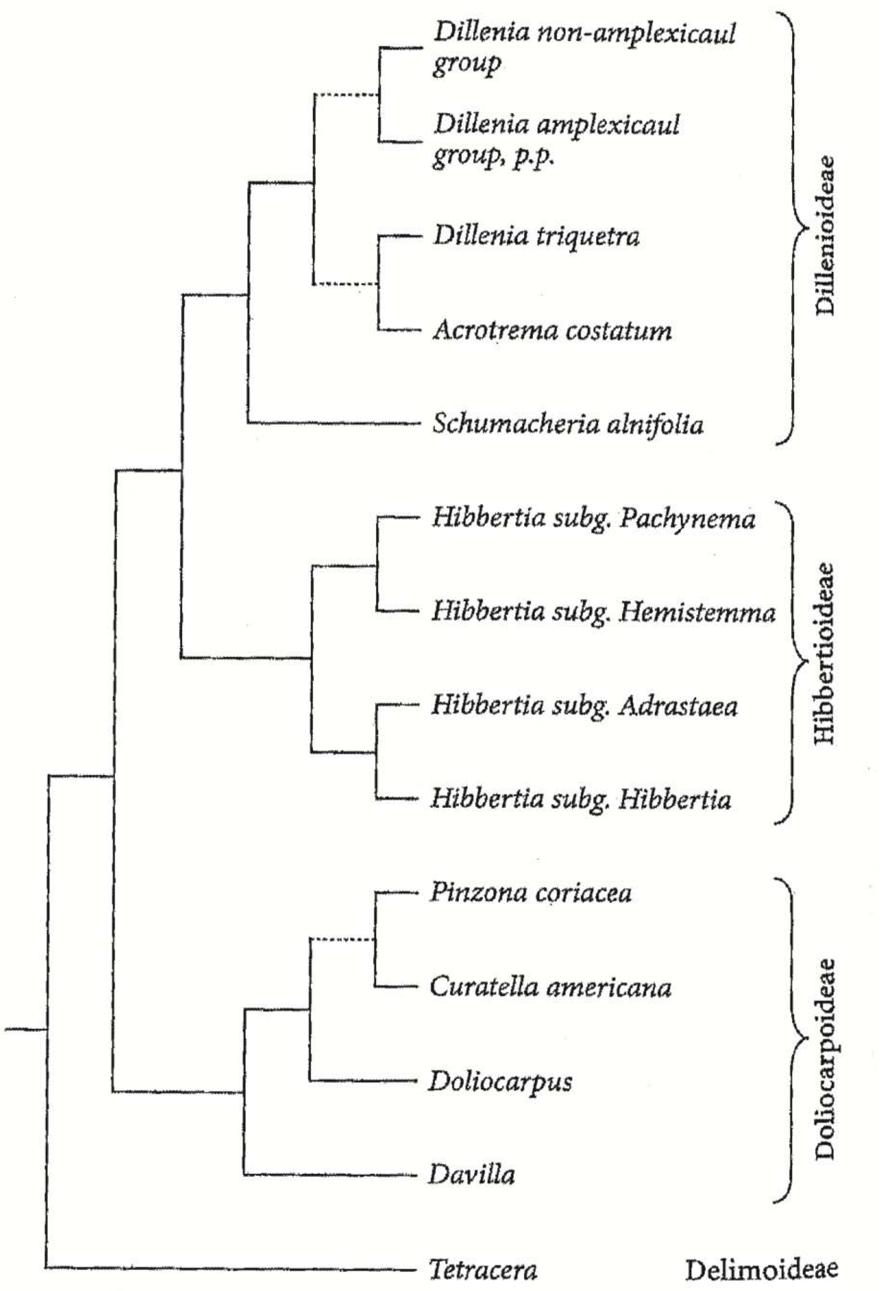

Figure 2. Dilleniaceae. Summary tree of relationships in Dilleniaceae from Horn (2007)

The Doliocarpoideae clade include the genera *Curatella*, *Davilla*, *Dliocarpus* and *Pinzona* as a sister clade. The Hibbertioideae consisted of *Hibbertia*, *Adrastaea* and *Pachynema* and was treated as an Old World clade.

1.6 Overall aims of this project

The present study aims to do a detailed systematic study of species relationships based on morphological and molecular data sets for the approximately 87 species within 16 informally recognised species groups (complexes) proposed by Toelken in the clade of *Hemistemma* recognised by Horn (2005) distributed mainly in eastern and southern Australia. A phylogenetic classification is needed to determine systematic relationships of species of *Hibbertia* within the clade, as previous studies did not cover all of the taxa, nor include many possibly useful morphological characters.

1.6.1 Intraspecific variation within groups/individuals

Hibbertia displays wide range of geographical distribution across Australia and occurs in numerous habitats. Morphological variation has always been problematic; for example, should species be distinguished on surface indumentum, or only in consideration with other morphological characters. Toelken (1998) recorded 15 different species and varieties of *Hibbertia* from SE Australia based on morphological characteristics within the SE Australian clade of subg. *Hemistemma* of Horn (2005). Toelken (1998, 2000, 2010, pers. comm. 2009) proposed 16 informal species groups and these form the basis for this study (Table 1). In *Hibbertia*, variation has been reported in leaf venation patterns (Rury and Dickison 1977) and morphological characteristics of *Hibbertia* were also reported by Dickison *et al.* (1978) for 27 species.

Systematic relationships within this clade are unclear and in depth research has been presented on members of the SE Aust. subg. *Hemistemma* clade and species relationships within this clade remain unclear. What little has been done used only morphological characters (Dickison 1967, 1968, 1969, 1970a, 1970b; Dickison *et al.* 1978; Dickison *et al.* 1982; Toelken 1998, 2000, 2010). As a result, additional characters especially anatomical and cuticular characteristics have not been studied. Thus, this study is a contribution towards a better understanding of *Hibbertia* subgenus *Hemistemma* based on examining new sets of characters and combined them into molecular phylogenetic analyses. The informally define groups of Toelken (1998, 2000, 2010, pers. comm. 2009) and their currently recognised morphological characteristics are listed in Table 1.

Table 2. Informal species groups within the SE Australian clade of *Hibbertia* subg. *Hemistemma* and their characters is show. MS indicates unpublished manuscript names of Toelken (after Horn 2005).

NOTE:
This table is included on pages 1-8 - 1-11 of the print copy of the thesis held in the University of Adelaide Library.

1.6.2 Surface indumentum

The most significant morphological features which have been used to distinguished *Hibbertia* species are surface indumentum and stamens characters (Toelken 1998, 2000, 2010). Toelken found that hair types are useful to distinguish between different *Hibbertia* species. For example, *H. aspera* subsp. *aspera* young adaxial leaf surfaces possess multi-angulate stellate hairs and the abaxial surface has few multi-angulate radially stellate hairs. In *H. cinerea* the leaf surface bases multi-angulate stellate hairs and few hooked hairs.

Black (1922) included *H. cinerea* as a variety of *H. sericea* based on the dense indumentum of stellate hairs. However, all hairs in the *Sericea* group have a tuberculate base which is an aggregation of epidermal cells on the base of the trichome (Toelken 1998).

Systematic studies are needed to solve species relationships within and between the groups, combined with phylogenetic relationships between the groups.

1.6.3 Leaf Venation

Leaf morphology and venation patterns of *H. acicularis* were investigated by Stebbins and Hoogland (1976) who noted that the venation is dichotomous, arising from the mid-vein. The size and the shape of the areoles are variable and these patterns are different from Western Australian species. *Hibbertia* has three different polymorphic leaf venation patterns according to Rury and Dickison (1977): weakly brochidromous stout, brochidromous and craspedodromous.

1.6.4 Anatomy

Ananda and Das (1979) noted that sclereids were absent in *H. aspera* leaves and that this could support a relationship with *H. hypericoides* (DC.) Benth. The presence, distribution and nature of sclereids might also be useful in species-level taxonomic studies. They also mentioned that “dilated tracheoids” form at the veinlet ending single or in clusters in many *Hibbertia* species, especially *H. serpyllifolia* R.Br., while “foliar sclereids” and spheroidal sclereids are present in 31 *Hibbertia* taxa including *H. bracteata* (DC.) Benth. and *H. vestita* A.Cunn. ex.Benth.

Dickison *et al.* (1978) examined the angular pores in the xylem elements, noting that porate xylem elements occur in most *Hibbertia* species, excluding *H. exutiacies*, *H. huegelli* and *H. scandens*, which are characterised by rounded pores. They did not formally categorise species according to these features, but recommended further morphological and anatomical studies.

Prychid (1999) noted that calcium oxalate crystals are widespread in flowering plants with three main crystals types: druses, styloid and raphides. Two main crystal types are present in *Hibbertia*, with raphides crystals and druses (Dickison 1968). The druses crystals in *H. tetrandra* and *H. cuneiformis* are present in the gynoecium. This character along with opposite or oblique forms of gynoecia features within bicarpellate flowers might be indecisive to depends on to separate the species or include them in one distinct group.

Ananda and Das (1979) also examined the ramiform sclereids inside in the mesophyll of the ericoid leaf of *H. pedunculata*, as well as the sclerotised epidermis of the lamina, as well as epidermal sclereids in *H. acicularis*. They suggested that these features in the epidermal tissue of the lamina and anatomical characteristics of *H. acicularis* could be phylogenetically informative.

1.6.5 Floral Features

Dickison (1968) described the carpels for five *Hibbertia* species, including *H. exutiacies* (*Exutiacies* group) and *H. cuneiformis*. He pointed that the bisexual flowers of *Hibbertia* species have 1, 2, 3, 5 or 10 carpels, with an apocarpous gynoecium, noting that the indumentum varied from glabrous to pubescent, but that more studies on morphology were needed to resolve species relationships. Inflorescence morphology of *H. cuneiformis* was also investigated by Stebbins and Hoogland (1976) who recommended more studies, especially on the reproductive system.

According to Toelken (1998), the *Aspera* group possesses anthers which arranged in a fan-shape in one side of ovary, with short styles just protruding from under them. The leaves are flat, scarcely recurved with central vein visible, but not thickened. Fasciculate hairs are present. Toelken also stated that the number of stamens (in one cluster) differs from one species to another, and the flowers are stalked. However, he did not mention any numbers of stamens for other *Hibbertia* species and other morphological characters such as anthers dehiscence, fusion of the androecium, as well as synstemony. Similarly, gynoecial characteristics do not seems to have been recorded uniformly. In the molecular study by Horn (2005), *H. empetrifolia* (DC) Hoogland subsp. *empetrifolia* was the only included representative of the *Aspera* group, so further morphological and anatomical studies are needed to provide more detailed understanding for species diversity in this and other groups informally proposed by Toelken and to determine the range of flora; characteristics.

Flowers of *H. humifusa* F.Muell. are single, sympodial, arranged along the branches, stalked (mainly pedunculate) and the plant is decumbent with soft branches that are not flanged (APNI 2007). According to Toelken (1995), *H. humifusa* with three subspecies:

a) *H. humifusa* F.Muell subsp. *humifusa*
b) *H. humifusa* subsp. *erigens* Toelken
c) *H. humifusa* subsp. *erigens* Toelken

These three *H. humifusa* subspecies were separated on the basis of surface indumentum, but would be important to use other features such as leaf cuticular and DNA studies to define taxon boundaries more precisely.

One of the most significant features in *Pedunculata* group is the androecium. The 15–25 stamens are diffuse and prostrate (rarely erect) surround the hairy ovaries and are accompanied by one or two small staminodia (Bentham 1863). Owing to variation in the calyx as well as the plant size, *H. platyphylla* and related species are difficult to recognise from other, but this may be in part of problems with difficulties in determining the identity of collected *Hibbertia* specimens (Toelken 2000). Surface indumentum characters may help to as well as characters such as anatomical and DNA to solve such problems.

In conclusion, different species of *Hibbertia* have been extensively treated by de Candolle (1824), Bentham (Bentham 1863), Stebbins and Hoogland (1974, 1976), Bernhardt (1984, 1986), Dickison (1967a, 1968, 1970a, 1970b, 1978, 1982) mostly providing general descriptions and geographical locations in Australia. Up to 126 species of *Hibbertia* were studied in Western Australia by Wheeler (2002a, 2002b, 2002c, 2002d, 2003, 2004a, 2004b) based on morphological features. Toelken made significant contribution in recognising different species, subspecies and verities of *Hibbertia* (1998, 2000, 2010), and proposed 16 informal species groups within the SE Australian *Hemistemma* clade based on morphological characters (pers. comm. 2009). However, phylogenetic relationships and character evolution are poorly understood and need to investigate in detail.

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Chapter 2

A morphological phylogeny of *Hibbertia* Andrews subg. *Hemistemma* (Thouars) J.W.Horn (Dilleniaceae) from south-eastern Australia

Abstract

Phylogenetic relationships among 87 of the 93 taxa of the SE Australian clade of *Hibbertia* subgenus *Hemistemma* were studied using morphological (vegetative and floral) characters to test the monophyly and relationships, assess taxon boundaries and character evolution. The majority of the taxa are resolved into seven distinct clades, but with high levels of homoplasy and low bootstrap support. These clades can be defined mainly on hair characteristics (especially, mixed hairs presence, length and branching), leaf characters (especially, leaf margin, glabrous and villous leaf surfaces), anther and filament character (especially, stamen number, clusters and position). Despite high levels of homoplasy and low supports for the main clades, there was support for some of smaller monophyletic lineages within them, but none of the 16 informal species groups proposed by Toelken (unpubl.) was supported. In addition, several taxa previously recognised as subspecies did not form monophyletic species lineages, often falling instead within well separated clades or groups: *H. glebosa* subsp. *oblongata* (Clade F) and *H. glebosa* subsp. *glebosa* (Clade G), *H. empetrifolia* subsp. *radians* close to *H. truncata* (Clade G), *H. aspera* subsp. *pilosifolia* was located as a sister to *H. decumbens* + *H. glaciifera* (Clade F) and *H. aspera* subsp. *aspera* close to *H. notabilis* (Clade F).

Introduction

The Guinea Flower (*Hibbertia* Andrews, Dilleniaceae) with ~380 species, most undescribed, is distributed mainly in Australia, but extends to Madagascar, Malaysia, Fiji, New Caledonia and New Guinea (Tucker and Bernhardt 2000; CHAH 2008). *Hibbertia* is one of 11 genera within the family: *Acrotrema* Jac, *Curatella* Loefl., *Davilla* Vand., *Didesmandra* Stapf., *Dillenia* L., *Doliocarpus* Rol., *Hibbertia* Andrews, *Pachynema* R.Br.ex DC., *Pinzona* Mart.& Zucc., *Schumacheria* Vahl, and Tetracera (Horn 2005, 2007).

Hibbertia also has four subgenera, based on indumentum (vestiture) and floral features: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn (Horn 2005, 2007). Toelken (1998, 2000, 2010) described species and subspecies of *Hibbertia* within the SE Australian groups: *Aspera* and *Sericea* based mainly on morphology and surface indumentum features. Toelken also proposed 16 informal species groups within the SE Australian clade of *Hibbertia* subgenus *Hemistemma* described by Horn (2005) (Table. 1). However, many of the species, subspecies and varieties in these proposed groups are undescribed and their phylogenetic relationships are poorly understood. The need for comprehensive study of these groups is thus essential.

The representative of subgenus *Hemistemma* are characterised as follows: perennial shrubs to medium size trees; leaves are ericoid with fasciculate hairs; flowers are usually terminal or axillary; calyx of 5 sepals, free or sometimes fused; corolla often (3–5) petals, free; androecium, c. (1–) 5–100 (–300) stamens, partly staminodal, either polysymmetric or monosymetric; gynoecium of 1–5 (–10) carpels, glabrous or pubescent; ovules 1–25 per carpel; seeds often 1–8, aril sub-fleshy (Horn 2005).

Previous studies of *Hibbertia* species have dealt with the taxonomic identification based on morphological features. de Candolle (1824) used habit of the inflorescence to classify the Dilleniaceae into two tribes Delimeae and Dilleniae, and Bentham (1863) used a wide range of characters to define the 67 *Hibbertia* species he recognised and associated with their geographical distribution in Australia. Subsequently, Dickison (1968) described carpel features for five Australian *Hibbertia* species (*H. cuneiformis* (Labill.) Gilg., *H. dentata* R.Br., *H. exutiacies* Wakef., *H. scandens* (Willd.) and *H. tetrandra* (Lindl) Gilg.), describing the gynoecium surface indumentum as “glabrous to densely pubescent” and the slender style as attached to the “swollen ovary”. This agreed with Wakefield’s (1957) description of *H. australis* and *H. stricta,* when he used surface indumentum, androecium and gynoecium characters, as well as illustrating their distribution and intra-specific variation.

Table 1. Informal species groups recognised by Toelken (1998, 2000, 2010, pers. comm. 2009) within the south-eastern Australian clade of *Hibbertia* subg. *Hemistemma* recognised by Horn (2005).

No.	Group	**Species**
1	Australis	*H. australis* N.A.Wakef.; *H. glebosa* MS; subsp. *glebosa*; subsp. *oblongata* (J.M.Black) MS; *H. tenuis* Toelken
2	Acicularis	*H. acicularis* R.Br. ex DC.; *H. oligastrota* MS; *H. caespitulosa* MS; *H. cuneifolia* MS; *H. protuberans* MS; *H. pusticulata* MS; *H. woronarana* MS
3	Aspera	*H. appressa* Toelken; *H. aspera* DC.; subsp. *aspera;* subsp. *pilosifolia* Toelken; *H. cinerea* (R.Br. ex DC.) Toelken; *H. decumbens* Toelken; *H. empetrifolia* (DC.) Hoogl.; subsp. *empetrifolia*; ssp. *radians* Toelken; subsp. *uncinata* Toelken; *H. hirta* Toelken; *H. hirticalyx* Toelken; *H. notabilis* Toelken; *H. pallidiflora* Toelken; *H. rhynchocalyx* Toelken; *H. truncata* Toelken
4	Bracteata	*H. bracteata* (R.Br. ex DC.) Benth.; *H. nitida* (R.Br. ex DC.) Benth.
5	Cistoidea	*H. cistoidea* (Hook.) C.T.White; *H. covenyana* Conn; *H. semota* MS; *H. glaciifera* MS
6	Exutiacies	*H. cistiflora* N.A.Wakef.; *H. exutiacies* N.A.Wakef.; *H. oxycraspedotus* MS; *H. tetranthera* MS
7	Hermaniifolia	*H. acaulothrix* MS; *H. hermanniifolia* DC.; subsp. *hermanniifolia*; ssp. *recondita* MS; *H. hexandra* C.T.White; *H. spathulata* N.A.Wakef.; *H. reticulata* MS
8	Humifusa	*H. humifusa* F.Muell.; subsp. *debilis* Toelken; subsp. *erigens* Toelken; subsp. *humifusa*
9	Pedunculata	*H. corifolia* Sims; *H. dispar* MS; *H. expansa* MS; *H. florida* MS; ssp. *angustinervis* MS; ssp. *florida; H. fruticosa* MS; *H. pedunculata* R.Br. ex DC.; *H. porcata* MS
10	Serpyllifolia	*H. serpyllifolia* R.Br. ex DC.
11	Riparia	*H. riparia* (R.Br. ex DC.) Hoogland
12	Rufa	*H. obtusibracteata* Toelken; *H. pilifera* MS; *H. rufa* N.A.Wkef.; *H. surcularis* MS
13	Sericea	*H. crinita* Toelken; *H. paeninsularis* J.M.Black; *H. platyphylla* Toelken; subsp. *halmaturina* Toelken; subsp. *major* (J.M.Black) Toelken; subsp. *platyphylla*; *H. praemorsa* Toelken; *H. sericea* (R.Br. ex DC.) Benth.; var. *densiflora* (Hook.) Benth.; var. *scabrifolia* J.M.Black; var. *sericea*; *H. sessiliflora* Toelken; *H. simulans* Toelken; *H. superans* Toelken; *H. villifera* Tepper ex Toelken; *H. hirsuta* (Hook.) Benth.; *H. patens* Toelken
14	Stricta	*H. apoda* MS; *H. devitata* MS; *H. setifera* MS; *H. stricta* (R.Br. ex DC.) F.Muell.
15	Strigosa	*H. calycina* (DC.) N.A.Wakef.*;* *H. hendersonii* S.Reynolds; *H. horrida* MS; *H. strigosa* MS; *H. synandra* F.Muell.
16	Vestita	*H. boweniensis* MS; *H. erectinervis* MS; *H. hypsophylloides* MS; *H. vestita* A.Cunn. ex Benth.

The intraspecific variation in the genus, especially in floral features is often presented by: 1) floral symmetry with sepals and petals are arising in quincucial helices in radially symmetry (Tucker and Bernhardt 2000); and 2) stamen characters such as 3–4 stamens attached to only one "Pedestal" (north-eastern coastal population in New South Wales of *H. fasciculata* R.Br. ex DC.) *vs.* 0–12 stamens, each on one "Pedestal" (south-eastern Australian populations of *H. fasciculata* R.Br. ex DC.) with the reduction of stamen number due to reduction of all plant parts (Tucker and Bernhardt 2003). Toelken (1998) also separated the *H. aspera*–*H. empetrifolia* complex into two series, based on stamen number: *H. empetrifolia* (4–9) and *H. aspera* (9–15). In contrast, unlike some of the other *Hibbertia* species, the number of stamens “may vary considerably”, especially in *H. sericea* and other related species (Toelken 2000).

In recent years, several new *Hibbertia* species have been recognised and morphological studies published such as Wheeler (2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b), including a key to 126 Western Australian species. Toelken and Wheeler (2002) also investigated three Tasmanian *Hibbertia* species based on morphology: *H. procumbens* (Labill.) DC, *H. hemignosta* (Steud.) J.R.Wheeler and *H. hibbertoides* (Steud in Lehm.) J.R.Wheeler and Toelken’s (2010) treatment of *H. devitata* Toelken, *H. setifera* Toelken and *H. glebosa* Toelken was also mainly based on morphology and surface indumentum. Nevertheless, the majority of the SE Australian species of *Hibbertia* subgenus *Hemistemma* species are still poorly known.

Toelken’s 16 informal SE Australian species groups can be recognised morphologically as follows:

***Australis* group**: Leaves with no or short axillary tufts, margins prominent, revolute, central vein bulging (no undersurface visible) and typically with rounded. Flowers single, ± pedunculate; bracts usually close to flower.

Acicularis group**:** Plant glabrous and diffuse, leaves narrow to linear, margins recurved.

***Aspera* group:** leaves flat, scarcely recurved, central vein visible, fasciculate hairs usually present. Flowers stalked; bracts on upper third. Anthers fan-shaped, on one side of ovary; styles short.

***Bracteata* group**: Leaves with pronounced axillary tuft, flat, margins slightly recurved, leaf base flanged, central vein scarcely raised; hairs simple only. Flowers single, terminal on fasciculate branches; stamens in one cluster.

***Cistoidea* group**: Leaves with axillary tufts absent or almost so, leaf bases scarcely flanged, margins prominent, revolute. Flowers pedunculate; calyx lobes broad, without ridges, hairs fasciculate and simple hooked; stamens in one cluster.

Exutiacies group: Leaves spine-tipped, bases flanged, margins revolute; hairs simple, fasciculate. Flowers single, sessile on fasciculate branches; bracts 3 or more, subtending the flowers; stamens in a dorsal cluster; ovary hirsute to tomentose.

***Hermaniifolia* group**: Leaves oblanceolate or spathulate, margins ± revolute and discrete from raised central vein; hairs mainly fasciculate. Flowers single along branches (sympodial), pedunculate to sessile; stamens surrounding ovaries.

***Humifusa* group**: Plants decumbent with soft branches. Leaf bases not flanged, axillary tufts of fine simple hairs present, margins revolute, abaxial surface usually visible. Flowers single, sympodial, along branches, pedunculate; calyx ridged and recurved.

***Pedunculata* group**: Leaves bases scarcely decurrent, margins revolute, discrete; hairs simple. Flowers single, pedicellate and/or pedunculate; bracts linear-lanceolate, position variable; stamens surrounding hairy ovary.

***Riparia* group**: Leaves with revolute margins, undersurface not visible; axillary hair tufts pronounced, decurrent along flanges of leaf bases; hairs fasciculate and straight simple. Flowers single, sessile, terminal; bracts small; calyx ± ridged.

Rufa* group:* Leaves linear to lanceolate, apex ± acute, margins recurved, lamina glabrous. Flowers axillary; staminal filaments connate.

***Sericea* group**: Leaves usually with pronounced axillary tufts, bases scarcely flanged, margins revolute; hairs simple or simple and fasciculate (rarely only fasciculate). Flowers single in terminal clusters; bracts single, recurved. Outer calyx lobes ridged, margins recurved.

***Stricta* group:** Stems flanged. Leaf axillary tufts short or absent, leaf margins revolute, central vein bulging; hairs on leaves and calyx mostly short, stellate; flowers single, sessile, terminal; bract single, leaf-like, small, not recurved; fasciculate hairs on calyx rarely overtopped by hooked simple hairs.

***Strigosa* group**: Branches flanged. Leaf axillary tufts present, leaf margins prominently revolute. Flowers single, sessile, often surrounded by larger leaves, bract not visible; calyx hairs fasciculate; stamens 8–15.

***Vestita* group:** Leaves often with axillary tufts only below flowers, margins revolute, exposed undersurface glabrous; hairs simple, spreading. Flowers single, sessile, terminal, large; pistils often 3; stamens usually more than 25, surrounding carpels.

Although Horn (2005) undertook a preliminary investigation of character evolution in the Dilleniaceae, he sampled only 23 taxa within the SE Australian *Hemistemma* clade. As a result, no detailed studies exist for the phylogenetic significance of characters for the majority of SE Australian taxa, many of which are new or only recently described. In addition, the circumscription of intraspecific categories and relationships within the clade are also largely ambiguous. Accordingly, this study focuses on Toelken 16 informal groups and aims to:

a) Assess the monophyly of Toelken's proposed 16 informal species groups.
b) To determine if there are any distinct lineages within the SE Australian clade of *Hibbertia* subg. *Hemistemma*.
c) Assess intra-specific variation and character evolution within the clade.

Materials and methods

Taxon sampling

Ninety-three *Hibbertia* taxa, representing SE Australia were examined within the 16 informal proposed groups of Toelken (Tab. 1), using samples from SA herbarium specimens, collected from different allocation within SE Australia (Tab. 2).

Morphological studies

Herbarium samples were reconstituted by using 2 drops of detergent for 10–15 minutes for dry leaves and flowers. The samples were then placed under a dissecting light microscope (Zeiss model 1968), examined at ×10 magnification and photographed with a digital camera (Panasonic type Lumix model FS42 2005). SEM investigations were also conducted at Adelaide Microscopy, using both XL20 and XL30 type/ Philips (manufactured in 1994 by Philips in Eindhoven, The Netherlands), following standard preparation techniques. Surface indumentum terminology follows Hewson (1988).

Outgroup

For the outgroup, *H. ancistrophylla* J.W.Wheeler from Western Australia was included in the analysis, based on its position in a sister Clade, following Horn (2005)

Data analysis

A total of 105 morphological characters were coded into discrete states (Tab. 3), some binary, but most were multistate and/or polymorphic due to intra-specific variation in vegetative and floral characters. For each species, five measurements were taken from mature parts of the specimens, and the mean and range of values were recorded. Morphological matrix was created (see Appendix 1). The data were analysed using WinClada version 1.00.08 (Nixon 2002) with the retention option, 1000 replicates (TBR on, hold 10 trees per step.). Jackknife support was calculated with the same options, but TBR off.

Table 2. List of species observed with their voucher information

Taxon	Collector and state	Herbarium	Accession no.
H. acaulothrix	J.B. Briggs 2080, NSW	NSW	386
H. acicularis	B.M. Overton 1125, SA (KI);	AD	99014093
	H.C. Beauglehole 43835, Vic.	AD	98621183
H. ancistrophylla	J.W.Horn 4049, WA	AD	158577
H. apoda	collector unknown 3886, NSW	CANB	168185
H. appressa	A.M. Buchanan 1295, Tas.	HO	97456
H. aspera	H. Tryon 415915, Qld	BRI	358626
H. aspera subsp. *aspera*	R.S. Dick CL7a, Qld	BRI	253673
H. aspera subsp. *pilosifolia*	R.J. Henderson, L. Durrington , P. Sharpe 1040, Qld	BRI	182059
	J.R. Gelford 3370, Qld	BRI	054757
H. australis	D.J. Whibley 9655, SA	AD	98824292
H. boweniensis	P. Hind , J. Forlonge 2647, NSW	NSW	254914
	P. Hind 2401, Qld	NSW	254862
H. bracteata	R.D. Hoogland 12233, NSW	NSW	131
H. caespitulosa	van Reyen 9338, Qld	BRI	059659
H. calycina	G. Pulley 476, NSW	CANB	056574
H. cinerea	R. Bates 1409, SA	AD	98938203
	M. Kenny s.n., Sept. 1975 (SA, AD)	AD	NA
H. cistiflora	C. Burgess s.n., Jul. 1963 (NSW, AD)	CANB	004753
H. cistoidea	collector unknown 4652, Qld	BRI	133172
H. corifolia	H. Salasoo 1810, NSW	NSW	496
H. covenyana	R. Caveny, S.K. Roy 9042, NSW	CANB	00404379
H. crinita	Ian Abbott 475, SA	AD	171780
	D.J.E.Whibley 3817, SA	AD	97242022
H. cuneifolia	T. Tame 1127, NSW	NSW	47
H. decumbens	C. Burgees s.n., Nov. 1970 (NSW, AD)	CANB	036200
H. devitata	A.R.R. Higginson s.n., Oct. 1956 (SA, AD)	AD	966072887
H. dispar	R. Bates 10636 Vic.	AD	98801233
H. empetrifolia subsp. *empetrifolia*	R. Pullen 4123, NSW	AD	96620012
	Michael Evans 2509, SA	AD	unknown
H. empetrifolia subsp. *radians*	Lindley D. Williams 6005, SA	AD	9843585
	D.N.Kraehenbuehl 211, SA	AD	NA
H. empetrifolia subsp. *uncinata*	R.D. Hoogland 12243, NSW	NSW	218
H. erectinervis	collector unknown s.n., Nov. 1953 (NSW, AD)	CANB	126265
	J.M. Taylor 2413, NSW	CANB	8410978
H. expansa	J.R. Jelford 2547, NSW	CANB	037098

	J.L. Boorman 663, NSW	NSW	515
H. exutiacies	H.M. Cooper s.n., Oct. 1941 (SA, AD)	AD	7317115
	E.H. Ising s.n., Oct. 1959 (SA, AD)	AD	97029187
H. florida subsp. *florida*	J.M. Fox 87/125, NSW	CANB	00406335
H. florida subsp. *angustinervis*	R. Coveny, NSW	NSW	535
H. florida subsp. *florida*	collector unknown s.n., no date, NSW	AD	1086480
H. fruticosa	R. Coveny 8877, NSW	NSW	529
	K. Hill 2769, NSW	NSW	533
H. glaciifera	R.W. Johnson 2497, Qld	BRI	339654
	M. Lazarides 6953, Qld	BRI	051433
H. glebosa	M. Hyde 105, SA	AD	119336
H. glebosa subsp. *glebosa*	A.G. Spooner 8302, SA	AD	98326221
	R.V. Southcott s.n., Oct. 1960 (SA, AD)	AD	97323349
H. glebosa subsp. *oblongata*	B.M. Overton 2569, SA (KI)	AD	99649112
H. hendersonii	R. J. Henderson, L. Durrington , P. Sharpe 1059, Qld	BRI	182353
	C.H. Gittins 384, Qld	BRI	1
H. hermanniifolia subsp. *hermaniifolia*	G.L. Stebbins A–63, NSW	NSW	255007
H. hermanniifolia subsp. *recondite*	A.C. Beauglehole 37140, Vic.	CANB	237920
H. hexandra	R.D. Hoogland 11807, NSW	CANB	205127
H. hirsuta	R.D. Hoogland 11748, NSW	CANB	200389
	W.M. Curtis s.n., Nov. 1952 (TAS, AD)	HO	3114
H. hirta	I. Telford , H. Streimann 3808, NSW	CANB	056123
H. hirticalyx	W. M. Curtis s.n., Nov. 1974 (TAS, AD)	HO	29265
	A.E. Orchard 5860, TAS	HO	98430192
H. horrida	J.R. Jelford 2589, NSW	CANB	037408
H. humifusa subsp. *debilis*	H.C. Beauglehole 38020, Vic.	HO	64375
H. humifusa subsp. *erigens*	R. Thomas 583, Vic.	MEL	2019490
H. humifusa subsp. *humifusa*	A.C. Beauglehole 69828, Vic.	MEL	49799
H. hypsophylloides	L.G. Adams 3478, Qld	BRI	430130
H. nitida	collector unknown 26599, NSW	NSW	263
H. notabilis	David E. Albrecht 1037, Vic.	MEL	673624
H. obtusibracteata	G. Jackson 3008, SA (KI)	AD	99051222
H. oligastrota	J.L. Clarkson 5879, Qld	BRI	359875
H. oxycraspedotus	L.G. Adams , K. Paijmans 3780, NSW	CANB	381942
H. paeninsularis	B.M. Overton 2600, SA	AD	99733365
H. pallidiflora	R. Bates 7620, SA	AD	98649870
H. patens	G. Leiper 458082, Qld	BRI	447077
H. pedunculata	W. Forsyth, s.n., Jun. 1897 (NSW, AD)	AD	98142025
	J.M. Bechervaise s.n., Jan. 1948 (VIC, AD)	MEL	35577
H. pilifera	Michael Evans 2519, NSW	CANB	161485
H. platyphylla subsp. *halmaturina*	B.M. Overton 2498, SA	AD	99610194
H. platyphylla subsp. *major*	J.B. Weber 6166, SA	AD	97947114
H. platyphylla subsp. *platyphylla*	N. Donner 11095, SA	AD	98631006
H. porcata	H.C. Beauglehole 91076, Vic.	MEL	119768

H. praemorsa	P. Gilmour 5272, NSW	CANB	8504647
H. protuberans	N. Geary s.n., Feb. 1944 (BRI, AD), Qld	BRI	010716
H. pusticulata	I. Cowie 164, Qld	BRI	253649
H. reticulata	L.J. Brass 19339, Qld	QSL (CANB)	199556
	B.J. Conn 3787, Qld	AD	99542107
H. rhynchocalyx	J.B. Williams 603, NSW	NSW	95
H. riparia	E.N.S. Jackson 4291, SA	AD	4291
H. rufa	R.D. Hoogland 11917, Vic.	MEL	580142
	J.D. Briggs 692, NSW	NSW	833
H. semota	P.K. Latz 10646, NT	AD	99508051
	B.G. Thomson 3552, NT	AD	99507217
H. sericea var. *densiflora*	R.D. Hoogland 11910, Vic.	MEL	572143
H. sericea var. *scabrifolia*	R. Melville, P. Morris , A. Hicks 985, Vic.	NSW	403
H. sericea var. *sericea*	R.D. Hoogland 11888, Vic.	CANB	205548
	M.G. Corrick 8562, Vic	CANB	347698
H. serpyllifolia	P.R. Sharpe 3001, Qld	BRI	405505
	J.R. Jelford 3622, NSW	CANB	055004
H. sessiliflora	A.C. Beauglehole 38005, Vic.	MEL	517653
H. setifera	R. Bates 61074, SA	AD	155475
H. simulans	collector unknown s.n., Nov. 1918 (NSW, AD)	CANB (NSW)	
H. spathulata	N.G. Walsh , K.C. Norris 2209, Vic.	MEL	119822
H. stricta	J. Taylor, J. Rymer , R. Jackson 1251, NSW	CANB	8008298
H. strigosa	H.C. Beauglehole 3590, Vic.	MEL	695584
	A.C. Beauglehole 91572, Vic.	MEL	119619
H. superans	H. Streimann 053933, NSW	CANB	053933
H. surcularis	collector unknown 287, Vic.	MEL	1003813
H. synandra	B. Hyland 7065, Vic.	BRI	235206
H. tenuis	D.E. Murfet , R.L. Taplin 1910, SA	AD	99531091
H. tetranthera	R.D. Hoogland 12252, NSW	NSW	202
H. truncata	S.G. Harris 108, Vic.	HO	120083
H. vestita	W.J. McDonald 398283, Qld	BRI	375462
H. villifera	R. Bates 61095, SA	AD	155427
H. woronarana	R.D. Hoogland 12257, NSW	CANB (MEL)	572180

Table 3. List of morphological and leaf cuticular characters together with their character states used in the analysis

1	Habit: erect (0); procumbent (1); decumbent (2)
2	Plant height (m): 0.1–0.5 (0); 0.6–1 (1); ≥1.1 (2)
3	Simple hairs on stem: present (0); absent (1)
4	Hooked hairs on stem: present (0); absent (1)
5	Stellate hairs on stem: present (0); absent (1)
6	Indumentum on the stem surface: sparsely pubescent (0); densely pubescent (1); absent (2)
7	Basal hair cells: unspecialised (0); tuberculate (1)
8	Hair length (mm): 0.1–0.5 (0); 0.6–1 (1); 1.1–1.5 (2); >1.5 (3)
9	Hair branch number: unbranched (0); 1–2 (1); 3–4 (2); 5–7 (3); >7 (4)
10	Hair branch length: equal (0); semi-equal (1); unequal (2); absent (3)
11	Petiole indumentum: present (0); absent (1)
12	Simple hairs on petiole: present (0); absent (1)
13	Hooked hairs on petiole: present (0); absent (1)
14	Stellate hairs on petiole: present (0); absent (1)
15	Indumentum on petiole surface: sparsely pubescent (0); densely pubescent (1); absent (2)
16	Axillary hair tufts: present (0); absent (1)

17 Leaf blade shape: broadly obovate (0); obovate (1); narrowly obovate (2); oblanceolate(2); narrowly oblanceolate (3); linear-oblanceolate (4)
18 Leaf base: cuneate (0); acute (1); obtuse (2); flanged (3)
19 Leaf apex: acute (0), rounded (1); acuminate (2); obtuse (3); truncate-apiculate (4)
20 Leaf margin: entire (0); recurved (1); revolute (2)
21 Leaf lamina length (mm): <5–10 (0); 10.1–15 (1); 15.1–20 (2); >20.1 (3)
22 Leaf blade width: (mm): <1–5 (0); 5.1–10 (1); 10.1–15 (2); >15 (3)
23 Leaf mucro length (mm): absent (0); <0.5–0.8 (1); >0.9 (2)
24 Leaf mid-vein width (mm): <0.1–0.2 (0); 0.3 – 0.4 (1); > 0.4 (2)
25 Leaf adaxial midrib indumentum: present (0); absent (1)
26 Leaf abaxial midrib indumentum: present (0); absent (1)
27 Leaf adaxial surface indumentum: present (0); absent (1)
28 Simple hairs on leaf adaxial surface: present (0); absent (1)
29 Hooked hairs on leaf adaxial surface: present (0); absent (1)
30 Stellate hairs on leaf adaxial surface: present (0); absent (1)
31 Indumentum on the leaf adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
32 Leaf abaxial surface indumentum: present (0); absent (1)
33 Simple hairs on leaf abaxial surface: present (0); absent (1)
34 Hooked hairs on leaf abaxial surface: present (0); absent (1)
35 Stellate hairs on leaf abaxial surface: present (0); absent (1)
36 Indumentum on the leaf abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
37 Bract shape: linear (0); linear-subulate (1); linear-lanceolate (2); elliptic (3), oblanceolate (4), leaf like (5); lanceolate-elliptic (6)
38 Bract margin: flat (0); less recurved (1); more recurved (2)
39 Bract surface indumentum: present (0); absent (1)
40 Simple hairs on bract surface: present (0); absent (1)
41 Hooked hairs on bract surface: present (0); absent (1)
42 Stellate hairs on bract surface: present (0); absent (1)
43 Indumentum on bract surface: sparsely pubescent (0); densely pubescent (1); absent (2)
44 Bract length (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)
45 Bract width (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)
46 Flower position: terminal (0); lateral (1)
47 Flower pedicel: present (0);absent (1)
48 Outer sepal shape: ovate (0); ovate-elliptic (1); ovate-lanceolate (2); lanceolate (3); lanceolate-ovate (4); oblong-ovate (5); oblong-elliptic (6)
49 Outer sepal apex: acute (0); acuminate (1); rounded(2); mucronate (3); obtuse (4)
50 Outer sepal base : acute (0), acuminate (1), cuneate (2), rounded (3)
51 Outer sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
52 Outer sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
53 Outer sepal margin disposition: revolute (0); recurved (1); incurve (2)
54 Outer sepal margin cilia: absent (0); present (1)
55 Simple hairs on outer sepal adaxial surface: present (0); absent (1)
56 Hooked hairs on outer sepal adaxial surface: present (0); absent (1)
57 Stellate hairs on outer sepal adaxial surface : present (0); absent (1)
58 Indumentum on outer sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
59 Simple hairs on outer sepal abaxial surface: present (0); absent (1)
60 Hooked hairs on outer sepal abaxial surface: present (0); absent (1)
61 Stellate hairs on outer sepal abaxial surface : present (0); absent (1)
62 Indumentum on outer sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
63 Inner sepal shape: ovate (0); ovate-elliptic (1); lanceolate-ovate (2); oblong-ovate (3); oblong-obovate (4); absent (5)
64 Inner sepal apex: acute (0); acuminate (1); rounded (2); rounded-cuspidate (3); rounded-retuse (4); absent (5)
65 Inner sepal base: acute (0); acuminate (1); cuneate (2); rounded (3); absent (4)

66	Inner sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
67	Inner sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
68	Inner sepal margin disposition: revolute (0); recurved (1); incurve (2); absent (3)
69	Inner sepal margin cilia: absent (0); present (1)
70	Simple hairs on inner sepal adaxial surface: present (0); absent (1)
71	Hooked hairs on inner sepal adaxial surface: present (0); absent (1)
72	Stellate hairs on inner sepal adaxial surface : present (0); absent (1)
73	Indumentum on inner sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
74	Simple hairs on inner sepal abaxial surface: present (0); absent (1)
75	Hooked hairs on inner sepal abaxial surface: present (0); absent (1)
76	Stellate hairs on inner sepal abaxial surface: present (0); absent (1)
77	Indumentum on inner sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
78	Petal shape: ovate (0); obovate (1); spathulate (2); oblong- obovate (3); oblanceolate (4); narrowly obtriangular (5)
79	Petal length (mm): 1–5 (0), 5.1–10 (1), 10.1–15 (2), ≥15.1 (3)
80	Petal width (mm): 1–3 (0); 3–6 (1); 6.1–10 (2); >10.1 (3)
81	Stamen number: 3–5 (0); 5–10 (1); 10–15 (2); ≥15 (3)
82	Stamen arrangement: in one cluster (0); in two cluster (1); in three cluster (2); >three clusters (3)
83	Stamens cluster position: stamens cluster around carpel (0); stamens cluster around carpel and with another cluster (1); stamens cluster on one side of carpel (2); stamens cluster between the carpels (3).
84	Stamen length (mm): 1–3 (0); 3.1–6 (1); >6.1 (2)
85	Anther length (mm): 1–1.5 (0); 1.6–2 (1); 2.1–3 (2); >3.1 (3)
86	Anther width (mm): 0.2–0.5 (0); 0.6–0.8 (1); >0.9 (2)
87	Anther apex: acute (0); round (1); truncate (2); retuse (3)
88	Anther dehiscence: lateral slits (0); terminal pores (1); terminal pores and lateral slits (2); terminal pores and introrse slits (3); introrse slits (4)
89	Hairs type between anthers and petals: simple hairs (0); stellate hairs + simple hairs (1), mixture short and long simple hairs (2); glabrous (3)
90	Indumentum between anthers and petals: sparsely pubescent (0); densely pubescent (1); absent (2)
91	Filament fusion: free (0); basally connate (1); half connate (2); all connate (3)
92	Anther shape: linear (0); oblong (1); narrowly oblong (2); broadly oblong (3)
93	Pistil shape: uncompressed (0); compressed (1)
94	Style colour: yellow (0); red (1); greenish-yellow (2)
95	Style base: straight (0); recurved (1)
96	Style attachment to ovary: apical (0); lateral (1); semi lateral (2)
97	Stigma shape: capitate (0); discoid (1); linear (2)
98	Ovary shape: spherical (0); compressed (1); ovoid (2)
99	Simple hair on ovary: present (0); absent (1)
100	Stellate hairs on ovary: present (0); absent (1)
101	Indumentum on ovary surface: sparsely pubescent (0); densely pubescent (1); absent (2)
102	Seed shape: spherical (0); compressed (1); ovoid (2)
103	Seed colour: black (0) brown (1); brown-black (2); shiny black (3)
104	Seed dimension (mm): 1–1.5 (0); 1.6–2 (1); 2.1–2.5 (2); >2.5 (3)
105	Aril length: >1/3 of the seed (0); 1/3–1/2 of the seed (1)

Results

The analysis produced a single most parsimonious tree of L= 2178, CI= 11, RI= 43 (Fig. 1) consisting of 7 distinct clades, all with low Jackknife support. Toelken’s informal species groups (Tab. 1) were distributed on the tree (Fig. 1) as follows:

Hibbertia acicularis (*Acicularis* group) was basal and sister to the remainder of the ingroup. Two clades comprised each a single taxa, Clade A with *H. acicularis* that had 100% JK support and Clade C with *H. cistiflora* that had no support 4% JK.

Clade B with 8 taxa had no support but defined by the characters: absence of leaf mucro (23/0), absence of leaf adaxial surface indumentum (27/1; 30/1; 40/1.), leaves ovate-lanceolate (48/2), anther apex truncate (87/2), anthers with terminal pores and lateral slits (88/2) and semi-lateral style attachment to ovary (96/2). The species pair *H. nitida* and *H. obtusibracteata* (54% JK) was supported by the unique synapomorphy of an entire leaf margin (20/0). Similarly, three other sister relationships in this Clade: *H. caespitulosa* which was defined by the characters: presence of axillary hairs (16/0), acute leaf apex (19/0), absence of indumentum on bract surfaces (43/2), and 1–2.5 mm bract width (45/1); *H. woronarana* which was defined by the characters: >0.9 mm leaf mucro length (23/2), and presence of stellate hairs on leaf abaxial surface (35/0); and *H. pilifera* which was defined by the characters: presence of simple hairs on stem (3/0), sparsely pubescent stem indumentum (6/0), <0.5–0.8 mm leaf mucro length (23/1), presence of hooked hairs on leaf abaxial surface (34/0), and densely pubescent indumentum on bract surface (43/1).

Clade D was not supported (12% JK), and circumscribed by the characters: absence of stellate hairs on bract surface (42/1). Only 2 taxa were included in this Clade: *H. fruticosa* and *H. protuberans* and they share the homoplasious synapomorphies: absence of simple hairs on the leaf abaxial surface (33/1), linear to subulate bract shape (37/1), presence of hooked hairs on the bracts (41/0), absence of stellate hairs on the bract surface (42/1) and 1–2.5 mm bract length (44/1). Similarly, Clade E was not supported (8% JK) and comprised the two taxa: *H. tetranthera* and *H. oxycraspedotus*, defined by the homoplasious synapomorphies: absence of stellate hairs on bract surfaces (42/1) and absence of outer sepal margin cilia (54/0) (Fig. 1–3).

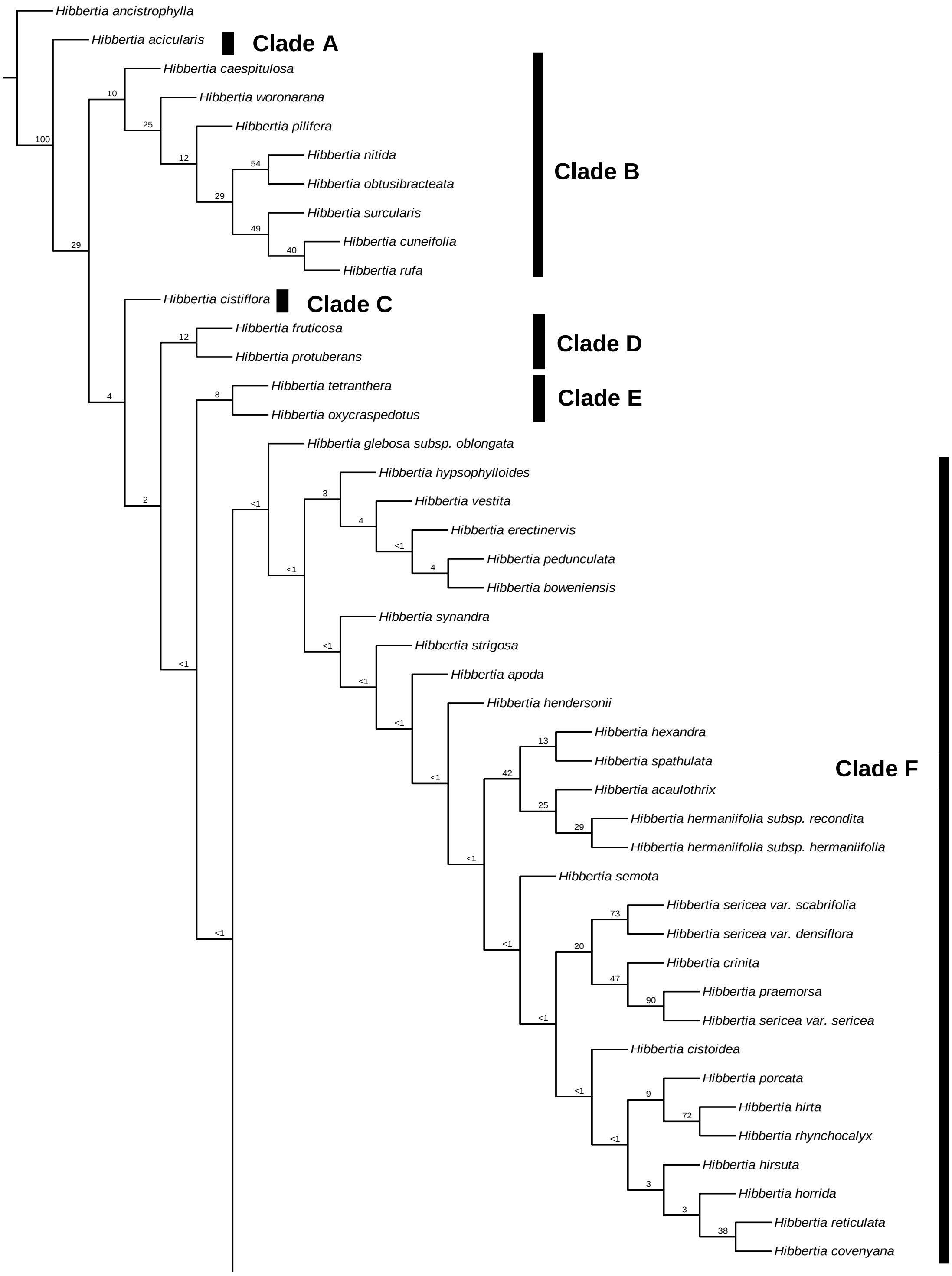

Figure 1. Single equally most parsimonious tree (L 2178; CI 11; RI 43) produced by a WinClada ratchet analysis of the morphological data (1000 reps, 10 trees held per rep) showing jackknife branch support values (1000 reps).

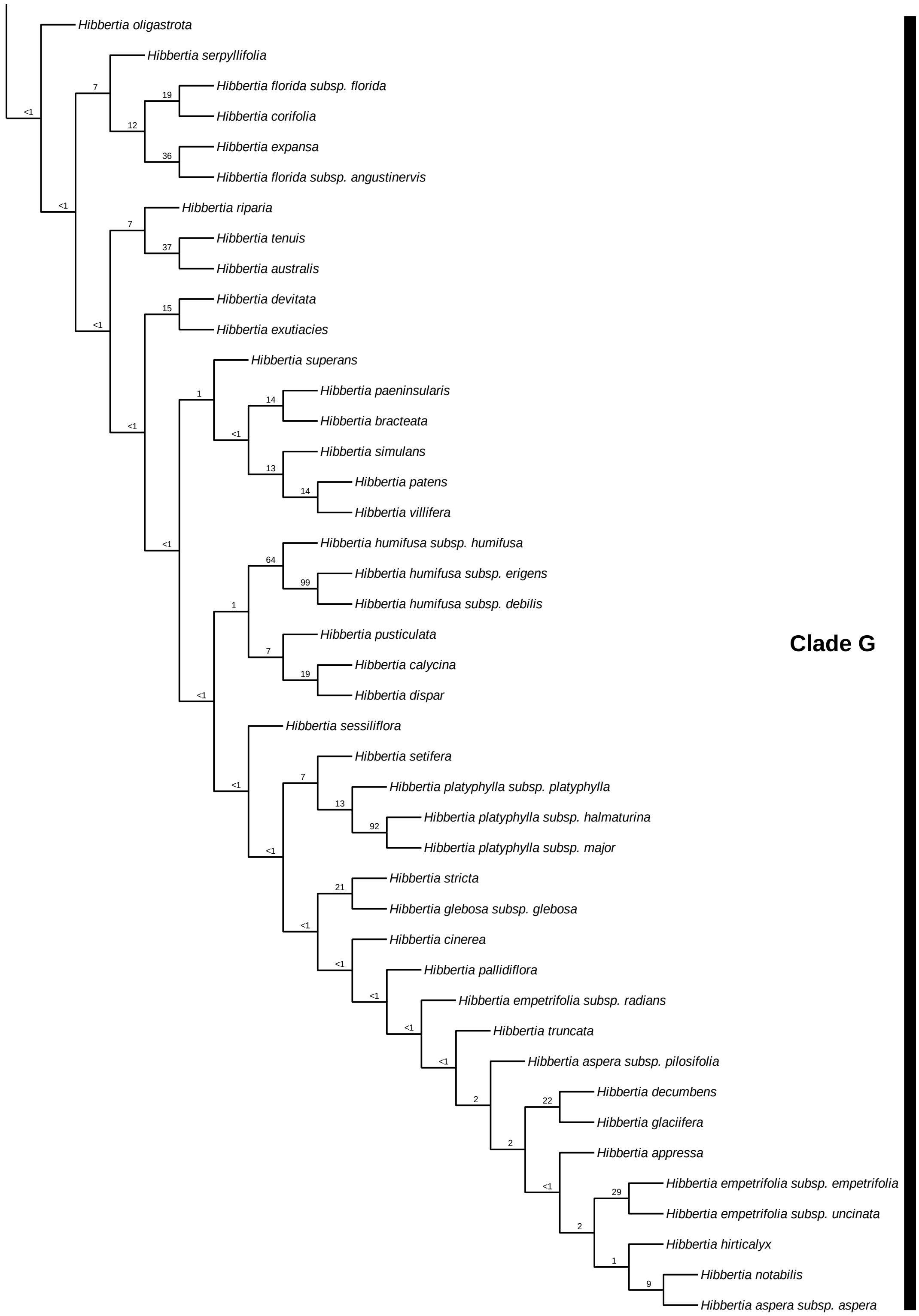

Fig. 1 Continued

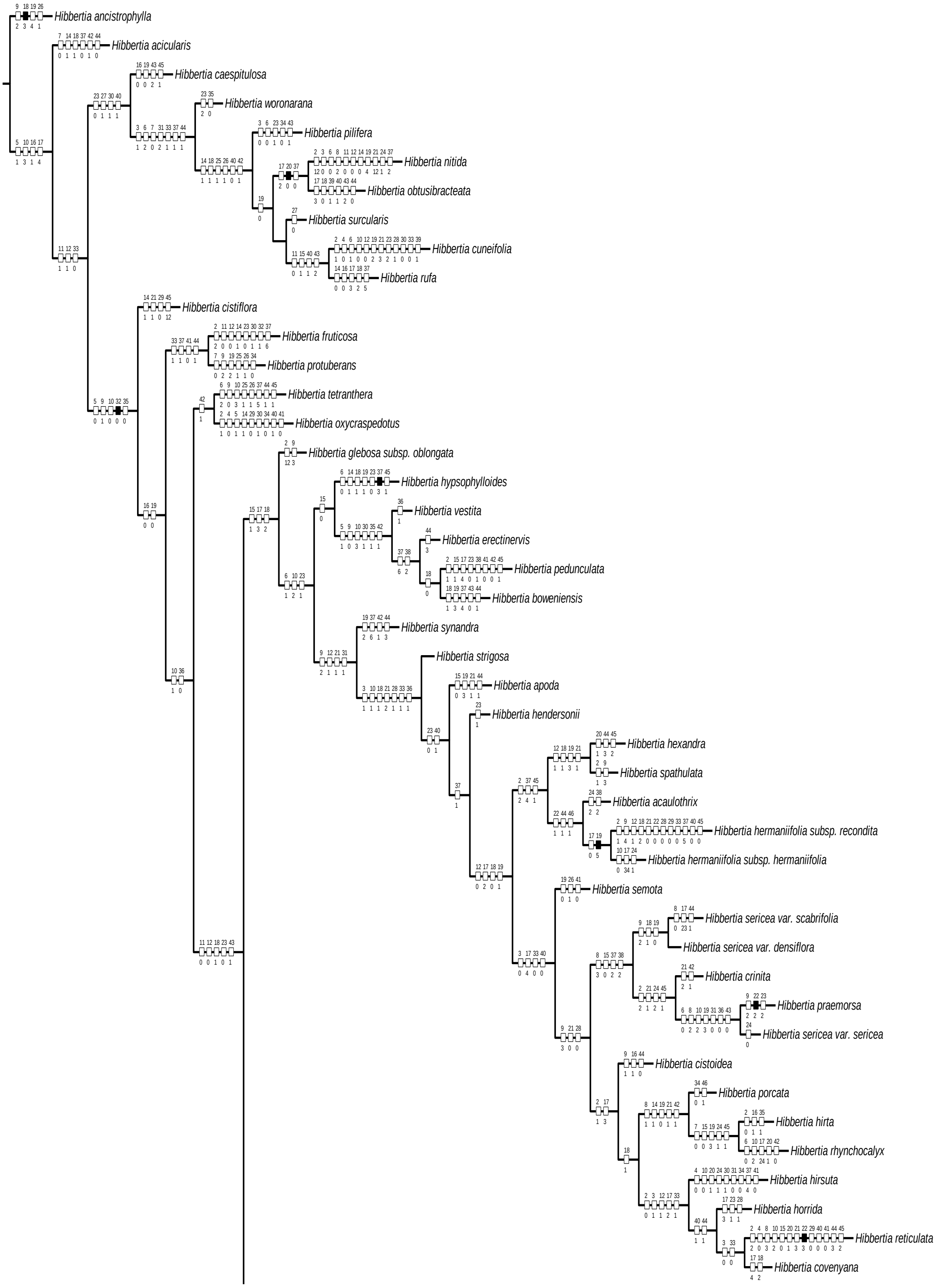

Figure 2. Single equally most parsimonious tree (L 2178; CI 11; RI 43) produced by a WinClada ratchet analysis of the morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for vegetation and indumentum characters (characters 1–46). Filled squares are unique synapomorphies; open squares indicate homoplasy.

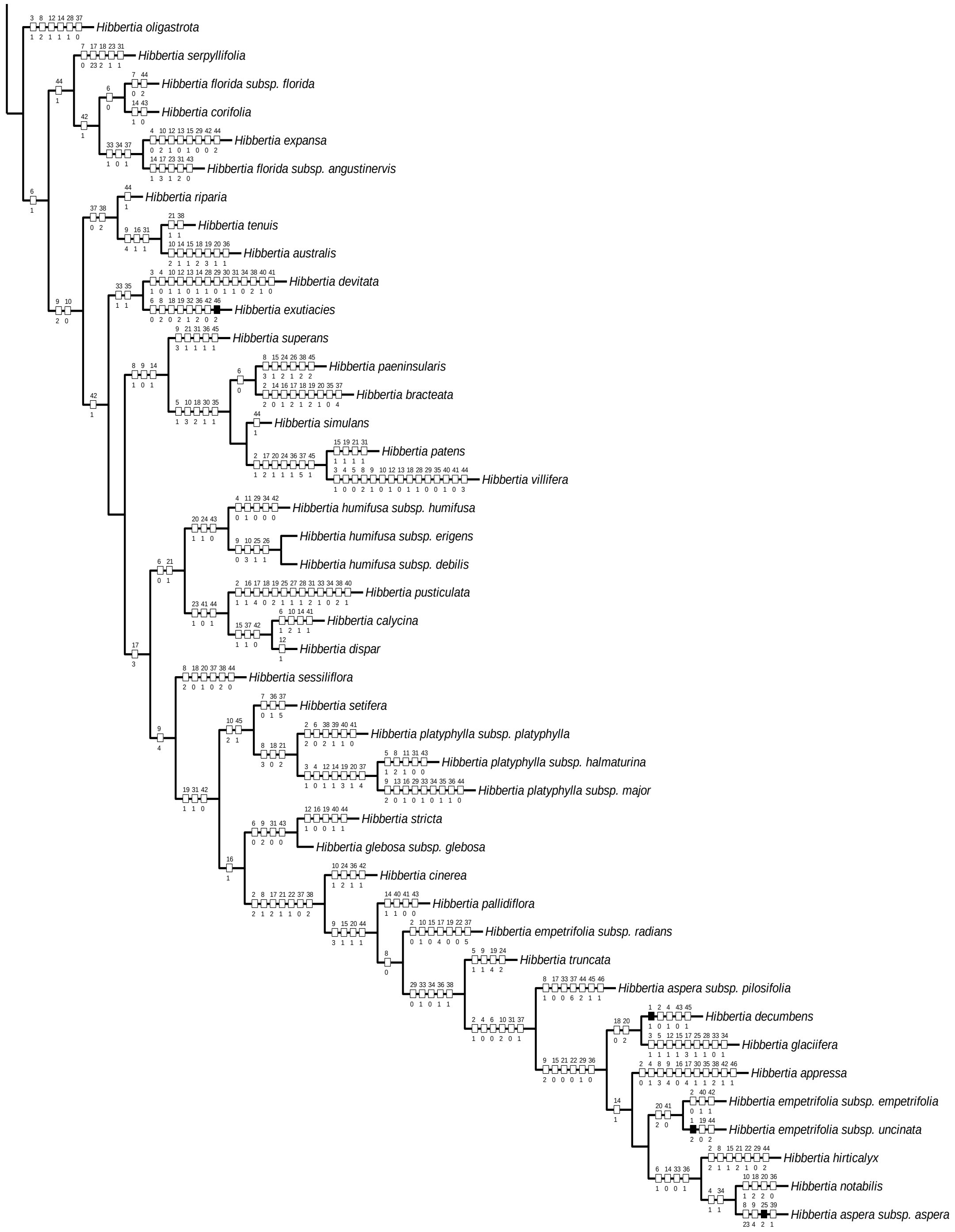

Fig. 2 Continued

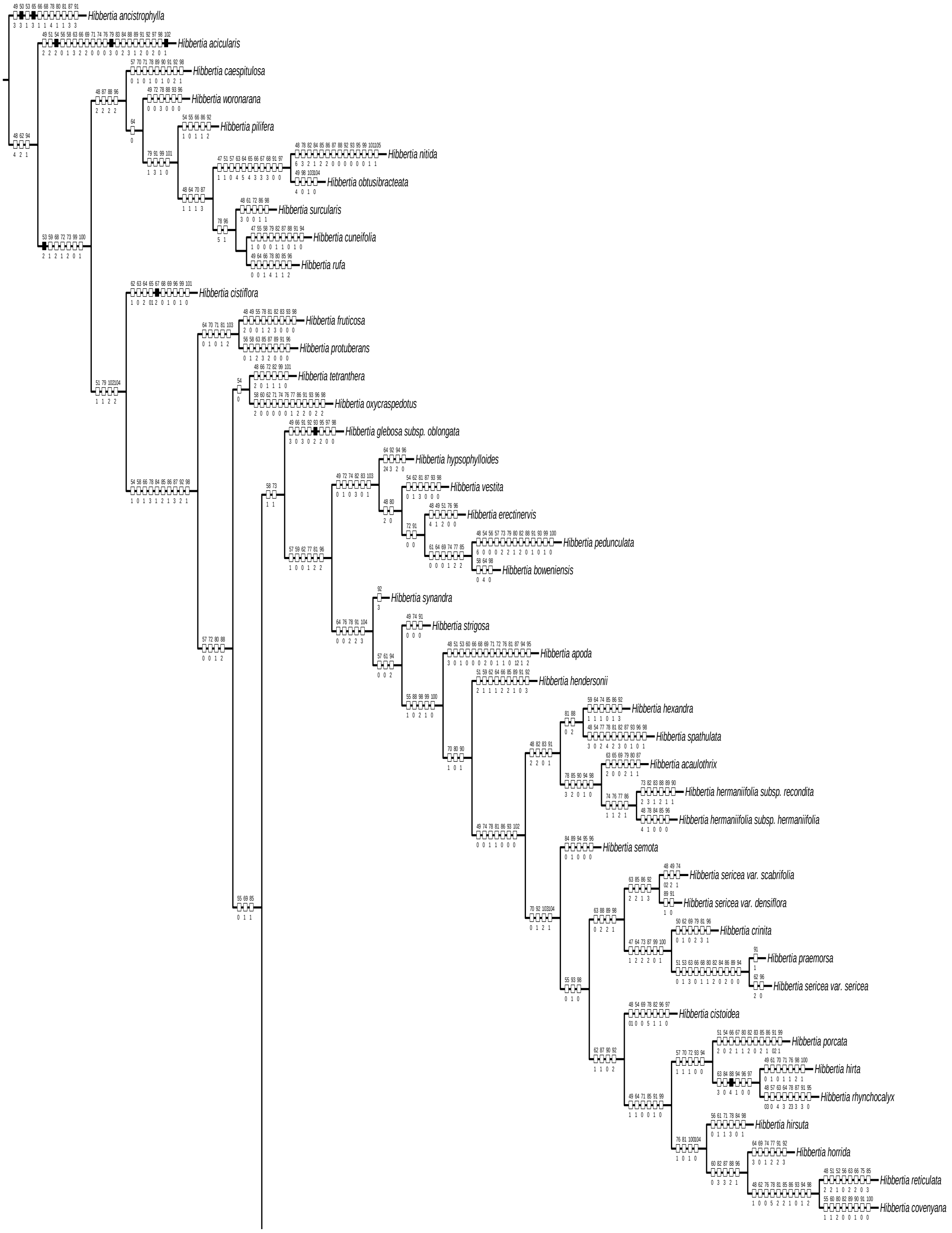

Figure 3. Single equally most parsimonious tree (L 2178; CI 11; RI 43) produced by a WinClada ratchet analysis of the morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for floral and seed characters (characters 47–105). Open squares indicate homoplasy.

Fig. 3 continued

The large Clade F was also not supported (<1% JK), but was defined mainly by a number of vegetative and indumentum characters (15/1; 17/3; 18/2; 58/1; 73/1). Five main sub-lineages (Fig. 1) can be defined by the homoplasious synapomorphies listed in Tab. 4.

Table 4. Main lineages within Clade F with characters and character state and taxa included (See Tab. 3)

Lineage	character/character state	taxa
F/1	15/1; 49/0; 72/1; 74/0; 82/3; 83/0; 103/1	*H. hypsophylloides, H. vestita, H. erctinervis, H. pedunculata, H. boweniensis*
F/2	22/2; 3/0; 27/1; 30/1; 37/4; 40/1; 45/1	*H. hexandra, H. spathulata, H. acaulothrix, H. hermaniifolia* subsp. *recondita, H. hermaniifolia* subsp. *hermaniifolia*
F/3	8/3; 15/0; 37/2; 19/0; 3/0; 88/2; 89/2; 98/1	*H. sericea* var. *scabrifolia, H. sericea* var. *densiflora, H. crinita, H. praemorsa, H. sericea* var. *sericea*
F/4	18/1; 49/1; 64/1; 71/0; 85/0; 91/1; 99/0	*H. porcata, H. hirta, H. rhynchocalyx, H. hirsuta, H. horrida, H. reticulata, H. covenyana*
F/5	2/0; 3/1; 12/1; 17/2; 23/1; 76/1; 81/0; 100/1; 104/0	*H. hirsuta, H. horrida, H. reticulata, H. covenyana*

The first lineage (F/1: 3% JK) was supported by the unique synapomorphy of elliptic of bract shape (37/3), but only the terminal pair of *H. pedunculata* + *H. boweniensis* shared a large number of features: cuneate leaf base (18/0), stellate hairs on the outer sepal abaxial surface (61/0), acute inner sepal apex (64/0), absence of ciliate inner sepal margins (69/0), absence of simple hairs on the inner sepal abaxial surface (74/1) and >6.1 mm anther length (85/2).

In F/2 (42% JK), the pair formed by *H. hermaniifolia* subsp. *recondita* and subsp. *hermaniifolia* was supported by the unique synapomorphy: obtuse leaf apex, as well as sharing the homoplasious synapomorphies: broadly obovate leaf shape, absence of simple hairs, stellate hairs on the inner sepal abaxial surfaces and 0.6–0.8 mm anther width.

The third lineage F/3 demonstrated a close relationship between *H. praemorsa* and *H. sericea* var. *sericea* (90% JK), circumscribed by numerous morphological characters (Fig. 3). In contrast, the relationship between *H. sericea* var. *scabrifolia* and var. *densiflora* was only moderately supported (75% JK), sharing the homoplasious synapomorphies: 3–4 hair branches, acute leaf base and apex, lanceolate–ovate inner sepal shape, 2.1–3 mm anther length and broadly oblong anther shape (Fig. 4).

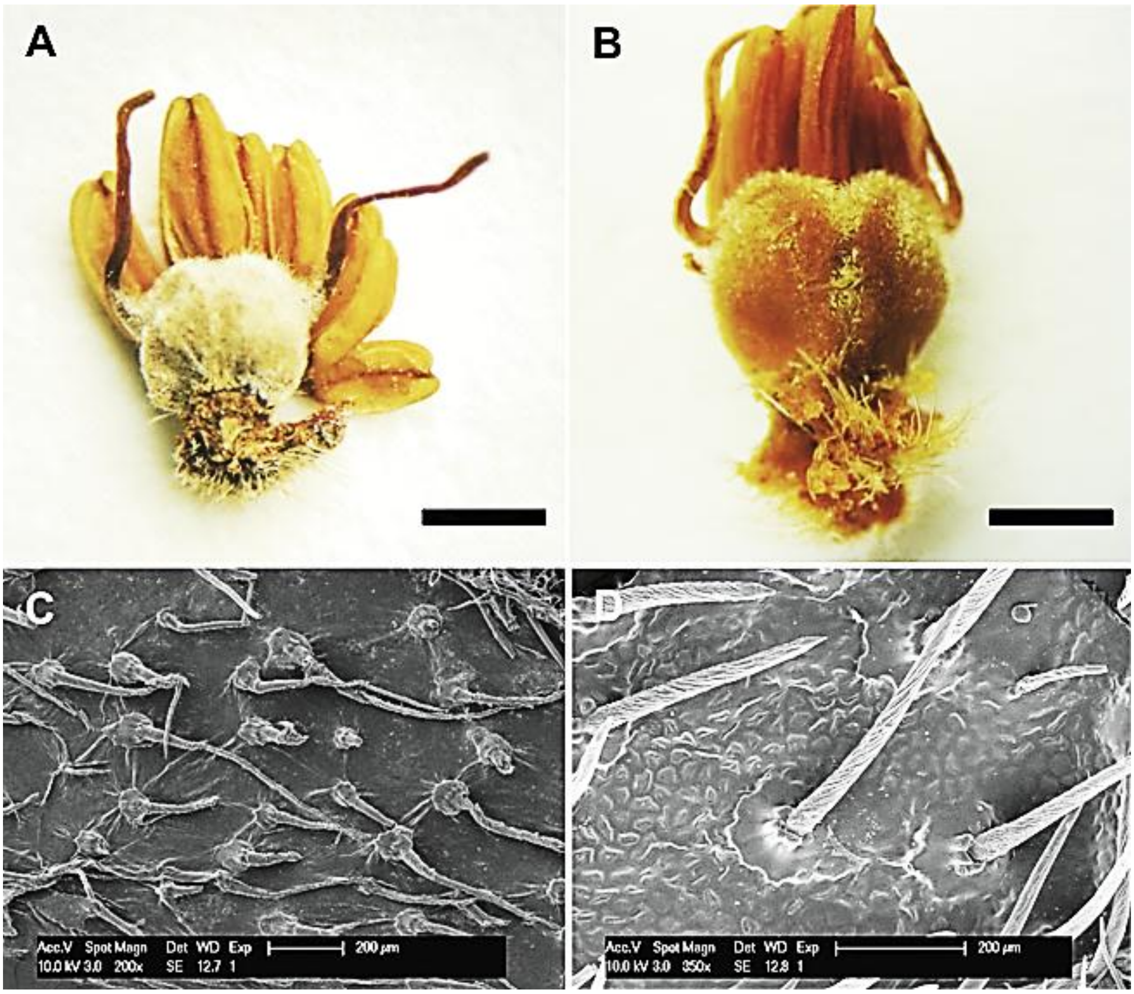

Figure 4. A & D Stamens, pistils and leaf indumentum of *H. sericea* var. *densiflora* (MEL572143) and var. *scabrifolia* (MEL403). A) Stamens with broadly oblong anthers, recurved semi-lateral style of *H. sericea* var. *densiflora*. B) Stamens with broadly oblong anthers, recurved semi-lateral style of *H. sericea* var. *scabrifolia*. C) Leaf adaxial surfaces displaying stellate hairs and mixture short and long simple hairs of *H. sericea* var. *densiflora*. D) Leaf adaxial surfaces displaying only short and long simple hairs of *H. sericea* var. *scabrifolia*. (Scale bars: A & B = 1.5 cm; C–D as indicated)

The fourth lineage F/4 comprised a close related species pair (*H. hirta* and *H. rhynchocalyx*) supported by the unique synapomorphy of introrse anther dehiscence by slits (88/4) and the homoplasious synapomorphies: rounded-cuspidate inner sepal apex, 1–3 mm stamen length, apical style attachment to ovary (63/3; 84/0; 96/0; 97/0) respectively. The character state >15 mm leaf blade width was the unique synapomorphy for the *H. reticulata* + *H. covenyana* within the fifth lineage, D/5.

Clade G (<1% JK) could be defined by the homoplasious synapomorphies: stem densely pubescent (6/1), ovate-elliptic outer sepal shape (48/1), stamens in two cluster (82/1), lateral anther dehiscence by slits (88/0), densely pubescent indumentum between the anthers and petals (90/1) and ovoid ovary shape (98/2), with *H. oligastrota* sister to the remainder of the Clade. In addition, there are four main lineages in this Clade (Tab. 5).

Table 5. Main lineages within Clade G with characters and character state and taxa included (See Tab. 3)

Lineage	character/character state	taxa
G/1	44/1; 78/2; 81/1; 82/3; 83/0; 103/1	*H. serpyllifolia, H. florida subsp. florida, H. corifolia, H. expansa, H. florida subsp. angustinervis*
G/2	8/1; 9/0; 14/1;	*H. superans, H. paeninsularis, H. bracteata, H. simulans, H. patens, H. villifera*
G/3	6/0; 21/1;	*H. humifusa subsp. humifusa, H. humifusa subsp. erigens, H. humifusa subsp. debilis, H. pusticulata, H. calycina, H. dispar*
G/4	19/1; 31/1; 42/0	*H. sessiliflora, H. setifera, H. platyphylla subsp. platyphylla subsp. halmaturina, H. platyphylla subsp. major, H. stricta, H. glebosa subsp. glebosa, H. cinerea, H. pallidiflora, H. empetrifolia subsp. radians, H. truncata, H. aspera subsp. pilosifolia, H. decumbens, H. glaciifera, H. appressa, H. empetrifolia subsp. empetrifolia, H. empetrifolia subsp. uncinata, H. hirticalyx, H. notabilis, H. aspera subsp. aspera*

In this Clade, the first lineage G/1 had a species pair: *H. florida* subsp. *florida* + *H. corifolia* that share the homoplasious synapomorphies: sparse stem pubescence (6/1), absence of simple hairs (99/1), but presence of stellate hairs on the ovary (100/0). In contrast, the relationship between *H. expansa* and *H. florida* subsp. *angustinervis* was defined by the homoplasious synapomorphies: hooked hairs on leaf abaxial and outer sepal adaxial surfaces (34/0; 56/0), rounded inner sepal apex (64/2) and oblong- obovate petal shape (78/3). The morphological differences between *H. florida* subsp. *florida* and subsp. *angustinervis* illustrated in Fig. 5 and summarised in Tab. 6.

Table 6. A comparison of character state differences between *H. florida* subsp. *florida* and subsp. *angustinervis*

Characters	*H. florida* subsp. *florida*	*H. florida* subsp. *angustinervis*
Hair bases	Unspecialised	Tuberculate
Leaf abaxial surface indumentum	Long and short hairs + stellate hairs abaxial	Stellate hairs + hooked simple hairs
Bract surface indumentum	pubescent to glabrescent	densely pubescent
Outer sepal shape	Ovate–elliptic	Lanceolate
Outer sepal abaxial surface indumentum	mixture short and long simple hairs	Glabrous
Stamen number	8–12	12–25
Anther apex	Acute	Round & truncate
Anther shape	Narrowly oblong	Broadly oblong
Hairs between stamen and petals	Absent	Present

Figure 5. Androecium, gynoecium of *H. florida* subsp. *florida* and subsp. *angustinervis.* A) >three clusters stamens around carpel, lateral slits anther dehiscence, semi lateral style with recurved base of *H. florida* subsp. *florida* (AD*1086480*). B) >three clusters stamens around carpel, lateral slits anther dehiscence and semi lateral style with recurved base of *H. florida* subsp. *angustinervis* (NSW*535*). (Scale bar: A & B= 2 mm)

Hibbertia paeninsularis and *H. bracteata* formed the second lineage (G/2) united by the homoplasious synapomorphies: sparsely pubescent indumentum on stem (6/0), simple hairs on outer sepal abaxial surface (59/0) and ovate inner sepal shape (63/0). Similarly, the poorly supported pair *H. patens* + *H. villifera* shared the homoplasious synapomorphies: narrowly obovate leaf shape (17/2) and recurved leaf margin (20/1).

The third lineage (G/3) included all of the *H. humifusa* subspecies, with *H. humifusa* subsp. *erigens* and subsp. *debilis* forming a strongly supported pair (99% JK). Although the *H. calycina* + *H. dispar* pair was not supported (19% JK), one unique synapomorphy defined their relationship: terminal anther dehiscence by pores (88/1). The fourth lineage (G/4) was large and comprised numerous different taxa, including all the *H. platyphylla* subspecies, with strong support (92% JK) for *H. platyphylla* subsp. *halmaturina* + subsp. *major*, with subsp. *platyphylla* as their sister. In contrast, *H. empetrifolia* subsp. *radians* from South Australia did not form a monophyletic lineage with the terminal pair formed by subsp. *empetrifolia* + subsp. *uncinata*, instead forming a terminal pair with *H. truncata* (Fig. 6B; Tab. 7).

Discussion

Toelken's proposed groups

Rather than separating into the 16 informal species groups proposed by Toelken (1998, 2000, 2010, pers. comm. 2009), the SE Australian members of *Hibbertia* subgenus

Hemistemma resolved into 7 clades, as well as the two taxa *H. glebosa* subsp. *oblongata* (*Australis* group) and *H. oligastrota* (*Acicularis* group) as part of Clade F. Some of these clades included many taxa belonging to the same proposed groups, but none of Toelken's groups were recovered (Tab. 8).

Table 7. A comparison of character and character states between *H. empetrifolia* subsp. *empetrifolia* and *H. empetrifolia* subsp. *radians*

Character	*H. empetrifolia* subsp. *empetrifolia*	*H. empetrifolia* subsp. *radians*
Hair branch length	Unequal	Semi-equal
Petiole indumentum	Short + long simple hairs only	Stellate hairs + simple hairs
Leaf adaxial indumentum	Stellate hairs + hooked simple hairs	Stellate hairs + simple hairs
Bract indumentum	Hooked simple hairs	Stellate hairs + simple hairs
Outer sepal margin cilia	Present	Absent
Stamen position	Around carpel	On one side of carpel
Hairs between anthers and petals	Stellate hairs + simple hairs	Mixture short and long simple hairs
Filament fusion	Half connate	Basally connate
Style base	Straight	Recurved
Style attachment to ovary	Semi lateral	Style attachment to ovary apical

Figure 6. Androecium and gynoecium of *H. devitata* and *H. empetrifolia* subsp. *radians.* A) *H. devitata* stamens on one side of carpel, basally connate, lateral slits dehiscence anther, semi-lateral style with recurved base (AD*966072887*). B) *H. empetrifolia* subsp. *radians* stamens on one side of carpel, basally connate, lateral slits dehiscence anther, apical style with recurved base (AD*9843585*). (Scale bars: A = 1.5 mm; B = 2 mm)

Morphological relationships and phylogeny

There was considerable intra-specific variation and variability for many of the morphological characters between the taxa of *Hibbertia* subgenus *Hemistemma* taxa in this analysis (Table 3), mirroring the character plasticity suggested by Cronquist (1988) and Horn (2005) for the genus. This might be due to ecological factors, especially for indumentum features (e.g. simple, mixed, stellate and hooked hairs) on the aerial axes serving as possible adaptations for reducing water loss in these species (Toelken 1998, 2000, 2010).

Although some of the differences in characters and character states between the studied taxa are of interest, such as those of the androecium, several vegetative, indumentum and floral characters are considered to be useful for distinguishing between *Hibbertia* taxa. For example, *H. cuneifolia* and *H. rufa* (Clade A) share the petiole indumentum, a densely pubescent petiole surface and glabrous bracts, whereas *H. fruticosa* and *H. protuberans* (Clade B) shared the floral and seed character states: 1–5 mm inner sepal length, 5–10 stamens and brown-black seed colour (Fig. 3; Tab. 8).

The nature of the simple hairs (with a tuberculate base) and hooked hairs seen in this study are comparable with those seen by Toelken (1998). It is notable that simple hairs which have a tuberculate base are longer than hooked hairs and are also different from stellate hairs that have 2–7 branches (Toelken 1998). However, these characters are the some of the most homoplasious among the taxa studied (see Tabs. 4–7). Absence of surface indumentum, particularly on leaf adaxial surfaces is apparently a diagnostic feature for several species groups e.g. *H. woronarana*, *H. rufa* and *H. surcularis* (Clade B) and *H. cistiflora* (Ch. 5), but as this feature is shared with the outgroup (*H. ancistrophylla*) it would seem to be both homoplasious and a reversal.

Most *Hibbertia* stems possess both simple and stellate hairs, but hooked hairs are present intermixed with simple hairs in several taxa including: *H. cuneifolia* and *H. nitida* (Clade B); *H. oxycraspedotus* and *H. tetranthera* (Clade E), *H. reticulata* (Clade F), *H. empetrifolia* subsp. *empetrifolia* and subsp. *uncinata*, *H. platyphylla* subsp. *halmaturina* and subsp. *major* and *H. villifera* (Clade G; see also Ch. 5: App. 2).

Leaf margin recurved or revolute is an important leaf adaxial surface character (Toelken 2010) and used widely within SE *Hibbertia* subgenus *Hemistemma* for defining taxa and affinities in the group (Hotchkiss 1954; Dickison 1970; Rury and Dickison 1977; Toelken 1998, 2000, 2010). This character was a synapomorphy for the terminal pair comprised of *H. nitida* + *H. obtusibracteata*; but was also a shared homoplasious character state (recurved) for the *H. patens* + *H. villifera* pair.

Table 8. Comparison of the clades recovered in this study for *Hibbertia* subg. *Hemistemma* with Toelken's informal species groups

Clade	Taxa	Toelken's group
A	*H. acicularis*	Acicularis
B	*H. caespitulosa, H. woronarana, H. cuneifolia.*	Acicularis
B	*H. rufa*	Rufa
B	*H. nitida*	Bracteata
C	*H. cistiflora*	Exutiacies
D	*H. fruticosa*	Pedunculata
D	*H. protuberans*	Acicularis
E	*H. tetranthera, H. oxycraspedotus*	Exutiacies
F	*H. glebosa* subsp. *oblongata*	Acicularis
F	*H. hypsophylloides, H. vestita, H. erctinervis, H. boweniensis,*	Vestita
F	*H. pedunculata*	Pedunculata
F	*H. synandra, H. strigosa, H. hendersonii*	Strigosa
F	*H. apoda*	Stricta
F	*H. hexandra, H. hermaniifolia* subsp. *recondita, H. hermaniifolia* subsp. *hermaniifolia, H. spathulata, H. acaulothrix, H. reticulata*	Hermaniifolia
F	*H. semota, H. cistoidea, H. covenyana*	Cistoidea
F	*H. sericea* var. *scabrifolia, H. sericea* var. *densiflora, H. crinita, H. praemorsa, H. sericea* var. *sericea, H. hirta, H. rhynchocalyx, H. hirsuta, H. horrida,*	Sericea
F	*H. porcata,*	Pedunculata
G	*H. oligastrota*	Acicularis
G	*H. serpyllifolia*	Serpyllifolia
G	*H. florida* subsp. *florida, H. corifolia, H. expansa, H. florida* subsp. *angustinervis, H. dispar,*	Pedunculata
G	*H. riparia*	Riparia
G	*H. tenuis, H. australis*	Australis
G	*H. devitata, H. setifera, H. glebosa* subsp. *glebosa*	Stricta
G	*H. exutiacies*	Exutiacies
G	*H. superans, H. paeninsularis, H. simulans, H. patens, H. villifera, H. sessiliflora, H. platyphylla* subsp. *platyphylla, H. platyphylla* subsp. *halmaturina, H. platyphylla* subsp. *major,*	Sericea
G	*H. bracteata*	Bracteata
G	*H. humifusa* subsp. *humifusa, H. humifusa* subsp. *erigens, H. humifusa* subsp. *debilis*	Humifusa
G	*H. pusticulata*	Acicularis
G	*H. calycina*	Strigosa
G	*H. stricta*	Stricta
G	*H. cinerea, H. pallidiflora, H. empetrifolia* subsp. *radians, H. truncata, H. aspera* subsp. *pilosifolia, H. decumbens, H. glaciifera, H. appressa, H. empetrifolia* subsp. *empetrifolia, H. empetrifolia* subsp. *uncinata, H. hirticalyx, H. notabilis, H. aspera* subsp. *aspera*	Aspera
G		

The most variable floral features in *Hibbertia* are the androecium and gynoecium characters (Dickison 1970; Tucker and Bernhardt 2000), many of which have been regarded as "primitive" in the angiosperms (Stebbins 1974; Stebbins and Hoogland 1976; Horn 2005). Stamen number is highly variable within and between *Hibbertia* species: e.g. 2 stamens in *H. hirsuta*; 27– 30 in *H. vestita* (See Ch. 6: App. 1) and anther dehiscence is also quite variable. Horn (2005, 2007) reported only one type of anther dehiscence (pores) within subgenus *Hemistemma*, but from the present study it is clear that numerous species have different anther dehiscence characters (Fig. 7; see also Ch. 6: App. 1), with both terminal pore and lateral slit dehiscence present in nearly all taxa within Clade A, except for *H. woronarana* and *H. nitida* which are characterised by lateral slits anther dehiscence.

There were eight species seen with anther dehiscence by both terminal pores and lateral slits, including *A. caespitulosa* (Fig. 7A), *H. boweniensis* and *H. appressa*; lateral slits occurred in 10 taxa, including *H. oligastrota* (Fig. 7B), *H. pusticulata, H. protuberans, H. apoda, H. hendersonii, H. acaulothrix, H. hermaniifolia* subsp. *recondita* and *H. hermaniifolia* subsp *hermaniifolia, H. porcata* and *H. hirsuta*.

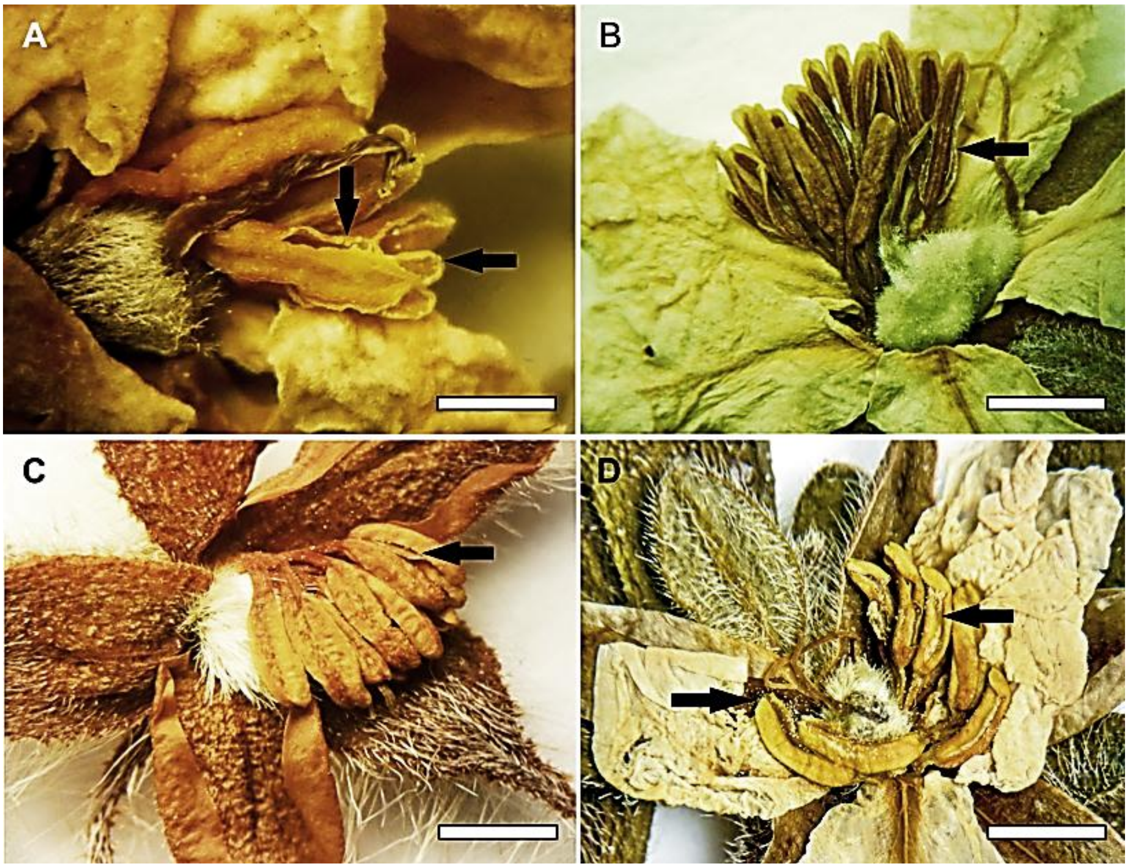

Figure 7. Anther dehiscence types of *Hibbertia* species, A) *H. caespitulosa* (AD106483) showing anther dehiscence by both terminal pores and lateral slits; B) *H. oligastrota* showing anther dehiscence by lateral slits only (QSL359875); C) *H. hirta* showing anther dehiscence by introrse slits (CANB056123); D) *H. praemorsa* showing anther dehiscence by terminal pores and introrse slits (CANB8504647). (Scale bars: A–B = 2 mm; C–D = 1.3mm)

Conclusions

Morphological analysis of the SE Australian Clade of *Hibbertia* subg. *Hemistemma* shows that although there are seven main lineages, the 16 species groups as currently circumscribed by Toelken (unpubl.) are not supported. The taxa of the *Aspera* group species treatment by Toelken (1998) are placed within Clades G, together with those of the *Sericea* group specie treated by Toelken (2000). The circumscriptions of these clades need further investigation with molecular data to confirm their composition and accuracy.

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Appendix 1. Nexus file of the morphology matrix for the characters and character states listed in Tab. 3.

#NEXUS

BEGINDATA;

DIMENSIONS NTAX= 87 NCHAR = 135;

FORMAT DATATYPE = STANDARD GAP = - MISSING =? SYMBOLS = "0123456789";

MATRIX

Hibbertia ancistrophylla
0012102100111013420010010121532101220003(15)330010(24)(23)20(13)1310102(15)(23)424011020003013233101112010????20010000111100(01)(01)0000010000101000010000001010

Hibbertia acaulothrix
024110(24)10010105012(12)1020004104142031(12)(01)102(12)021021311(23)20010201411(01)3211201201013212011210011????20100(01)(01)00(01)1100001200100(01)(01)00011000(01)(01)30001010

Hibbertia acicularis
0032000300211101220010000321530102200002422200100233122001020232300002000301220111120122133020011000110100010000010101000000010011001101

Hibbertia apoda
004(12)102100310011321000000410412103110002312001131(01)2102000122140211002111(12)013222112212010????200100001111001001000100(01)(01)00110000130001010

Hibbertia appressa
0012134200211041110000000032002120201010120000101243322001011242111(01)2200012132221000?2000????200100(01)(01)00000000110010010100100001020001010

Hibbertia aspera subsp. *aspera*
01111(23)4(02)0011(14)1(68)111(01)00010012011111111000301200111(12)(14)(23)3320010(12)(12)142(45)00(01)02000(23)3132230(01)12?001(01)2311200110(01)(01)011100100200011(01)(01)000110001021001001

Hibbertia aspera subsp. *pilosifolia*
01(01)2113200102191111100000(01)20(01)16101121102312001114313200101224250001200010132220000?00000321200100(01)(01)01100010020010100100010010030010010

Hibbertia australis
0011104200104172310000000110110201120000102101111431121011012421110121210013211001110000233020011000101000100200011001011100100210100(01)(01)

Hibbertia boweniensis
003(12)1(01)0300211011320010000320324202210002(12)02102122121421020111423102301213213202111210000????2001000(01)(01)10100001000010(01)(01)(01)0001000(01)(01)100(01)(01)010

Hibbertia bracteata
02321(01)030021115121(01)0000003(23)03(23)4102(12)2000211210112121454333046453510(12)221211002122(01)112?2000211020010000101100100100010000101000100100100(01)(01)

Hibbertia caespitulosa
00321003013210(17)00200000(01)132(01)(35)(23)2103321002212002033431120020(12)0242100002000(23)200102111211001????20011000101100(01)0(01)000100(01)(01)0000100(01)0(01)20001010

Hibbertia calycina
001110(12)2002030110210100001201(23)1101(12)10002(12)0210211(12)12(12)021020(12)11(23)2(24)101020210102(12)02121211000????20011001001(01)00(01)0(01)000100(01)0(01)00100001010010010

Hibbertia cinerea
0211114100113151121102000110110202120003212001110120020010012421111020112313211101010000032020011101001000100300101(01)(01)(01)001000100210111(01)1

Hibbertia cistiflora
001(24)1010014311(12)0221010000(15)(23)0(14)(23)21012(23)(12)0024121020434102(01)0201434432100020000013213111012122????0001000(01)(01)1110000110001001101110010110001010

Hibbertia cistoidea
011(12)1011001(01)2130120000000110111101100000(01)0210201111102(01)0201111(01)5(01)0(12)12110101322212110001 0????20100(01)(01)000110000120010010100100000130001010

Hibbertia corifolia
00(13)(12)1011002110(12)1020000000120122102210002142002121431021 0211(13)1421112301113013211111211010????2001000(01)(01)010000(01)(01)200011001001(01)00001(23)0001010

Hibbertia covenyana
0(01)(13)110(34)(01)0030104212000000 0(13)1011110311000011210213 1(12)011210212(13)1(12)0512(23)021213200002(01)111120(01)0????20100(01)0(01)010100(01)0(01)200100(01)0(01)00110000130001001

Hibbertia crinita
0211133100101020122002000110112202021010400101110110221020312111202021102202021121111000021020100 10(01)(01)11100010300100(01)(01)001110101030010001

Hibbertia cuneifolia
0102000000203101223020111 43(01)(35)(23)1114310010112002022431120020053425000120001013213(01)01112122????2001000(01)(01)11100(01)0(01)000100(01)(01)00010000(01)(01)30001001

Hibbertia decumbens
101110(123)20011(23)1(69)0(13)(12)00(01)00001202211012(01)10020(01)20020(01)2123020020312(24)(12)1(01)0(12)020001003200000000003????20100(01)(01)0011000(01)(01)0200100(01)0(01)0(01)11000(01)(01)100000(01)(01)

Hibbertia devitata
00211021000140(12)102000000001002(23)200120003(02)1200101243(02)020000202424000220112001 11(12)00122000022202001000010100010020 01000(01)(01)0(01)100010020001010

Hibbertia dispar
0012102(01)003(02)10110210100001201(23)110(02)1100023121021(02)(12)1210200211(13)22(12)210002101(01)013213121211000????2001100(01)(01)0(01)000001100010(01)0(01)0010000(01)(01)300010(01)(01)

Hibbertia empetrifolia subsp. *empetrifolia*
00(12)210(234)200212161120000000220221100110002212001 1(01)2023320020222425 00(012)2000200012200002200000210200 1000(01)(01)11100100200010(01)(01)000110010021001110

Hibbertia empetrifolia subsp. *radians*
001110(234)100110141011000000110125201210001012101012331220020030111010020001002011001011000021020010000111100100100100011001000100 20010010

Hibbertia empetrifolia subsp. *uncinata*
21(01)210(12)20021216102000000 0(01)2022110(01)1200032120021(01)21233200201124250 00(12)20003013222(01)00022000????20101101001100000200101100010100010110010(01)(01)

Hibbertia erectinervis
00311003001110(14)20200100003(12)03(12)(36)2021300024122021202(12)(12)1210211302131023011132132021(12)1011000????2001000(01)(01)(01)11001(01)(01)200010(01)(01)00(01)1100101200100(01)(01)

Hibbertia expansa
00(13)11(01)1200(12)020(24)1(01)20000000(03)(12)022110(12)1200021121020(012)14312211211(13)242310(01)30110(03)0132121112120(01)12120200100001011OO(01)0(01)200010(01)(01)001110000130001010

Hibbertia exutiacies
0012122000112020220OO?000121532101220210112001012435543330464531110221212000111121110000233120012OO(01)(01)010001001001000(01)(01)00100010000001010

Hibbertia florida subsp. *angustinervis*
00(13)(12)101100213011020010000(13)302211022100023121020(02)2431(24)210211(23)243(23)10230001(12)003213111211011????200100001(01)110000100001000101110000120001001

Hibbertia florida subsp. *florida*
0012001100(12)110210200(01)0000(13)20(34)22102120002112(01)020222214200211(13)14220013011100132(012)2111212010????2001000(01)(01)11100(01)0(01)200011001001100(01)0(01)30001001

Hibbertia fruticosa
02(13)(24)1010002110(01)002(01)0000003(23)143610(02)(23)1000220210212(23)431021020(12)0(23)4211023012130132120111100002220200100001O1(01)000011000100(01)(01)0(01)110000100010001

Hibbertia glaciifera
014210(12)20030311012(01)0001004(23)012110111000202200210 1(01)102200201(01)1113001020112013203000020000 03(12)020100(01)(01)00(01)1100110100010011001 10100120??????

Hibbertia glebosa subsp. *glebosa*
001210200011111112000000012012210122020231200110102122001100142100002000221321111101200O????2001000101110001020010000100110001030010O(01)(01)

Hibbertia glebosa subsp. *oblongata*
0(12)121(01)3(01)0010(34)0(12)202(01)000(01)00120122101120000(246)3210211143(12)120021011423(01)1002111321323011210000O????2001000(01)(01)111000111000100010(01)110000110001001

Hibbertia hendersonii
004110(02)100302011022010000 4104(12)(13)1031(12)0002(24)12202131311122021(12)4130(24)1020212130011031212120112330201001O(01)(01)1110010030010000100100000130001O(01)(01)

Hibbertia hermaniifolia subsp. *hermaniifolia*
024110(123)0003040(01)052(23)(12)0100041041410311110240210213 11(12)1021021141421101200013013212011010010033020100(01)(01)0001100(01)0(01)(12)00100(01)0(01)001(01)00001(23)0001001

Hibbertia hermaniifolia subsp. *recondita*
01411041003010925200000000(04)1011510(03)11010220210213 11(23)102102(01)14342(23)1013112132011(01)2011210010????20100(01)(01)0001100(01)0(01)200100(01)0(01)001(01)0000130001001

Hibbertia hexandra
024110(124)(01)00302051(13)1(12)(01)0000041041410313200(23)202102131321121021(23)4131110020101321321302121201O????20100(01)(01)0001100(01)0(01)200100100001(01)0000130001010

Hibbertia hirsuta
002110(123)00030208111(01)0010003202(12)410(01)(12)20002(46)12(01)021(03)12(12)1121021(1
2)(13)12(12)3100020001013212121211000020020100(01)0(01)(01)01100100200100(01)0(01)00110
10(01)(01)30001001

Hibbertia hirta
00(13)1(01)(12)(13)(01)0021(34)131(13)2(01)001000(13)003011020(23)(12)0004021021202131210
2103(01)211(01)010200014132120110020000????2010110100110(01)010(23)0010110101110010021
010110

Hibbertia hirticalyx
02(01)1112200102151112100000(012)20(012)1110112000031200101243332001011242211(01)020
0010132220000?00012321200100(01)(01)010100010200101(01)(01)00(01)110001011001001

Hibbertia horrida
004110(123)(12)003010111200100004104111031100024121021(03)1(03)113210201214211003210
032132231211100000????2001000(01)(01)01(01)00100(01)00100(01)0(01)001100001300100(01)(01
)

Hibbertia humifusa subsp. *debilis*
00121(01)030011301101(01)00111032012210222(01)00020200211012212002021111(14)(12)2(02)0
2(01)00200010111121200121(23)12001100(01)(01)10100100100100101000000010031100101

Hibbertia humifusa subsp. *erigens*
00121(01)030011301101(01)00111032012210222(01)00020200211012212002021111(14)(12)2(02)0
2(01)00200010111111200121(23)12001100(01)(01)1010010010010110100110010021001101

Hibbertia humifusa subsp. *humifusa*
00(01)21(02)(123)0011(012)(02)(01)1(01)(01)(01)(01)0(01)(01)00(01)(012)(02)(01)(012)(12)(12)(01
)(01)1(12)(012)(01)002(01)(01)(12)(01)(01)1111111(12)111111100112(01)(23)(123)121200010111
121200121(23)120010001111100100100010(01)00110001021001010

Hibbertia hypsophylloides
001210120021201112(01)000000120123101121002(14)02102121221(24)2102113121311230111321
3213121011000????2001010(01)(01)11100100200010(01)(01)0001100001200010(01)(01)

Hibbertia nitida
0(12)3202033011(24)1514(01)(12)0011115315321022(15)0(01)1261210103343454333046453310(0
1)2212200132000102?2000033120010000111100100000100001101000100001010(01)(01)

Hibbertia notabilis
011(12)10(23)10011(12)15212000000012012110101000041200211112322002021211310102000101
3222110000013????2001100(01)(01)00100100100101100001000(01)10031001101

Hibbertia obtusibracteata
00540003014241100000000111531530114300013142102033434443330464532100020003213203111
2001220100200100(01)(01)01110010000001001101110010000001001

Hibbertia oligastrota
004212(12)10031100102(012)000000420120101120003112102112121121021312423112121110001
102111102001????2001000(01)(01)11100(01)0(01)10010000(01)(01)11100(01)(01)030001001

Hibbertia oxycraspedotus
01(03)2101(12)014220(12)0020010000020(05)(23)2100220002(34)12102033021121020(12)(01)(12)
(12)1311002122(23)213222011212000????2001100(01)(01)11100(01)0(01)1000110110(01)0100010
100010(01)(01)

Hibbertia paeninsularis
00301303002010420200020103(02)03(02)220202200341210102(01)(24)(23)0121020130421120020
01020012(13)101210000030120010000110100100100010(01)(01)000110010010010001

Hibbertia pallidiflora
02111130002041511111000001101202012100001100010124332200100124230010200020132100210100000321200100(01)(01)00100010020010000100100010010010010

Hibbertia patens
0131110300203052111001000300305102021002112201120221121011230213212221212002001111010000????2010000(01)(01)10100010200100(01)(01)0001000010200010(01)(01)

Hibbertia pedunculata
01311003001010200200000003(12)032610(02)1210026021020(012)1(24)(23)10210202(13)2323212201213013212011211011212020010000111100010100100(01)(01)000100001020001001

Hibbertia pilifera
003200030142112122001011153143110211000221200212(12)43102102023(12)422100020012213232111212122????2001100(01)(01)010000010000110(01)(01)00100000100010010

Hibbertia platyphylla subsp. *halmaturina*
000112421153501031200000012012410122121010210111112102001021242122212111201321111112000003202001001(01)(01)11001100200010(01)(01)000100010030100010

Hibbertia platyphylla subsp. *major*
002113220001311031200000021001410010100010211110101102101020201122222112301321111112000003202001000(01)(01)1100010020001000100010010030001001

Hibbertia platyphylla subsp. *platyphylla*
02121(03)420011(24)0(01)0(01)2200(02)000(13)(12)0(13)(23)(12)220(12)2(12)00(02)(03)(04)(12)(1
2)(01)01102(14)(13)(01)12(13)(012)(01)(01)(12)1(12)(13)21(12)(01)2121(12)(12)(02)(01)(13)(12)(1
2)11(01)111000103202001100101100010010001110101110010020001010

Hibbertia porcata
01(13)111(13)(01)00200011021000000(13)10(13)01102120103412202021(12)(12)11221211(13)1(1
2)1(14)(01)112012110132(02)20012100100210200100001011001002001000(01)(01)001100001200
01010

Hibbertia praemorsa
0231120300211060(135)1010100030030510202100031210202020202102023(12)4212000212133132120112100002121200120(01)(01)011100100200101(01)1000110010(01)310010(01)(01)

Hibbertia protuberans
00(45)(24)002(01)003(12)10(01)022(01)010110(45)(23)02(23)110(03)210002412102101432021020(
12)02423101021312000(12)02111011000????200100001111001(01)(01)0000100010(01)110000100
0010(01)(01)

Hibbertia pusticulata
011(24)10(12)0001141(07)022101010143012220011000230210210112(24)02102021142110002121000010211021(02)000????2001000(01)(01)1100010010001001100100011010001010

Hibbertia reticulata
02(01)113(13)200311051113300000(01)1011(12)10(01)1320032122121(01)(01)(01)(01)21220211(0
1)(01)(01)(01)510(23)32(12)3132132120(01)1112000????20100(01)(01)0011100(01)0(01)200100(0
1)(01)0001100001300100(01)(01)

Hibbertia rhynchocalyx
0(12)120(01)(23)20021(23)0(67)(01)(34)1(012)(01)010001101(01)1101121000(03)12(01)021(12)01
1432102121(01)11(23)(01)010200034132320100000(01)1????2010111000100010020010110000110
000031001110

Hibbertia riparia
00(13)(12)102(01)001110(24)1020000000120120201210002(34)12102012431(03)2001021242000012010321323311121000003002001000(01)(01)01000100100010(01)(01)000110010021001001

Hibbertia rufa
00540003003000120200000111531535104310002102002043431021020(23)54424110020103213233111212122????200100001(01)1100001000100(01)0(01)00100000120001001

Hibbertia semota
001110(12)(01)00(12)030(02)002(12)00001041011(15)10(01)1(12)00024021021311(12)1021021(12)1111110(13)02010(23)001021000012010????20(01)(01)0(01)(01)00(01)11001002001001100010000013000101(01)

Hibbertia sericea var. *densiflora*
0(01)111(13)(12)(01)00(12)1302102(01)000000(13)10(13)0220(12)02000(23)(34)021021(12)0(12)(23)2021021(123)(13)0(12)(12)1(01)01021213201103121211011????201000(01)(01)010100(01)0(01)100100(01)0(01)0000000(01)(01)30001010

Hibbertia sericea var. *scabrifolia*
0011102100(12)110(18)1020000000310(13)1220(12)110002(02)221021(12)012(02)021021(12)(12)0301(01)010212132020231(12)1211011????201000010(01)0100100200100(01)(01)0000100010300100(01)(01)

Hibbertia sericea var. *sericea*
0212123200112020321000000120122201221012402001110123220011212111111122012220012110101100002102010110101100010030010100100100000031010010

Hibbertia serpyllifolia
00110011001(12)10(16)2020010000110122101(12)1000(23)(12)1210201(23)431020021(12)3(23)2(12)21013010130132031(02)1212000????20010000101100(01)0(01)1000100(01)(01)01110010000001001

Hibbertia sessiliflora
00111240001110100100 0?00012012020220000240210111113102101023241110022000120203101101000003102001000(01)(01)11000100100010101000000(01)0030001010

Hibbertia setifera
0013004200111031120000000110115101121000402001111112020000011421111220212013202111010000????200100001(01)100010020001200100110010030001010

Hibbertia simulans
0031110300212002020000000320322102110002412101120431221011330211111221112002032111011000????2001200(01)(01)01100100100101010001100100210010(01)(01)

Hibbertia spathulata
0141103(01)00304081321000000041041(34)1031210033021020311210210213411241023011002132121(12)1011010????20100(01)(01)0001101001300100(01)(01)000100000130001010

Hibbertia stricta
001(12)102000311011020000000(14)(12)0122103(12)10000(146)12102111(01)2142002114142(25)10002111(23)2132131(12)1011010????20010001011100(01)0(01)20010010100100010030001010

Hibbertia strigosa
004110(12)(01)00301011022010000410 4(12)21010200014021021(12)0(12)(23)1021021(12)(13)01(12)2112021113213202121211000????2001000(01)(01)(01)01001(01)(01)(12)0001101100010011011001010

Hibbertia superans
00111130002100010210000001101121021210023121011214311210103304211112210120020211111100002120200110(01)(01)00100010020001100100100000130001010

Hibbertia surcularis
00540003014211210200001103(23)153(12)10221000231200204333(12)120020253425100020013213233111111122????20010001011100001000100(01)0(01)00100000120001001

Hibbertia synandra

00(13)110(12)(02)003(01)10122210100003(12)0126102(01)3000(23)41210212(01)2(12)1021021(12)30302112021113213223111211000????2001100(01)(01)(01)1100001200001011001000001100010 01

Chapter 3

A molecular phylogeny of the south-eastern Australian clade of *Hibbertia* Andrews subg. *Hemistemma* (Thouars) J.W.Horn (Dilleniaceae)

Abstract

Phylogenetic relationships among 87 of the 93 taxa representatives of the south-eastern Australian clade of *Hibbertia* subg. *Hemistemma* (Thouars) J.W.Horn, were assessed by using nuclear rDNA (ITS) and intergenic spacer regions of cpDNA (*trn*L–F) sequence data. When data were analysed separately, phylogenetic relationships based on ITS data analyses were poorly resolved, with *trn*L–F was slightly better. The combined molecular data produced 7 clades: five clades form a grade of species pairs, but two large, distal clades (F and G) were recognisable, the last clade with two subclades. The rDNA (ITS) and cpDNA (*trn*L–F) data sets were useful to enhance the resolution of the infrataxa relationships. Phylogenetic relationships are summarised, taxon delimitation and evolution are discussed.

Introduction

Hibbertia (Dilleniaceae: Hibbertioideae) is a genus of ~380 species (many new and undescribed), chiefly in Australia, some extending to Madagascar, Malaysia, and Fiji (CHAH 2008). Four subgenera (*Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn) are recognised based on floral features such as stamen number, fertility, fusion and arrangement, carpel and ovule number (Horn 2005, 2007). However, these features vary considerably, in particular those of the androecium (Horn 2005).

Recent molecular analyses of Dilleniaceae by Horn (2005) placed all SE Australian *Hibbertia* taxa from subgen *Hemistemma* within a single clade. Subgenus *Hemistemma* consists of ~160 species mostly from southern Australia and 16 informal groups species within the SE Australian clade (Ch. 2: Tab. 1) have been proposed by Toelken (1998, 2000, 2010 and pers. comm. 2009). Accordingly, a major goal of this study is to test the phylogenetic coherence of Toelken's informal groups and to investigate species relationships within the SE clade *Hibbertia* subg. *Hemistemma.*

There have been numerous morphological taxonomic studies on *Hibbertia*; Bentham (1863) recognised 41 species, expanding on the description of 19 species from eastern Australia by de Candolle (1817). Additional studies include those wood anatomy (Dickison 1967, 1979), pollen morphology (Dickison *et al.* 1982), carpels (Dickison 1968), leaf anatomy (Dickison 1970) and leaf venation (Rury and Dickison 1977). In addition, many species have also been described based on features such as surface indumentum (vestiture) (Toelken 1995, 1998, 2000, 2010), floral ontogeny (Tucker and Bernhardt 2000) and stamen number (Tucker and Bernhardt 2003). Morphological studies using these types of characters have also been used for the description of numerous new Western Australian species (Wheeler 2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b)

Hibbertia is shows significant variation in morphological characters (Horn 2005, 2007), particularly floral characteristics such as variation in gynoecium number (Gilg and Werdermann 1925; Hoogland 1952; Endress and Igersheim 1997). Thus, molecular analysis may be useful to elucidate character variation and evolution between closely related taxa using nrDNA and cpDNA sequence data (Soltis and Soltis 1998).

Chloroplast DNA (cpDNA) regions are utilised widely to problems in systematics (Drábková *et al.* 2004), specifically, the use of non-coding regions such as the *trn*L intron and *trn*L–F intergenic spacer (Taberlet *et al.* 1991; Stanford *et al.* 2000) especially at lower taxonomic levels (Gielly and Taberlet 1994). However, studies using cpDNA to determining phylogenetic relationships in *Hibbertia* are few.

The ITS region (internal transcribed spacer) of the nuclear rDNA genes (rDNA) is also used widely in phylogenetic studies. ITS1, 5.8S and ITS2 region are each approximately ~700 bases in length and can be amplified by using the polymerase chain reaction (PCR) (Cox *et al.* 1997; Cameron *et al.* 1999), and ITS has been utilised widely to illustrate species relationships.

Accordingly, we used ITS and non-coding cpDNA (*trn*L–F intergenic spacer) sequence data. Our study aimed to achieve the following:

1) To investigate the phylogenetic lineages and relationships of species of SE Australian *Hibbertia* subgenus *Hemistemma*.

2) To test the monophyly of species of *Hibbertia* subg. *Hemistemma* based on rDNA (ITS) and cpDNA (*trn*L–F) in compare and combined.

Materials and methods

Eighty-seven of the 93 taxa representing the SE Australian clade of *Hibbertia* subgenus *Hemistemma* recognised by Horn (2005) were included in the present study (Table 1). *Hibbertia ancistrophylla* J.Wheeler from Western Australia was used as the outgroup.

Table 1. List of *Hibbertia* taxa and specimens and their voucher information used in molecular analysis

Taxon	Collector and state	Herbarium	Accession no.
H. acaulothrix	J.B. Briggs 2080, NSW	NSW	386
H. acicularis	H.C. Beauglehole 43835, Vic.	AD	98621183
H. acaulothrix	J.B. Briggs 2080, NSW	NSW	386
H. ancistrophylla	J.W.Horn 4049, WA	AD	158577
H. apoda	collector unknown 3886, NSW	CANB	168185
H. appressa	A.M. Buchanan 1295, Tas.	HO	97456
H. aspera subsp. *aspera*	R.S. Dick CL7a, Qld	BRI	253673
H. aspera subsp. *pilosifolia*	R.J. Henderson, L. Durrington , P. Sharpe 1040, Qld	BRI	182059
H. australis	D.J. Whibley 9655, SA	AD	98824292
H. boweniensis	P. Hind , J. Forlonge 2647, NSW	NSW	254914
H. bracteata	R.D. Hoogland 12233, NSW	NSW	131
H. caespitulosa	van Reyen 9338, Qld	BRI	059659
H. calycina	G. Pulley 476, NSW	CANB	056574
H. cinerea	R. Bates 1409, SA	AD	98938203
	M. Kenny s.n., Sept. 1975 (SA, AD)	AD	NA
H. cistiflora	C. Burgess s.n., Jul. 1963 (NSW, AD)	CANB	004753
H. cistoidea	collector unknown 4652, Qld	BRI	133172
H. corifolia	H. Salasoo 1810, NSW	NSW	496
H. covenyana	R. Caveny, S.K. Roy 9042, NSW	CANB	00404379
H. crinita	Ian Abbott 475, SA	AD	171780
	D.J.E.Whibley 3817, SA	AD	97242022
H. cuneifolia	T. Tame 1127, NSW	NSW	47
H. decumbens	C. Burgees s.n., Nov. 1970 (NSW, AD)	CANB	036200
H. devitata	A.R.R. Higginson s.n., Oct. 1956 (SA, AD)	AD	966072887
H. dispar	R. Bates 10636 Vic.	AD	98801233
H. empetrifolia subsp. *empetrifolia*	R. Pullen 4123, NSW	AD	96620012
H. empetrifolia subsp. *radians*	Lindley D. Williams 6005, SA	AD	9843585
H. empetrifolia subsp. *uncinata*	R.D. Hoogland 12243, NSW	NSW	218
H. erectinervis	collector unknown s.n., Nov. 1953 (NSW, AD)	CANB	126265
H. expansa	J.R. Jelford 2547, NSW	CANB	037098
H. exutiacies	H.M. Cooper s.n., Oct. 1941 (SA, AD)	AD	7317115

H. florida subsp. *angustinervis*	R. Coveny, NSW	NSW	535
H. florida subsp. *florida*	collector unknown s.n., no date, NSW	AD	1086480
H. fruticosa	R. Coveny 8877, NSW	NSW	529
	K. Hill 2769, NSW	NSW	533
H. glaciifera	R.W. Johnson 2497, Qld	BRI	339654
H. glebosa subsp. *glebosa*	R.V. Southcott s.n., Oct. 1960 (SA, AD)	AD	97323349
H. glebosa subsp. *oblongata*	B.M. Overton 2569, SA (KI)	AD	99649112
H. hendersonii	R. J. Henderson, L. Durrington , P. Sharpe 059, Qld	BRI	182353
H. hermanniifolia subsp. *hermaniifolia*	G.L. Stebbins A–63, NSW	NSW	255007
H. hermanniifolia subsp. *recondite*	A.C. Beauglehole 37140, Vic.	CANB	237920
H. hexandra	R.D. Hoogland 11807, NSW	CANB	205127
H. hirsuta	R.D. Hoogland 11748, NSW	CANB	200389
	W.M. Curtis s.n., Nov. 1952 (TAS, AD)	HO	3114
H. hirta	I. Telford , H. Streimann 3808, NSW	CANB	056123
H. hirticalyx	A.E. Orchard 5860, TAS	HO	98430192
H. horrida	J.R. Jelford 2589, NSW	CANB	037408
H. humifusa subsp. *debilis*	H.C. Beauglehole 38020, Vic.	HO	64375
H. humifusa subsp. *erigens*	R. Thomas 583, Vic.	MEL	2019490
H. humifusa subsp. *humifusa*	A.C. Beauglehole 69828, Vic.	MEL	49799
H. hypsophylloides	L.G. Adams 3478, Qld	BRI	430130
H. nitida	collector unknown 26599, NSW	NSW	263
H. notabilis	David E. Albrecht 1037, Vic.	MEL	673624
H. obtusibracteata	G. Jackson 3008, SA (KI)	AD	99051222
H. oligastrota	J.L. Clarkson 5879, Qld	BRI	359875
H. oxycraspedotus	L.G. Adams , K. Paijmans 3780, NSW	CANB	381942
H. paeninsularis	B.M. Overton 2600, SA	AD	99733365
H. pallidiflora	R. Bates 7620, SA	AD	98649870
H. patens	G. Leiper 458082, Qld	BRI	447077
H. pedunculata	W. Forsyth, s.n., Jun. 1897 (NSW, AD)	AD	98142025
	J.M. Bechervaise s.n., Jan. 1948 (VIC, AD)	MEL	35577
H. pilifera	Michael Evans 2519, NSW	CANB	161485
H. platyphylla subsp. *halmaturina*	B.M. Overton 2498, SA	AD	99610194
H. platyphylla subsp. *major*	J.B. Weber 6166, SA	AD	97947114
H. platyphylla subsp. *platyphylla*	N. Donner 11095, SA	AD	98631006
H. porcata	H.C. Beauglehole 91076, Vic.	MEL	119768
H. praemorsa	P. Gilmour 5272, NSW	CANB	8504647
H. protuberans	N. Geary s.n., Feb. 1944 (BRI, AD), Qld	BRI	010716
H. pusticulata	I. Cowie 164, Qld	BRI	253649
H. reticulata	B.J. Conn 3787, Qld	AD	99542107
H. rhynchocalyx	J.B. Williams 603, NSW	NSW	95
H. riparia	E.N.S. Jackson 4291, SA	AD	291
H. rufa	R.D. Hoogland 11917, Vic.	MEL	580142
	J.D. Briggs 692, NSW	NSW	833
H. semota	P.K. Latz 10646, NT	AD	99508051
	B.G. Thomson 3552, NT	AD	99507217

H. sericea var. *densiflora*	R.D. Hoogland 11910, Vic.	MEL	572143
H. sericea var. *scabrifolia*	R. Melville, P. Morris , A. Hicks 985, Vic.	NSW	403
H. sericea var. *sericea*	R.D. Hoogland 11888, Vic.	CANB	205548
H. serpyllifolia	P.R. Sharpe 3001, Qld	BRI	405505
H. sessiliflora	A.C. Beauglehole 38005, Vic.	MEL	517653
H. setifera	R. Bates 61074, SA	AD	155475
H. simulans	collector unknown s.n., Nov. 1918 (NSW, AD)	CANB (NSW)	710
H. spathulata	N.G. Walsh , K.C. Norris 2209, Vic.	MEL	119822
H. stricta	J. Taylor, J. Rymer , R. Jackson 1251, NSW	CANB	8008298
H. strigosa	A.C. Beauglehole 91572, Vic.	MEL	119619
H. superans	H. Streimann 053933, NSW	CANB	053933
H. surcularis	collector unknown 287, Vic.	MEL	1003813
H. synandra	B. Hyland 7065, Vic.	BRI	235206
H. tenuis	D.E. Murfet , R.L. Taplin 1910, SA	AD	99531091
H. tetranthera	R.D. Hoogland 12252, NSW	NSW	202
H. truncata	S.G. Harris 108, Vic.	HO	120083
H. vestita	W.J. McDonald 398283, Qld	BRI	375462
H. villifera	R. Bates 61095, SA	AD	155427
H. woronarana	R.D. Hoogland 12257, NSW	CANB (MEL)	572180

DNA extraction

Total DNA was extracted from herbarium material (leaves and stems) following the standard protocol for the Nucleospin Plant system (Machery-Nagel GmbH & Co, Düren, Germany).

PCR reactions

The whole ITS region (including 5.8 S) was amplified separately using two primers. The forward primer sits in the beginning of the 5.8S nuclear ribosomal region and was designed based on alignments between major fungal and land plant groups, to avoid amplification of epiphytic or contaminating fungi. The reversed primer was ITS4 28S TCCTCCGCTTATTGATATGC (White *et al.* 1990), and both were amplified according to the protocol in Table 2 for each 1 µl of template DNA.

Amplification profile

The amplification profile for using the relevant primers: 35 cycles was 94°C for 1 min to denature the DNA; 60°C for 1 min to anneal the primers; and 72°C for 2 min to polymerize of the new strand. At the end of 35 cycles, the temperature was held at 20°C for 1 min.

Table 2. Reaction volumes used in PCR reactions for each 1 µl of template DNA.

Reagents	Quantities (µl)
PCR water	6
PCR Buffer, 10 x*	1.25
Betaine	1
MgCl2	0.5
dNTP	1
Primer 1	1
Primer 2	1
HotStarTaq DNA	0.25

Table 3. Forward and reverse primers used (White *et al.* 1990; Taberlet *et al.* 1991)

Primers	Sequence 5′–3′
*trn*L–F spacer forward (E)	GGTTCAAGTCCCTCTATCCC
*trn*L–F spacer reversed (F)	ATTTGAACTGGTGACACGAG
ITS4 forward (A1854)	ACGACTCTCGGCAACGGATA
ITS4 reversed (P312)	TCCTCCGCTTATTGATATGC

Sequencing

The standard sequencing protocol was applied using the forward and reversed primers and Big Dye Terminator V3.1 (Life Technologies Ltd, Carsland, California, USA). Sequencing reactions were analysed on an Applied Biosystem 3730*xl* Genetic Analyser (Life Technologies Ltd).

Outgroup

For the outgroup, *H. ancistrophylla* J.W.Wheeler from Western Australia was included in the analysis, based on its position in a sister Clade, following Horn (2005).

Phylogenetic analyses

Initial DNA sequences were aligned using the default options in BioEdite (Hall 1999), and finalised using CLUSTAL X version 2.0 (Larkin *et al.* 2007). The molecular matrices were analysed separately and in combination using WinClada version 1.00.08 (Nixon 2002) with the retention option, 1000 replicates (TBR on, hold 10 trees per step.). Jackknife support was calculated with the same options but TBR off. The analysis produced 107 equally most parsimonious trees for the combined ITS and *trn*L–F data (L 5692; CI 36; RI 47; 245 uninf). Jackknife support of 50–74% is considered low, 75–84 moderate, and >85% high (Hassan *et al.* 2005).

Results

The ITS sequences were successfully obtained for all 87 samples, but for the *trn*L–F intergenic region sequences, 11 taxa were not able to be sequenced after several attempts due to DNA degradation of old herbarium specimens after multiple attempts (*H. bracteata, H. corifolia, H. hendersonii, H. florida* subsp. *florida, H. obtusibracteata, H. reticulata, H. simulans, H. sericea* var. *densiflora, H. sessiliflora, H. stricta* and *H. woronarana*). For the ingroup taxa, ITS length ranged from 350–400 bp and *trn*L–F varied from 380–460 bp. In total, 1,102 bp were aligned for the combined analysis.

The ITS region data was analysed and recovered 1367 equally parsimonious trees (L 1613; CI 45; RI 68) and a strict consensus tree is shown in Fig. 1. The cladogram of the ITS region was poorly resolved, with terminal clades all placed in an unresolved basal polytomy. However, 7 clades were very most weakly supported. The two clades A and B each comprised two taxa; with Clade C consisting of *H. paeninsularis* as sister to a *H. devitata* + *H. simulans*.

Clade D contained two sister groups: *H. oxycraspedotus* + *H. pusticulata* and *H. empetrifolia* subsp. *radians* + *H. decumbens* (Fig. 1).

All taxa within Clade E were arranged in an unresolved polytomy, whereas Clade F consisted mostly of taxa arranged in three lineages. The first lineage comprised *H. covenyana* and *H. glebosa* subsp. *oblongata* as sisters to a *H. australis* + *H. sericea* var. *scabrifolia*; the second comprised *H. cistiflora* + *H. platyphylla* subsp. *platyphylla* and *H. humifusa* subsp. *debilis* + *H. humifusa* subsp. *erigens* with *H. fruticosa* as a sister; and the third comprised H. villifera + *H. sericea* var. *sericea* with H. sericea var. sericea and *H. synandra* as sister relationships (Fig. 1).

Clade G divided into two subclades: G1 and G2. Within subclade G1, three lineages formed a polytomy: the first with two groups, each with two taxa; the second also comprised two groups, one of which contained infrataxa from two different species *H.*

empetrifolia subsp. *empetrifolia* + *H. aspera* subsp. *pilosifolia*); the third lineage comprised *H. hermaniifolia* subsp. *hermaniifolia* + *H. hexandra* and *H. hermaniifolia* subsp. *recondita* + *H. hexandra*. In contrast, subclade G2 contained 14 taxa, all in an unresolved polytomy (Fig. 1).

The *trn*L–F analyses (Figs. 3 and 4) produced 2077 most parsimonious trees of length 3058, consistency index 41 and retention index 57. The *trn*L–F strict consensus tree was different from that of the ITS gene region, with two taxa (*H. platyphylla* subsp. *major* and *H. covenyana*) placed in basal polytomy below a further 26 taxa, also arranged in an unresolved polytomy (mostly as species pairs). However, the *trn*L–F results show better support for several taxa phylogenetic relationships, with 10 clades present in the strict tree (Fig. 3). These 10 clades consisted of five small species-pairs (A–E), four small multispecies clades (F–I) and a larger lineage (J).

Clade A consisted of *H. cistiflora* + *H. semota* with strong support (89% JK) and the remaining small clades were moderately supported (77%, 73, 78%, and 78% JK respectively) (Fig. 3). Within Clade F, *H. cuneifolia* was a sister to the strong supported group *H. empetrifolia* subsp. *radians* + *H. paeninsularis* (81% JK). Clade G (four taxa) was strong supported taxa Clade G that was strong supported (99% JK), with *H. appressa* (85% JK) sister to the moderately supported group (73% JK) (*H. hirticalyx* +*H. pedunculata*) above *H. nitida*.

Five taxa within Clade H were arranged in two pairs above *H. apoda*, but only one of the pairs received moderate support (*H. oligastrota* + *H. erectinervis*; 77% JK). Six taxa within Clade I, *H. tenuis* (78% JK) and *H. surcularis* (78% JK) were proximate to the strong supported pair *H. riparia* + *H. hexandra* (84% JK) all above a polytomy with *H. empetrifolia* subsp. *empetrifolia* and *H. sericea* var. *scabrifolia* distal to four basal taxa . Clade J was the largest consisted of 18 taxa arranged in two divergent lineages. The first (J1) consisted of six taxa, only one pairs received strong support *H. truncata* + *H. boweniensis* (100% JK) with *H. glebosa* subsp. *oblongata*, *H. platyphylla* subsp. *halmaturina*, *H. strigosa*, and *H. hirsuta* were placed as sister (100%; 99%; 93%; and 99% JK respectively). The second (J2) consisted of two strong supported pairs: (*H. glebosa* subsp. *glebosa* + *H. notabilis*; 89% JK) with *H. acicularis* as a sister (95% JK) and (*H. pallidiflora* + *H. rhynchocalyx*; 90% JK) with sisters *H. humifusa* subsp. *debilis*, *H. aspera* subsp. *aspera*, *H. florida* subsp. *angustinervis* (78%; 79% and 100% JK respectively) (Fig. 3).

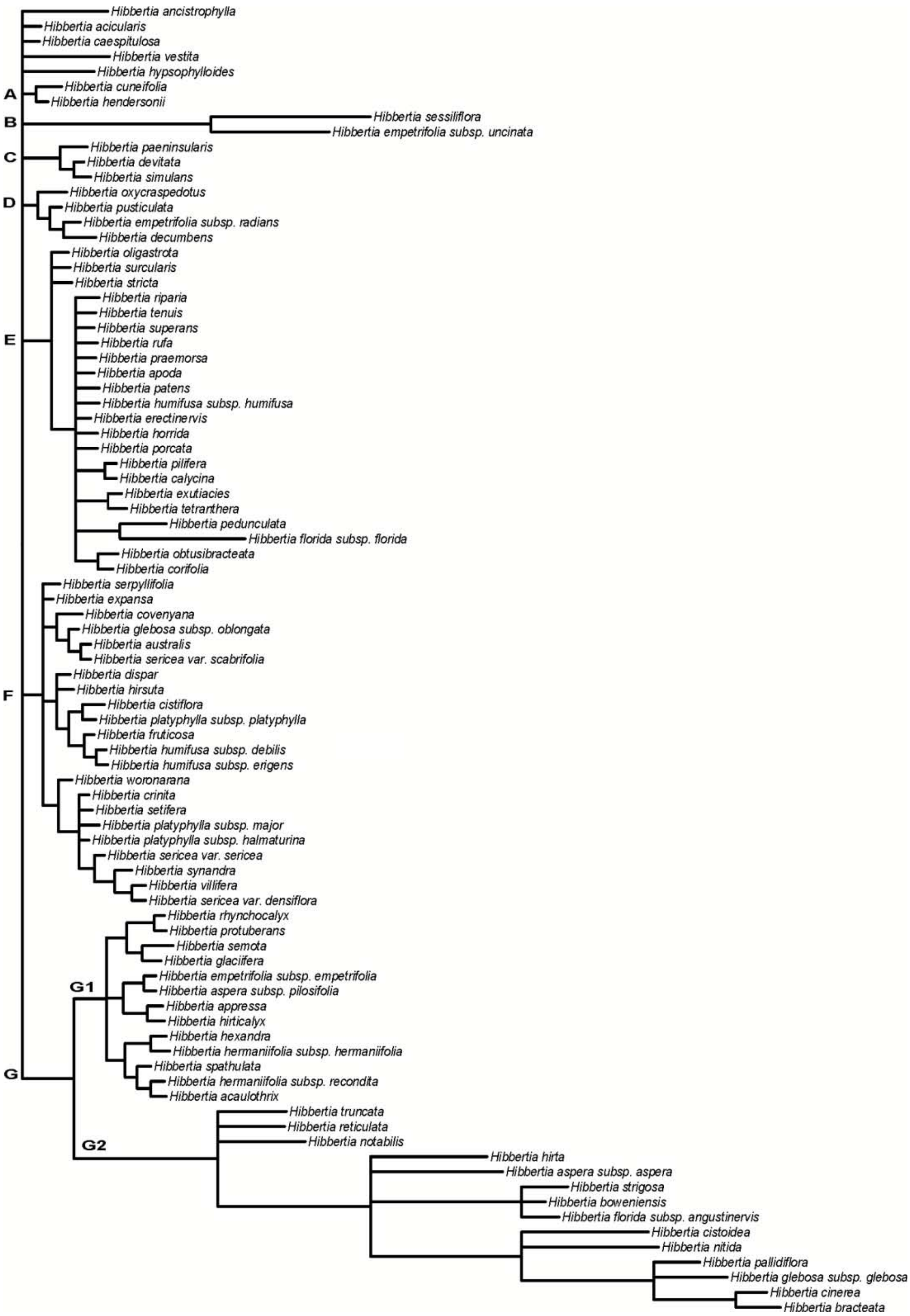

Figure 1. Strict consensus tree from 1367 equally most parsimonious trees (L 1613; CI 45; RI 68; 161 uninf) produced by a WinClada ratchet analysis of the ITS data (1000 reps, 10 trees held per rep) showing jackknife support values (1000 reps, 10 trees held per rep, TBR off).

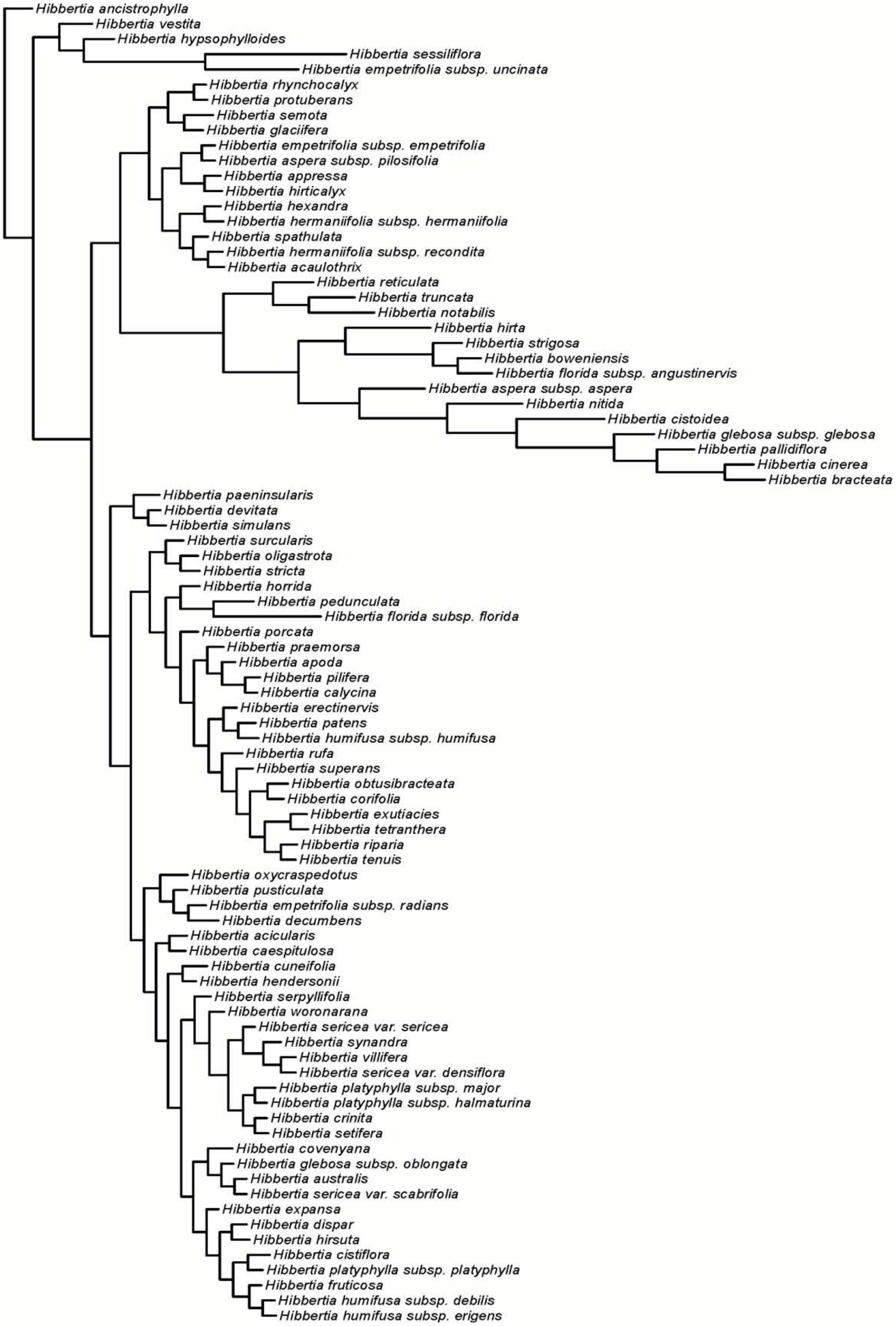

Figure 2. Randomly selected tree from 1367 equally most parsimonious trees (L 1613; CI 45; RI 68; 161 uninf) produced by a WinClada ratchet analysis of the ITS data (1000 reps, 10 trees held per rep) showing proportional branch lengths.

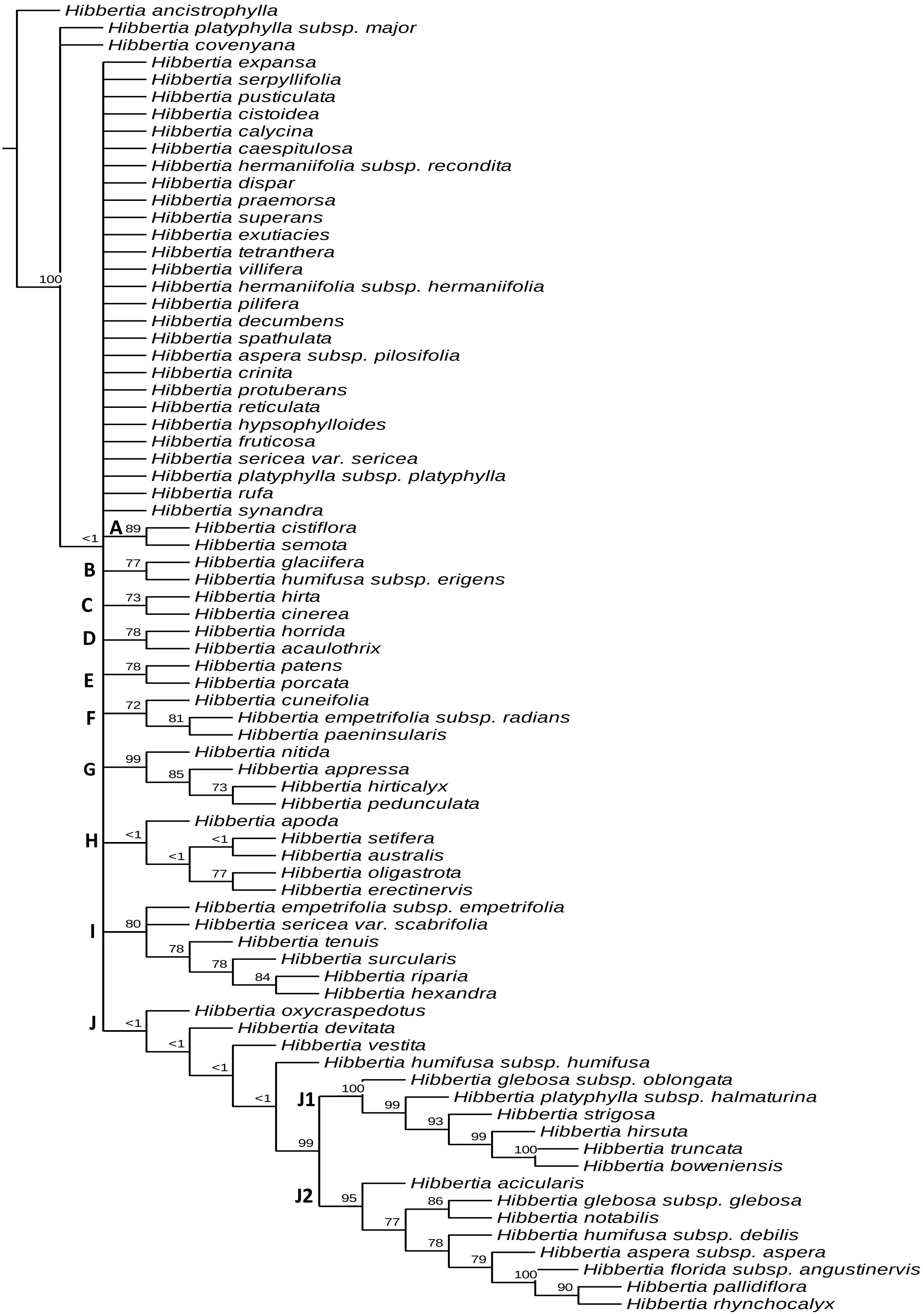

Figure 3. Strict consensus tree from 2077 equally most parsimonious trees (L 3058; CI 41; RI 57; 84 uninf) produced by a WinClada ratchet analysis of the *trn*L–F data (1000 reps, 10 trees held per rep) showing jackknife support values (1000 reps, 10 trees held per rep, TBR off).

Figure 4. Randomly selected tree from 2077 equally most parsimonious trees (L 3058; CI 41; RI 57; 84 uninf) produced by a WinClada ratchet analysis of the *trn*L–F data (1000 reps, 10 trees held per rep) showing proportional branch lengths.

The combined ITS and *trn*L–F analysis produced 107 equally most parsimonious trees of length 5692, consistency index 36 and retention index 47 (Figs. 5–6). Seven clades were obtained from the combined molecular analysis produced, four clades (A–D) consisted of pairs, but only Clade B was strong supported (94% JK) with moderately supported (*H. sericea* var. *scabrifolia* + *H. dispar*; 73% JK) within Clade D.

There were two lineages within Clade F. The first consisted of *H. cinerea* + *H. hirta* (70% JK) with three strong supported sisters: *H. cistoidea*, *H. nitida* and *H. reticulata* (99%; 82%; 100% JK respectively). The second contained 22 taxa, only three strong supported groups were formed: (*H. exutiacies* + *H. tetranthera*; 98% JK), (*H. pilifera* + *H. calycina*; 98% JK) and (*H. appressa* + *H. hirticalyx*; 95% JK) (Fig. 5).

Clade G consisted of 48 taxa arranged in two subclades: G1 and G2. The first (G1) comprised 9 taxa arranged in three groups (*H. glaciifera* + *H. semota*; 72% JK) with *H. protuberans* as a sister (69% JK), (*H. empetrifolia* subsp. *empetrifolia* + *H. aspera* subsp. *pilosifolia*; 97% JK), and (*H. hermaniifolia* subsp. *recondita* + *H. acaulothrix*; 72% JK) with *H. spathulata* as a strong supported sister (90% JK). The second (G2) was the major, consisted of 39 taxa, all arranged in two lineages, the first contained 6 taxa that *H. decumbens, H. crinita, H. sericea* var. *sericea*, *H. villifera* placed as sister to the low supported *H. synandra* + *H. sericea* var. *densiflora* (54% JK); the second consisted of 33 taxa, only 6 strong supported groups were formed: (*H. australis* + *H. covenyana*, 98% JK), (*H. sessiliflora* + *H. empetrifolia* subsp. *uncinata*, 100% JK) with one strong supported sister (88% JK) *H. rhynchocalyx*, (*H. boweniensis* + *H. strigosa*, 100% JK) with five sisters that only three of them strong supported *H. platyphylla* subsp. *halmaturina*, *H. simulans* and *H. truncata* (95%, 81%, and 100% JK respectively), (*H. humifusa* subsp. *debilis* + *H. acicularis*, 92% JK), (*H. glebosa* subsp. *glebosa* + *H. bracteata*, 93% JK), (*H. pallidiflora* + *H. florida* subsp. *angustinervis* (94% JK) with three sisters *H. florida* subsp. *florida*, *H. aspera* subsp. *aspera* and *H. glebosa* subsp. *oblongata* (75%, 95%, and 89% JK respectively) (Fig. 5).

Figure 5. Strict consensus tree from 107 equally most parsimonious trees (L 5692; CI 36; RI 47; 245 uninf) produced by a WinClada ratchet analysis of the combined ITS and *trn*L–F data (1000 reps, 10 trees held per rep) showing jackknife support values (1000 reps, 10 trees held per rep, TBR off).

Figure 6. Randomly selected tree from 107 equally most parsimonious trees (L 5692; CI 36; RI 47; 245 uninf) produced by a WinClada ratchet analysis of the combined ITS and *trn*L–F data (1000 reps, 10 trees held per rep) showing proportional branch lengths.

Discussion

Toelken's groups

Based on both rDNA (ITS) and cpDNA (*trn*L–F) data sets, the analysis resolved the 16 informal groups species of *Hibbertia* subg. *Hemistemma* of Toelken within 7 clades, indicating that Toelken's proposed groups are not supported by this analysis (see Table 4).

The species from the 16 informal groups of Toelken were placed into 7 clades resulted from the morphological analysis (see Ch. 2: Fig. 1), *Aspera* groups species were located within Clade G, but from molecular analysis, the species of *Aspera* group located within different phylogenetic lineages with members of the *Aspera* group located in clades F and G and mixed in with several other groups. Subclade G1 incorporated 5 of Toelken groups, but mainly were members of the *Aspera* and *Hermaniifolia* groups. Similarly, Subclade G2 contained 14 different informal groups species, but included numerous *Aspera* and *Sericea* groups species (see Fig. 5; Tab. 4).

Toelken did suggest a morphological similarity between *H. devitata* and *H. setifera* from the *Stricta* group, and this relationship was weakly supported (57% JK) in Clade C resulted from molecular analysis. From molecular analysis, the three *H. humifusa* infrataxa (*Humifusa* group) were not monophyletic, instead allied with taxa within Clades B, F and G, but from morphological analysis, the three subspecies were arranged in monophyletic within Clade G with no support, but the relationships were strongly supported (*H. humifusa* subsp. *erigens* + *H. humifusa* subsp. *debilis*; 99% JK) (see Ch. 2: Fig. 1). The three *H. platyphylla* infrataxa (*Sericea* group) were also not monophyletic resulted from molecular analysis, instead allied with taxa within Clades B and G; but in morphological analysis cladogram were monophyletic (see Ch. 2: Fig. 1). Similarly for the infrataxa of *H. empetrifolia* (*Aspera* group) which allied with taxa in Clades F and G, and *H. aspera* infrataxa (*Aspera* group) allied with taxa in Clade G; but in morphological cladogram, *H. empetrifolia* subsp. *empetrifolia* + *H. empetrifolia* subsp. *uncinata* with no support, and *H. empetrifolia* subsp. *radians* was close to *H. truncata* (with also no support) (see Ch. 2: Fig. 1). Accordingly, from the results, Toelken's informal assumed groups are not supported.

Table 4. Comparison of the combined analysis datasets with Toelken's informal groups species.

Clade	Taxa	Toelken group
A	*H. hypsophylloides*	Vestita
A	*H. hendersonii*	Strigosa
B	*H. platyphylla* subsp. *platyphylla*	Sericea
B	*H. humifusa* subsp. *erigens*	Humifusa
C	*H. devitata, H. setif*era	Stricta
D	*H. sericea* var. scabrifolia	Sericea
D	*H. dispar*	Pedunculata
E	*H. paeninsularis*	Sericea
E	*H. caespitulosa*	Australis
F	*H. empetrifolia subsp. radians, H. cinerea, H. hirta, H. appressa, H. hirticalyx*	Aspera
F	*H. cistoidea*	Cistoidea
F	*H. nitida*	Bracteata
F	*H. reticulata, H. hexandra, H. hermaniifolia* subsp. *hermaniifolia*	Hermaniifolia
F	*H. exutiacies, H. tetranthera*	Exutiacies
F	*H. pilifera, H. rufa, surcularis*	Rufa
F	*H. calycina, H. horrida*	Strigosa
F	*H. praemorsa, H. patens, H. superans*	Sericea
F	*H. porcata, corifolia, H. pedunculata*	Pedunculata
F	*H. erctinervis*	Vestita
F	*H. humifusa* subsp. *humifusa*	Humifusa
F	*H. apoda*	Stricta
F	*H. riparia*	Riparia
F	*H. tenuis*	Australis
G: G1	*H. cuneifolia*	Australis
G: G1	*H. protuberans*	Acicularis
G: G1	*H. glaciifera, H. semota*	Cistoidea
G: G1	*H. empetrifolia* subsp. *empetrifolia, H. aspera* subsp. *pilosifolia*	Aspera
G: G1	*H. spathulata, H. hermaniifolia* subsp. *recondita, H. acaulothrix*	Hermaniifolia
G: G2	*H. decumbens, H. rhynchocalyx, H. empetrifolia* subsp. *uncinata, H. truncata, H. notabilis, H. aspera* subsp. *aspera, H. pallidiflora*	Aspera
G: G2	*H. crinita, H. sericea* var. *sericea, H. villifera, H. sericea* var. *densiflora, H. platyphylla* subsp. *major, H. sessiliflora, H. platyphylla* subsp. *halmaturina, H. simulans, H. hirsuta*	Sericea
G: G2	*H. synandra, H. strigosa*	Strigosa
G: G2	*H. fruticosa, H. expansa, H. florida* subsp. *florida, H. florida* subsp. *angustinervis*	Pedunculata
G: G2	*H. australis, H. glebosa* subsp. *glebosa, H. glebosa* subsp. *oblongata*	Australis
G: G2	*H. covenyana*	Cistoidea
G: G2	*H. serpyllifolia*	Serpyllifolia
G: G2	*H. pusticulata, H. acicularis*	Acicularis
G: G2	*H. cistiflora, H. oxycraspedotus, H. woronarana, H. oligastrota*	Exutiacies
G: G2	*H. vestita, H. boweniensis*	Vestita
G: G2	*H. stricta*	Stricta
G: G2	*H. obtusibracteata*	Rufa
G: G2	*H. humifusa* subsp. *debilis*	Humifusa
G: G2	*H. bracteata*	Bracteata

ITS and *trn*L–F analysis comparison

The ITS analyses tree is slightly congruent with *trn*L–F analysis tree, particularly taxa within clades B and G in the combined analysis. For example the closely related *H. sessiliflora* + *H. empetrifolia* subsp. *uncinata* was confirmed strong supported in the combined analysis (100% JK) within Clade G. In contrast, the relationship between the infrataxa of *H. humifusa* (*Humifusa* group) were not supported, but allied with other taxa, for example *H. humifusa* subsp. *debilis* close to *H. acicularis* with strong support (92% JK) both in Clade H, and *H. humifusa* subsp. *erigens* placed close to *H. platyphylla* subsp. *platyphylla* with strong support (94% JK) both in Clade B. However, both infrataxa (subsp. *debilis* and subsp. *erigens*) were related in Clade F resulted from ITS analysis, but separated in *trn*L–F analysis tree when placed in Clades B and J, and thus, evolution might be predictable.

Similarly, the positions of the *H. florida* infrataxa (*Pedunculata* group) in the combined analyses tree were also changed, especially when *H. florida* subsp. *angustinervis* placed close to *H. pallidiflora* (94% JK) with *H. florida* subsp. *florida* as a sister (74% JK) in the same Clade G, but separated in clades E and G in ITS analyses.

The ITS analysis tree revealed lower supports for taxa relationships, and the combined molecular analysis supported taxa relationships accurately. The *trn*L–F analysis tree gives a strong good signal for Clade G phylogenetic relationships, particularly the position of *H. truncata* and *H. boweniensis* and other terminal taxa (Figs. 3–5).

Conclusions

Phylogenetic analysis of the SE Clade of *Hibbertia* subg. *Hemistemma* using ITS and *trn*L–F produced seven clades. The ITS analyses is shown low support for taxa delimitation, but *trn*L–F is better. The combined molecular analysis resolved most taxa phylogenetic relationships, with strong support for several species groups and sister relationships. As taxa located in different clades (A–G) mixed in with other groups, it is concluded that all the 16 informal groups of Toelken (unpubl.) are not supported, except the similarity between *H. devitata* and *H. setifera* (*Stricta* group) that placed within Clade C, but with low support (Tab. 4). In addition, several infrataxa are not part of monophyletic species: *H. empetrifolia* infrataxa (subsp. *uncinata* and subsp. *radians*), *H. humifusa* infrataxa (subsp. *debilis* and subsp. *erigens*) and *H. platyphylla* infrataxa (subsp. *halmaturina* and subsp. *major*).

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Chapter 4

A combined molecular and morphological phylogeny of the south-eastern Australian clade of *Hibbertia* Andrews subg. *Hemistemma* (Thouars) J.W.Horn (Dilleniaceae)

Abstract

Hibbertia subg. *Hemistemma* (Thouars) J.W.Horn is one of four subgenera recognised by Horn (2005). The phylogeny within the subgenus is poorly understood and the taxonomic relationships require investigation, especially for the member of the SE Australian clade species. Molecular and morphological data for 87 taxa within the SE Australian clade (species, subspecies and varieties many undescribed) were used for phylogenetic study compared and combined amplified nuclear ITS gene regions and cpDNA (*trn*L–F intergenic spacer) as well as morphological analyses (vegetative-indumentum, floral and anatomical data). All taxa resolved within eight clades, only terminal clades received significant Jackknife support. Several infrataxa within more variable species did not form monophyletic lineages: *H. florida* subsp. *angustinervis* + *H. pallidiflora*, *H. glebosa* subsp. *glebosa* + *H. bracteata*, *H. humifusa* subsp. *debilis* + *H. acicularis*, *H. empetrifolia* subsp. *uncinata* + *H. sessiliflora*, *H. empetrifolia* subsp. *empetrifolia* + *H. aspera* subsp. *pilosifolia*. The characters: lack of indumentum on stem, revolute leaf margins, acuminate inner sepal bases, >15 mm leaf blade width, 10.1–15 mm leaf blade width, absent of inner sepal, ≥10.1 mm inner sepal width, and 3.1–4 abaxial epidermal cell length to width ratio were among the few morphological synapomorphies for Clade/taxon definition. Phylogenetic relationships and character evolution of the taxa are discussed.

Introduction

Hibbertia Andrew (Dilleniaceae: Hibbertioideae) includes four subgenera: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn, based mainly on floral characters (Horn 2005) and distributed mainly in Australia, but extending to Madagascar, Malaysia, Fiji, New Caledonia and New Guinea (Tucker and Bernhardt 2003; CHAH 2008).

Recent molecular phylogenetic studies of Horn (2005) utilised ITS nrDNA and *rp116* intron cpDNA sequence data for 118 Dilleniaceae taxa, producing four main clades representing the subfamilies Dillenioideae, Doliocarpoideae, Delimoideae and Hibbertioideae (Horn 2005). Within the Hibbertioideae clade (=*Hibbertia*) the four traditional subgenera were recovered but as two sister pairs: (*Hibbertia* and *Adrastaea*) + (*Pachynema* and *Hemistemma*) (Fig. 1A). Subgenus *Hemistemma* was itself divided into four clades representing two clade pairs: a Clade largely composed of species from south-west and northern Western Australia, the Northern Territory and Queensland which was sister to a tropical Australia and New Caledonian clade; and a temperate, mainly ericoid-leaved clade pair representing south-west Western Australia and subtropical eastern and south-east Australia (Fig. 1B), the latter hereafter referred to as the SE Aust. Clade.

NOTE:
This figure is included on page 4-2
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 1. Phylogenetic relationships of *Hibbertia* subgenera (A) and the clades within subg. *Hemistemma* (B) summarised from the molecular analyses of Horn (2005).

Toelken (1998, 2000, 2010) has described many *Hibbertia* species, subspecies and varieties from SE Australia, proposing 16 informal groups within the SE Clade (unpubl. pers. comm. 2009), mainly based on surface indumentum and floral characters (see Ch. 2: Tab. 1). The phylogenetic relationships of the SE Aust. Clade of *Hibbertia* subgenus *Hemistemma* and the validity of the proposed groups are poorly understood and more detailed study is essential.

The representatives of the SE Clade of subgenus *Hemistemma* can be characterised as follows: perennial shrubs to medium sized trees; leaves ericoid with fasciculate hairs;

flowers usually terminal, sometimes axillary; calyx of 5, free or sometimes fused sepals; corolla with (3–) 5 free petals; androecium of (1–) 5–100 (–300), partly staminodal, polysymmetric or monosymetric stamens; gynoecium with 1–5 (–10) glabrous or pubescent carpels; ovules 1–25 per carpel; seeds 1–8, aril sub-fleshy (Horn 2005, 2007). However, defining species and taxon boundaries and relationships within subgenus *Hemistemma* is problematic (Toelken 1998, 2000, 2010), and there have been no phylogenetic studies to evaluate character utility or evolution.

Taxonomically, *Hibbertia* is regarded as displaying the most variable character states among Dilleniaceae (Dickison 1969, 1970; Rury and Dickison 1977; Takhtajan 1997), especially in floral symmetry, with sepals and petals arising in radially arranged quincuncial helices (Tucker and Bernhardt 2000), as well as for staminal features (Bernhardt 1986; Rannala and Yang 1996; Tucker and Bernhardt 2003). Several studies have also conducted into character variation, including wood anatomy (Dickison 1967a), pollen morphology (Dickison 1967b), leaf anatomy (Dickison 1970), androecium and gynoecium morphology (Wakefield 1955, 1957) and leaf venation patterns (Rury and Dickison 1977). Indumentum (vestiture) characteristics such as simple and/or stellate hairs have also been considered significant in classification of *Hibbertia* with characters such as: simple unicellular hairs (Trichomes) with tuberculate base (an aggregation of epidermal cells on hair basis) and range of different fasciculate simple hairs to form the stellate hairs used to distinguish numerous *Hibbertia* species by Toelken (1995, 1998, 2010). Hair characters were also included in descriptions *Hibbertia* species and varieties in Western Australia (Toelken and Wheeler 2002; Wheeler 2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b). All of these descriptions and groups were established based on morphological features (mainly indumentum and floral characters) without any explicit evolutionary hypothesis.

Phylogenetic analyses place Dilleniaceae in the order Dilleniales (Thorne and Reveal 2007; APG III 2009) and sister to either Caryophyllales (Chase *et al.* 1993; Soltis *et al.* 2000; Soltis *et al.* 2003), or Vitaceae (Bernhardt 1996; Savolainen *et al.* 2000; Hilu *et al.* 2003). Members of Dilleniaceae show considerable intraspecific variation, but most genera are considered to be monophyletic (Chase *et al.* 2000; Savolainen *et al.* 2000). Intraspecific variation and taxon links particularly among closely related taxa could be solved potentially by using cpDNA sequencing (Soltis and Soltis 1998). Chloroplast

DNA (cpDNA) regions are utilised widely to address problems in systematics (Drábková *et al.* 2004), particularly when using non-coding regions such as the *trn*L intron and *trn*L–F intergenic spacer (Taberlet *et al.* 1991; Stanford *et al.* 2000), as well as the widely used ITS1–2 internal transcribed spacer (Hassan *et al.* 2005).

Accordingly, we used ITS sequence data, non-coding cpDNA (*trn*L–F intergenic spacer) and combined morphological and molecular data to assess the 16 informal species groups in the SE Clade proposed by Toelken (1998, 2000, 2010, pers. comm. 2009). The study aimed to achieve the following:

1) To investigate the phylogenetic relationships of the studied taxa based on combined morphological and molecular data sets.
2) To test the monophyly of the SE Australian clade of *Hibbertia* subg. *Hemistemma*.
3) To explore character evolution and taxon boundaries.

Materials and methods

In total, 87 of 93 taxa representing putative members of the SE Australian Clade of *Hibbertia* subgenus *Hemistemma* of Horn (2005) were included in the present study (for vegetative-indumentum morphology see Ch. 5: App. 1–2; for floral morphology see Ch. 6: App. 1–2 and for leaf cuticle data see Ch. 7: App. 1). *Hibbertia ancistrophylla* J.Wheeler from Western Australia was used as the outgroup. Specimens used in the present study are listed in Ch. 3: Table 1.

Morphological data

Morphological characters and character states used in this analysis are based on study of herbarium specimens for species with identified name or phrase name with collection number (see Ch. 2: Table 2). Herbarium samples were reconstituted by using 2 drops of detergent for 10–15 minutes for dry leaves and flowers. The samples were then placed under a dissecting light microscope (Zeiss model 1968), examined at ×10 magnification and photographed with a digital camera (Panasonic type Lumix model FS42 2005). SEM investigations were also conducted at Adelaide Microscopy, using both XL20 and XL30 type/ Philips (manufactured in 1994 by Philips in Eindhoven, The Netherlands), following standard preparation techniques. The final morphological data matrix included 135 characters that coded into discrete states (Table 1), some characters coded as binary variables, while most were multistate due to the variation in vegetative-indumentum and reproductive features.

Table 1. List of morphological and anatomical character with their character states

1 Habit: erect (0); procumbent (1); decumbent (2)
2 Plant height (m): 0.1–0.5 (0); 0.6–1 (1); ≥1.1 (2)
3 Simple hairs on stem: present (0); absent (1)
4 Hooked hairs on stem: present (0); absent (1)
5 Stellate hairs on stem: present (0); absent (1)
6 Indumentum on the stem surface: sparsely pubescent (0); densely pubescent (1); absent (2)
7 Basal hair cells: unspecialised (0); tuberculate (1)
8 Hair length (mm): 0.1–0.5 (0); 0.6–1 (1); 1.1–1.5 (2); >1.5 (3)
9 Hair branch number: unbranched (0); 1–2 (1); 3–4 (2); 5–7 (3); >7 (4)
10 Hair branch length: equal (0); semi-equal (1); unequal (2); absent (3)
11 Petiole indumentum: present (0); absent (1)
12 Simple hairs on petiole: present (0); absent (1)
13 Hooked hairs on petiole: present (0); absent (1)
14 Stellate hairs on petiole: present (0); absent (1)
15 Indumentum on petiole surface: sparsely pubescent (0); densely pubescent (1); absent (2)
16 Axillary hair tufts: present (0); absent (1)
17 Leaf blade shape: broadly obovate (0); obovate (1); narrowly obovate (2); oblanceolate(2); narrowly oblanceolate (3); linear-oblanceolate (4)
18 Leaf base: cuneate (0); acute (1); obtuse (2); flanged (3)
19 Leaf apex: acute (0), rounded (1); acuminate (2); obtuse (3); truncate-apiculate (4)
20 Leaf margin: entire (0); recurved (1); revolute (2)
21 Leaf lamina length (mm): <5–10 (0); 10.1–15 (1); 15.1–20 (2); >20.1 (3)
22 Leaf blade width: (mm): <1–5 (0); 5.1–10 (1); 10.1–15 (2); >15 (3)
23 Leaf mucro length (mm): absent (0); <0.5–0.8 (1); >0.9 (2)
24 Leaf mid-vein width (mm): <0.1–0.2 (0); 0.3 – 0.4 (1); > 0.4 (2)
25 Leaf adaxial midrib indumentum: present (0); absent (1)
26 Leaf abaxial midrib indumentum: present (0); absent (1)
27 Leaf adaxial surface indumentum: present (0); absent (1)
28 Simple hairs on leaf adaxial surface: present (0); absent (1)
29 Hooked hairs on leaf adaxial surface: present (0); absent (1)
30 Stellate hairs on leaf adaxial surface: present (0); absent (1)
31 Indumentum on the leaf adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
32 Leaf abaxial surface indumentum: present (0); absent (1)
33 Simple hairs on leaf abaxial surface: present (0); absent (1)
34 Hooked hairs on leaf abaxial surface: present (0); absent (1)
35 Stellate hairs on leaf abaxial surface: present (0); absent (1)
36 Indumentum on the leaf abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
37 Bract shape: linear (0); linear-subulate (1); linear-lanceolate (2); elliptic (3), oblanceolate (4), leaf like (5); lanceolate-elliptic (6)
38 Bract margin: flat (0); less recurved (1); more recurved (2)
39 Bract surface indumentum: present (0); absent (1)
40 Simple hairs on bract surface: present (0); absent (1)
41 Hooked hairs on bract surface: present (0); absent (1)
42 Stellate hairs on bract surface: present (0); absent (1)
43 Indumentum on bract surface: sparsely pubescent (0); densely pubescent (1); absent (2)
44 Bract length (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)

45 Bract width (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)
46 Flower position: terminal (0); lateral (1)
47 Flower pedicel: present (0);absent (1)
48 Outer sepal shape: ovate (0); ovate-elliptic (1); ovate-lanceolate (2); lanceolate (3); lanceolate-ovate (4); oblong-ovate (5); oblong-elliptic (6)
49 Outer sepal apex: acute (0); acuminate (1); rounded(2); mucronate (3); obtuse (4)
50 Outer sepal base : acute (0), acuminate (1), cuneate (2), rounded (3)
51 Outer sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
52 Outer sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
53 Outer sepal margin disposition: revolute (0); recurved (1); incurve (2)
54 Outer sepal margin cilia: absent (0); present (1)
55 Simple hairs on outer sepal adaxial surface: present (0); absent (1)
56 Hooked hairs on outer sepal adaxial surface: present (0); absent (1)
57 Stellate hairs on outer sepal adaxial surface : present (0); absent (1)
58 Indumentum on outer sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
59 Simple hairs on outer sepal abaxial surface: present (0); absent (1)
60 Hooked hairs on outer sepal abaxial surface: present (0); absent (1)
61 Stellate hairs on outer sepal abaxial surface : present (0); absent (1)
62 Indumentum on outer sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
63 Inner sepal shape: ovate (0); ovate-elliptic (1); lanceolate-ovate (2); oblong-ovate (3); oblong-obovate (4); absent (5)
64 Inner sepal apex: acute (0); acuminate (1); rounded (2); rounded-cuspidate (3); rounded-retuse (4); absent (5)
65 Inner sepal base: acute (0); acuminate (1); cuneate (2); rounded (3); absent (4)
66 Inner sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
67 Inner sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
68 Inner sepal margin disposition: revolute (0); recurved (1); incurve (2); absent (3)
69 Inner sepal margin cilia: absent (0); present (1)
70 Simple hairs on inner sepal adaxial surface: present (0); absent (1)
71 Hooked hairs on inner sepal adaxial surface: present (0); absent (1)
72 Stellate hairs on inner sepal adaxial surface : present (0); absent (1)
73 Indumentum on inner sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
74 Simple hairs on inner sepal abaxial surface: present (0); absent (1)
75 Hooked hairs on inner sepal abaxial surface: present (0); absent (1)
76 Stellate hairs on inner sepal abaxial surface : present (0); absent (1)
77 Indumentum on inner sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
78 Petal shape: ovate (0); obovate (1); spathulate (2); oblong- obovate (3); oblanceolate (4); narrowly obtriangular (5)
79 Petal length (mm): 1–5 (0); 5.1–10 (1); 10.1–15 (2); ≥15.1 (3)
80 Petal width (mm): 1–3 (0); 3–6 (1); 6.1–10 (2); >10.1 (3)
81 Stamen number: 3–5 (0); 5–10 (1); 10–15 (2); ≥15 (3)
82 Stamen arrangement: in one cluster (0); in two cluster (1); in three cluster (2); >three clusters (3)
83 Stamens cluster position: stamens cluster around carpel (0); stamens cluster around carpel and with another cluster (1); stamens cluster on one side of carpel (2); stamens cluster between the carpels (3).
84 Stamen length (mm): 1–3 (0); 3.1–6 (1); >6.1 (2)
85 Anther length (mm): 1–1.5 (0); 1.6–2 (1); 2.1–3 (2); >3.1 (3)
86 Anther width (mm): 0.2–0.5 (0); 0.6–0.8 (1); >0.9 (2)
87 Anther apex: acute (0); round (1); truncate (2); retuse (3)
88 Anther dehiscence: lateral slits (0); terminal pores (1); terminal pores and lateral slits (2); terminal pores and introrse slits (3); introrse slits (4)
89 Hairs type between anthers and petals: simple hairs (0); stellate hairs + simple hairs (1); mixture short and long simple hairs (2); glabrous (3)

90 Indumentum between anthers and petals: sparsely pubescent (0); densely pubescent (1); absent (2)
91 Filament fusion: free (0); basally connate (1); half connate (2); all connate (3)
92 Anther shape: linear (0); oblong (1); narrowly oblong (2); broadly oblong (3)
93 Pistil shape: uncompressed (0); compressed (1)
94 Style colour: yellow (0); red (1); greenish-yellow (2)
95 Style base: straight (0); recurved (1)
96 Style attachment to ovary: apical (0); lateral (1); semi lateral (2)
97 Stigma shape: capitate (0); discoid (1); linear (2)
98 Ovary shape: spherical (0); compressed (1); ovoid (2)
99 Simple hair on ovary: present (0); absent (1)
100 Stellate hairs on ovary: present (0); absent (1)
101 Indumentum on ovary surface: sparsely pubescent (0); densely pubescent (1); absent (2)
102 Seed shape: spherical (0); compressed (1); ovoid (2)
103 Seed colour: black (0) brown (1); brown-black (2); shiny black (3)
104 Seed dimension (mm): 1–1.5 (0); 1.6–2 (1); 2.1–2.5 (2); >2.5 (3)
105 Aril length: >1/3 of the seed (0); 1/3–1/2 of the seed (1)
106 Stomatal distribution: amphistomatic (0); hyperstomatic (1); hypostomatic (2)
107 Adaxial epidermal anticlinal cells small (<30 μm): absent (0); present (1)
108 Adaxial epidermal anticlinal cells small medium sized (30–50 μm): absent (0); present (1)
109 Adaxial epidermal anticlinal cells small large (>55 μm): absent (0); present (1)
110 Adaxial epidermal cell length to width ratio: 1–2 (0); 2.1–3 (1); 3.1–4 (2); >4.1 (3)
111 Adaxial epidermal anticlinal cell walls rounded: absent (0); present (1)
112 Adaxial epidermal anticlinal cell walls angular: absent (0); present (1)
113 Adaxial epidermal anticlinal cell walls undulate: absent (0); present (1)
114 Adaxial epidermal anticlinal cell walls sinuous: absent (0); present (1)
115 Adaxial epidermal anticlinal cell walls : thin (0); thick (1)
116 Adaxial epidermal anticlinal cell walls smooth: absent (0); present (1)
117 Adaxial epidermal anticlinal cell walls beaded: absent (0); present (1)
118 Adaxial epidermal anticlinal cell walls buttressed: absent (0); present (1)
119 Adaxial epidermal periclinal cell walls smooth: absent (0); present (1)
120 Adaxial epidermal periclinal cell walls granular: absent (0); present (1)
121 Adaxial epidermal periclinal cell walls papillate: absent (0); fine (1); prominent (2)
122 Adaxial epidermal periclinal cell walls striate: absent (0); present (1)
123 Adaxial epidermal trichome bases: absent (0); rare 1 trichome base /400 × (1); common 2–7 trichome bases/400 × (2); very common >10 trichome basses/400 × (3)
124 Adaxial epidermal vein cells forming areoles: absent (0); present (1)
125 Abaxial epidermal anticlinal cells small (<30 μm): absent (0); present (1)
126 Abaxial epidermal anticlinal cells small medium sized (30–50 μm): absent (0); present (1)
127 Abaxial epidermal anticlinal cells small large (>55 μm): absent (0); present (1)
128 Abaxial epidermal cell length to width ratio: 1–2 (0); 2.1–3 (1); 3.1–4 (2); >4.1 (3)
129 Abaxial epidermal anticlinal cell walls rounded: absent (0), present (1)
130 Abaxial epidermal anticlinal cell walls angular: absent (0), present (1)
131 Abaxial epidermal anticlinal cell walls undulate: absent (0); present (1)
132 Abaxial epidermal anticlinal cell walls sinuous: absent (0); present (1)
133 Abaxial epidermal anticlinal cell walls : thin (0); thick (1)
134 Abaxial epidermal anticlinal cell walls smooth: absent (0); present (1)
135 Abaxial epidermal anticlinal cell walls beaded: absent (0); present (1)
136 Abaxial epidermal anticlinal cell walls buttressed: absent (0); present (1)
137 Abaxial epidermal periclinal cell walls smooth: absent (0); present (1)
138 Abaxial epidermal periclinal cell walls granular: absent (0); present (1)
139 Abaxial epidermal periclinal cell walls papillate: absent (0); fine (1); prominent (2)
140 Abaxial epidermal periclinal cell walls striate: absent (0); present (1)
141 Abaxial epidermal trichome bases: absent (0); rare 1 trichome base /400 × (1); common 2–7 trichome bases/400 × (2); very common >10 trichome basses/400 × (3)
142 Abaxial epidermal vein cells forming areoles: absent (0); present (1)
143 Stomata small (<10 μm long): absent (0); present (1);
144 Stomata medium (10–20 μm long): absent (0); present (1);

145 Stomata large (>20 µm long): absent (0); present (1)
146 Stomata sunken: absent (0); present (1)
147 Elliptic stomata: absent (0); present (1)
148 Circled stomata: absent (0); present (1)

DNA extraction

Total DNA was extracted from herbarium material (leaves and stems) following the standard protocol for the Nucleospin Plant system (Machery-Nagel GmbH & Co, Düren, Germany), following the protocol of the manufacturer.

PCR

PCR reactions were carried out, using 12.5µl reactions (Table 2). The universal primers used in White *et al.* (1990) and Taberlet *et al.* (1991) were used in all reactions (Table 3). For ITS region, forward primers designed based on alignments between major fungal and land plant groups, to avoid amplification of epiphytic and contaminating fungi.

Table 2. Reaction volumes used in PCR reactions for ITS and cpDNA

Reagents	Quantities (µl)
PCR water	6
PCR Buffer, 10 x*	1.25
Betaine	1
MgCl2	0.5
dNTP	1
Primer 1	1
Primer 2	1
HotStarTaq DNA	0.25
Template	1
Total volume	12.5

Sequencing

The standard sequencing protocol was applied using the primers forward and reversed and Big Dye Terminator V3.1 (Life Technologies Ltd, Carsland, California, USA). Sequencing reactions were analysed on an Applied Biosystem 3730*xl* Genetic Analyser (Life Technologies Ltd).

Table 3. Forward and reverse primers used (White *et al.* 1990; Taberlet *et al.* 1991)

Primers	Sequence 5′–3′
trnL–F spacer forward (E)	GGTTCAAGTCCCTCTATCCC
trnL–F spacer reversed (F)	ATTTGAACTGGTGACACGAG
ITS4 forward (A1854)	ACGACTCTCGGCAACGGATA
ITS4 reversed (P312)	TCCTCCGCTTATTGATATGC

Amplification profile

The amplification profile for using the relevant primers: 35 cycles was 94C for 1 min to denaturant the DNA, 60ºC to 1 min. to anneal the primers and 72ºC for 2 min. for polymerization of the new strand. At the end of 35 cycles, temperature was held at 20ºC for 1 min.

Outgroup

For the outgroup, *H. ancistrophylla* J.W.Wheeler from Western Australia was included in the analysis, based on its position in a sister Clade, following Horn (2005).

Phylogenetic analyses

DNA sequences were aligned using the default options in BioEdite (Hall 1999) and refined using CLUSTAL X version 2.0 (Larkin *et al.* 2007). The molecular matrix and combined matrix were analysed using WinClada version 1.00.08 (Nixon 2002), with the retention option, 1000 replicates (TBR on, hold 10 trees per step.). Jackknife support was calculated with the same options but TBR off. Jackknife support of 50–74% is considered low, 75–84 moderate, and >85% high.

Results

The combined data matrix included 1,250 positions, 148 morphological and anatomical characters and 1,102 molecular positions, 980 characters were parsimony informative and 225 uninformative. A ratchet parsimonious search yielded 4 equally parsimonious trees with 8,448 steps, CI = 27, RI = 40. The final produced trees revealed from the combined analysis formed eight clades which is largely congruent with the molecular phylogenetic analysis alone.

The strict consensus tree revealed eight clades, but all clades received low bootstrap support, the major clades A, B and C, with five terminal clades placed into a polytomy (D, E, F, G and H). Two species proximal to all other ingroup taxa: *H. hypsophylloides* and *H. hendersonii*; each defined by several homoplasious features as illustrated in Tab. 4.

Table 4. Characters and their states used to define *H. hypsophylloides* and *H. hendersonii* (see Tab. 1)

H. hypsophylloides	*H. hendersonii*
9/1; 10/2; 14/1; 37/3; 45/1; 62/0; 64/2&4' 72/1; 78/3; 82/3; 83/0; 88/2; 98/99; 100/1; 135/1; 141/2	3/1; 12/1; 15/1; 19/0; 23/1; 28/1; 33/1; 44/1; 49/1; 51/2; 55/1; 59/1; 66/2; 70/1; 74/1; 89/1; 91/0; 102/2; 123/3

Two character states were used to define Clade A: 1–2 hair branch number (9/1) and absence of axillary hair tufts (16/1), no floral and leaf cuticular characters used to defined this Clade. Only one synapomorphy in this Clade: leaf blade width >15 mm (22/3). The *H. reticulata* (100% JK) placed as sister group (*H. cinerea* + *H. hirta*) (81% JK) supported.

Clade B was characterised by densely pubescent petiole surface (15/1), linear-oblanceolate leaf blade shape (17/4), not compressed pistil shape (93/0) and yellow and red style colour (94/0&1), but only one monophyletic subgroup (*H. hexandra* + *H. hermaniifolia* subsp. *hermaniifolia*) was strongly supported (95% JK).

All *H. sericea* infrataxa were placed within Clade C. This lineage was defined by: linear-lanceolate bracts (37/2), strongly recurved bract margins (38/2), ovate or ovate–

elliptic inner sepals shape (63/0&1), terminal pores and lateral slits anther dehiscence (88/2), half connate filament fusion (91/2), broadly oblong anther shape (92/3) and presence of abaxial epidermal anticlinal cell walls undulate (131/1) (Figs. 2–6).

The last five clades formed an unresolved polytomy (Fig. 2). However, only Clade D was moderately supported (69% JK) and consisted of the species pair *H. platyphylla* subsp. *platyphylla* + *H. humifusa* subsp. *erigens* (69% JK). There were united in part by the unique synapomorphy acuminate inner sepal base (65/1), sharing the homoplasious synapomorphy: 6.1–10 mm petal width (80/2), oblong anther shape (92/1), lateral style attachment (96/1), 2.1–3 abaxial epidermal cell length to width ratio (128/1), presence of abaxial epidermal anticlinal cell walls rounded (129/1) and common 2–7 trichome bases/400× on leaf abaxial epidermal surface (141/2) (Figs. 2–6).

Several characters defined Clade E: absence of axillary tuft hairs (16/1), cuneate leaf base (18/0), absence of leaf abaxial simple hairs (33/1), presence of leaf abaxial hooked hairs (34/0), 1–2.5 mm bract length (44/1), absence of outer sepal adaxial stellate hairs (57/1), free filaments (91/0), straight style base (95/0) and rare 1 trichome base /400× on leaf abaxial epidermal surface (141/1). However, only one pair received strong support (94% JK) was present within the clade: *H. empetrifolia* subsp. *empetrifolia* (SA) + *H. aspera* subsp. *pilosifolia* (QLD).

Nine taxa were placed within Clade F, which was circumscribed by the characters: linear-oblanceolate leaf blade shape (17/4), acuminate inner sepal apex (64/1), absence of adaxial stellate hairs on the inner sepals (72/1), simple hairs between the anthers and petals (89/0) and 1–1.5 mm seed dimension (104/0). Three unique synapomorphies occurred within this Clade: absence of stem indumentum (6/2), entire leaf margin (20/0) and absence of inner sepal (63/5) (Figs. 2–6).

Clade G comprised 20 taxa and was defined by the characters: 5.1–10 mm inner sepal length (66/1), retuse anther apex (87/3), compressed seed shape (102/2), brown seed colour (103/1), absence of abaxial epidermal periclinal cell walls granular (138/0) and presence of abaxial epidermal periclinal cell walls striate (140/1), and supported by the synapomorphy ovate petal shape (78/0). Amphistomatic stomata distribution (106/0) was a unique synapomorphy for the pair *H. cistiflora* + *H. oxycraspedotus.* Within Clade G, two strongly supported groups were also present: *H. riparia* + *H. tenuis* (94% JK) and *H. appressa* + *H. hirticalyx* (96% JK).

Figure 2. Strict consensus tree derived from 4 equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing jackknife branch support values (1000 reps).

Figure 3. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing proportional branch lengths

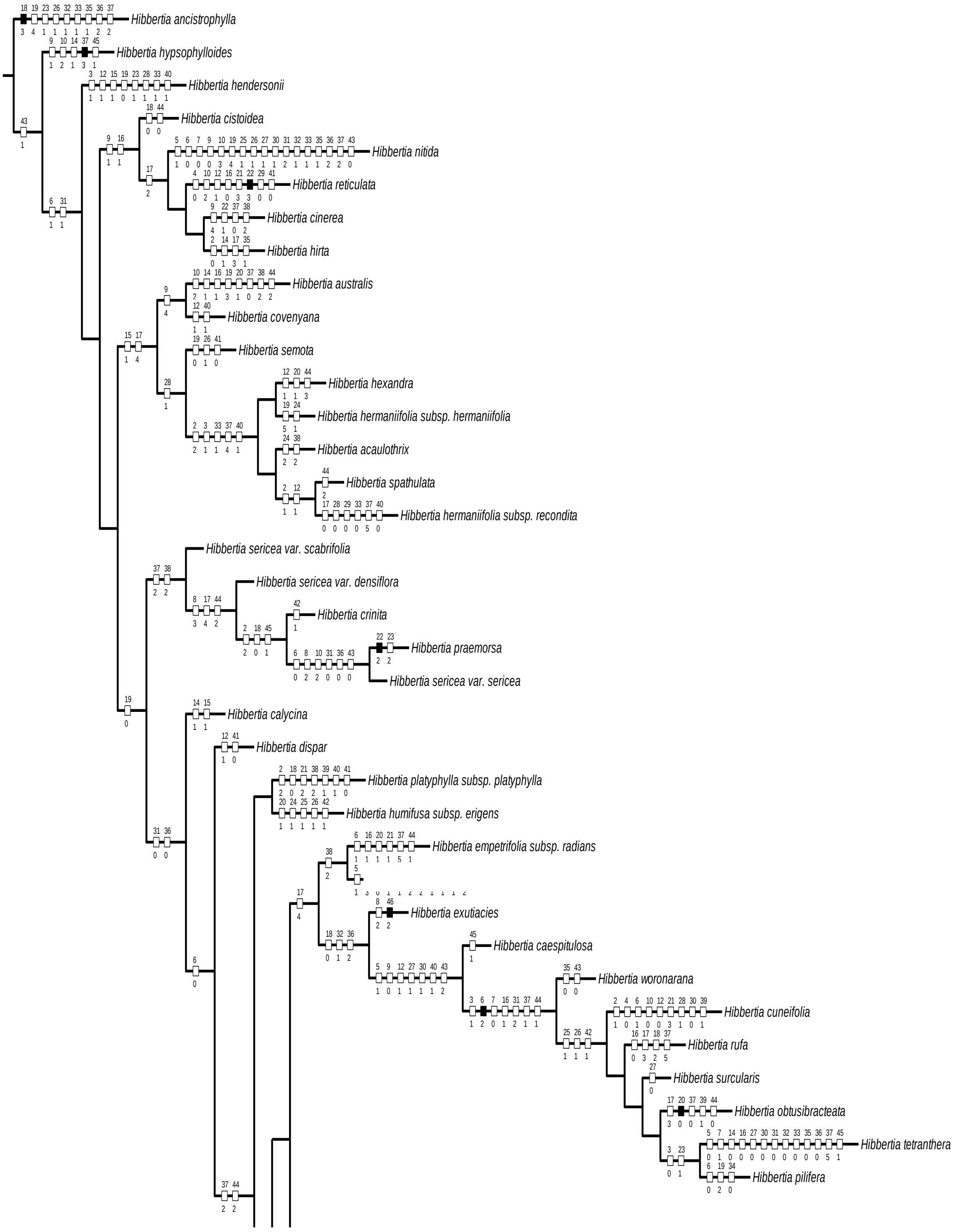

Figure 4. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for vegetation and indumentum characters (characters 1–46). Open squares indicate homoplasy.

Fig. 4 Vegetation and indumentum characters continued

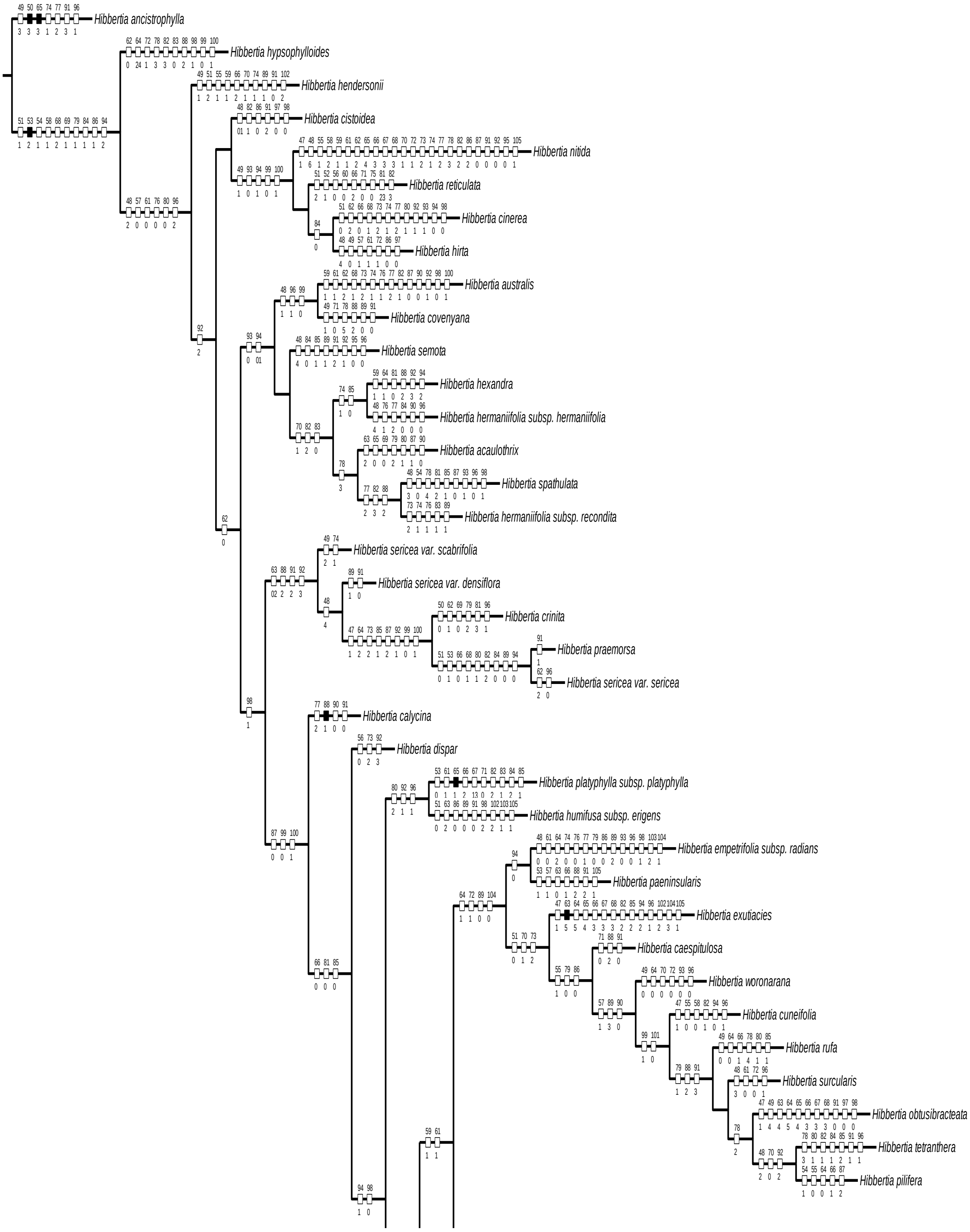

Figure 5. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for floral and seed characters (characters 47–105). Open squares indicate homoplasy.

Fig. 5 Floral and seed characters continued

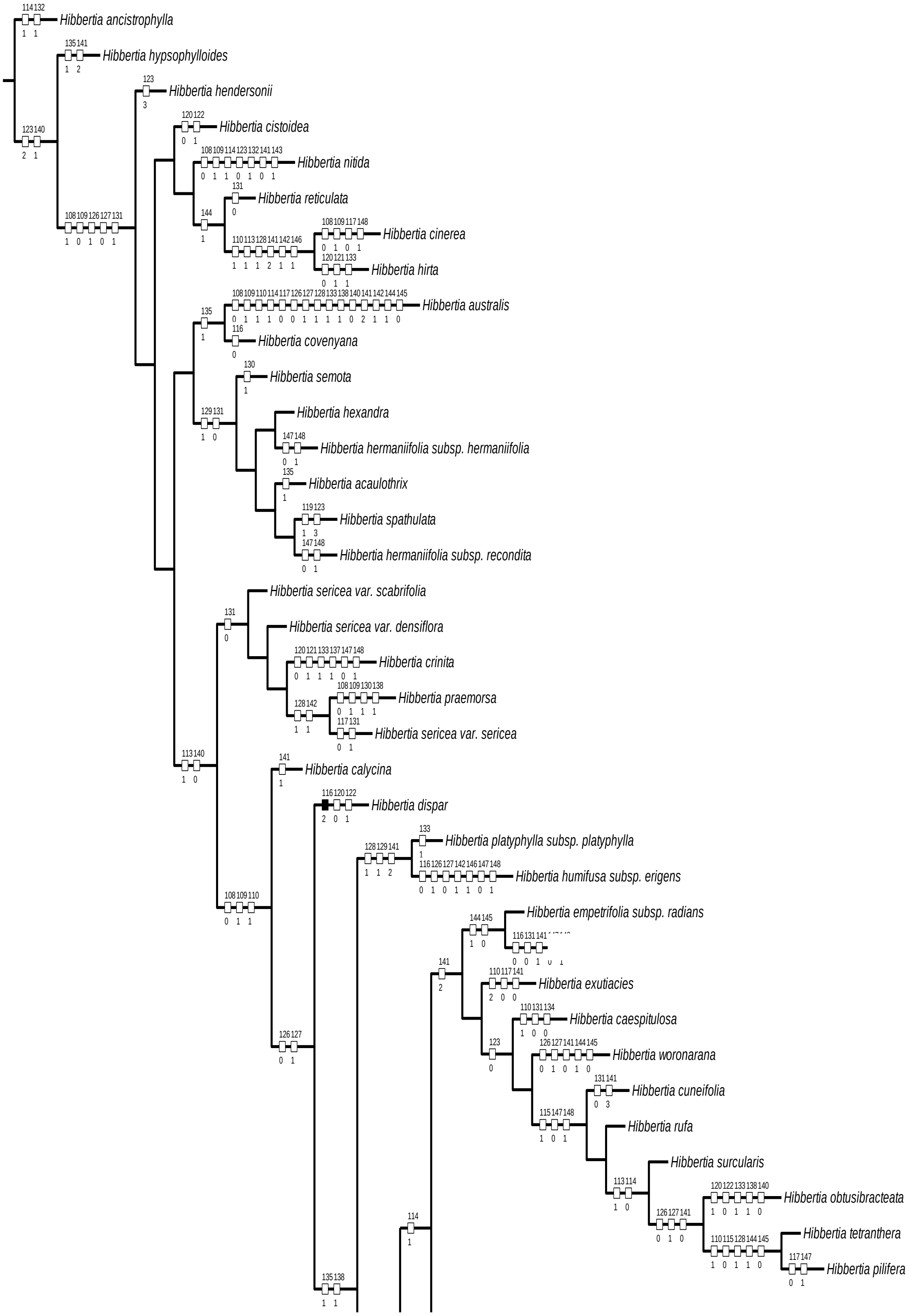

Figure 6. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for anatomical characters (characters 106–148). Open squares indicate homoplasy.

Fig. 6 Anatomical characters continued

Clade H was the largest in the tree, and was defined by the character presence of adaxial epidermal anticlinal cell walls beaded (117/1). Nevertheless, the relationships between some taxa within the clade were strongly supported, such as: *H. sessiliflora* + *H. empetrifolia* subsp. *uncinata* (100% JK), *H. boweniensis* + *H. strigosa* (100% JK), *H. humifusa* subsp. *debilis* + *H. acicularis* (96% JK), *H. glebosa* subsp. *glebosa* + *H. bracteata* (91% JK) and *H. pallidiflora* + *H. florida* subsp. *angustinervis* (92% JK) (Fig. 2).

Discussion

The combined morphological (vegetative-indumentum, floral and leaf cuticular characters) and molecular (ITS and *trn*L–F) phylogenetic analysis placed all species of Toelken's 16 informal proposed groups (unpubl.) within eight clades (Table 5).

Table 5. The resulted clades in compare with Toelken's groups species, arranged based on tree topology of combined morphological and molecular data sources

Clade	Taxa	Toelken informal groups
A	*H. cinerea, H. hirta*	*Aspera*
A	*H. cistoidea*	*Cistoidea*
A	*H. nitida*	*Bracteata*
A	*H. reticulata*	*Hermaniifolia*
B	*H. australis*	*Australis*
B	*H. covenyana, H. semota*	*Cistoidea*
B	*H. hexandra, H. hermaniifolia* subsp. *hermaniifolia, H.*	*Hermaniifolia*
B	*hermaniifolia* subsp. *recondita, H. acaulothrix, H. spathulata*	
C	*H. sericea* var. *densiflora, H. sericea* var. *scabrifolia, H. sericea*	*Sericea*
C	var. *sericea, H. praemorsa, H. crinita*	
D	*H. platyphylla* subsp. *platyphylla,*	*Aspera*
D	*H. humifusa* subsp. *erigens*	*Humifusa*
E	*H. pusticulata, H. protuberans*	*Acicularis*
E	*H. decumbens, empetrifolia* subsp. *empetrifolia, H. aspera* subs.	*Aspera*
E	*pilosifolia*	
E	*H. glaciifera*	*Cistoidea*
F	*H. exutiacies, H. caespitulosa, H. woronarana, H. tetranthera*	*Exutiacies*
F	*H. cuneifolia*	*Acicularis*

F	*H. rufa, H. surcularis, H. obtusibracteata, H. pilifera*	*Rufa*
G	*H. fruticosa, H. expansa, H. pedunculata, H. corifolia, H.*	*Pedunculata*
G	*porcata*	*Serpyllifolia*
G	*H. serpyllifolia*	*Exutiacies*
G	*H. cistiflora, H. oxycraspedotus*	*Stricta*
G	*H. apoda*	*Strigosa*
G	*H. horrida*	*Riparia*
G	*H. riparia*	*Australis*
G	*H. tenuis*	*Aspera*
G	*H. appressa, H. hirticalyx*	*Vestita*
G	*H. synandra, H. erectinervis*	*Sericea*
G	*H. superans, H. villifera, H. patens*	*Humifusa*
	humifusa subsp. *humifusa*	
H	*H. devitata, H. setifera, H. stricta*	*Stricta*
H	*H. platyphylla* subsp. *major, H. platyphylla* subsp. *halmaturina,*	*Sericea*
H	*H.*	
H	*simulans, H. sessiliflora, H. hirsuta*	*Vestita*
H	*H. vestita, H. boweniensis*	*Aspera*
H	*H. rhynchocalyx, H. empetrifolia* subsp. *uncinata, H. truncata,*	
H	*H. notabilis, H. aspera* subsp. *aspera, H. pallidiflora*	*Strigosa*
H	*H. strigosa*	*Acicularis*
H	*H. oligastrota, H. acicularis*	*Australis*
H	*H. glebosa* subsp. *glebosa, H. glebosa* subsp. *oblongata*	*Humifusa*
H	*H. humifusa* subsp. *debilis*	*Bracteata*
H	*H. bracteata*	*Pedunculata*
	H. florida subsp. *florida, H. florida* subsp. *angustinervis*	

In regards to taxa number within each proposed groups species by Toelken (unpubl.), three types of groups can be recognised: 1) groups composed of one taxon; 2) group composed of two taxa; and 3) groups composed of four or more taxa (Ch. 2: Tab. 1). The first type such as the *Riparia* and *Serpyllifolia* groups, in morphological analysis cladogram, *H. riparia* was placed (with no support, Clade G) as a sister group to *H. tenuis* + *H. australis,* and *H. serpyllifolia* was a sister to *H. florida* subsp. *florida* + *H. corifolia* (with no support) (see Ch. 2: Fig. 1); but in the combined analysis results (see

Fig. 2), *H. riparia* is placed close to *H. tenuis* (with strong support 94% JK) (Clade G), and *H. serpyllifolia* close to *H. fruticosa* (with no support). The second type includes pairs such as the *Bracteata* group, in morphological analysis cladogram (see Ch. 2: Fig. 1), *H. bracteata* was close to *H. paeninsularis* (with no support, Clade G), and *H. nitida* was close to *H. obtusibracteata* (with low support, 54% JK, Clade A); but in the combined analysis results, both *H. bracteata* and *H. nitida* are separated (see Fig. 2), the first associated with *H. glebosa* subsp. *glebosa* (91% JK) within Clade H, the second is a sister to *H. cistoidea* within Clade A. The third type of the informal groups species are the remainder which are the most variable regarding to taxon number. From the results, based on the combined morphological and molecular data sets analysis, Toelken's informal 16 groups are not supported

The *Australis* group species placed within Clade G resulted from morphological analysis tree (see Ch. 2: Fig. 1), but from the combined analysis, this groups species placed within three clades B, G and H, each associated with different taxa from different proposed groups (see Fig. 2); meanwhile, the *Acicularis* group species arranged in different clades (A, B, F and G) (see Ch. 2: Fig. 1); but here from the combined analysis arranged as: *H. pusticulata* and *H. protuberans* in Clade E with different correlation, *H. oligastrota* and *H. acicularis* in Clade H, and similarly for the remainder informal groups species (Tab. 5). Only one informal species group (*Hermaniifolia*) placed in Clade B associated with other member of species groups: *H. hermaniifolia* subsp. *hermaniifolia* + *H. hexandra* (95% JK) and *H. hermaniifolia* subsp. *recondita* + *H. spathulata* (53% JK) (Fig. 2). This means that none of the above described species groups are supported here.

Morphologically, the *Aspera* and *Sericea* species groups are the most variable for characters such as surface indumentum (simple, hooked and stellate hairs) (see Ch. 5) and floral features (see Ch. 6). These variations are due to taxa number included in each assumed group species and the geographical location within SE Australia. The phylogenetic tree obtained from the combined molecular and non-molecular datasets corroborate the eight clades, the *Aspera* species group located in 5 different clades based on molecular datasets, and the morphological characters for each Clade are as follows:

1. Clade A: 1–2 hairs branch number and absence of axillary hairs tuft.
2. Clade D: absent of axillary hairs tuft, acute leaf base, presence of hooked hairs on leaf abaxial surface, free filaments and straight style base.
3. Clade E: linear-oblanceolate leaf blade shape, absence of indumentum on inner sepal adaxial surface, absence of abaxial epidermal anticlinal cell walls undulate and striate.
4. Clade G: entire leaf margin, all connate filament fusion and not compressed ovary.
5. Clade H: presence of adaxial epidermal anticlinal cell walls beaded.

Meanwhile, the informal (*Sericea*) group species recovered in three clades based on molecular datasets and morphological characters for each Clade as follows:

1. Clade C: linear-lanceolate bract shape, less recurved bract margin, ovate and lanceolate-ovate inner sepal shape, terminal pores and lateral slits anther dehiscence, half connate filaments fusion and broadly oblong anther shape.
2. Clade G: absence of stellate hairs on bract surface and outer sepal adaxial surface, 1–5 mm inner sepal length, retuse anther apex, ovoid seed shape, black seed colour, absence of adaxial epidermal periclinal cell walls angular and presence of abaxial epidermal periclinal cell walls striate.
3. Clade H: recurved leaf margin, presence of abaxial epidermal anticlinal cells small medium (30–50 µm) absence of abaxial epidermal anticlinal cells small large (>55µm).

Although surface indumentum (simple, hooked and stellate) and floral characters are considered important in Toelken's classification of taxa in the SE Australian clade of *Hibbertia* subgenus *Hemistemma* Toelken (1995, 1998, 2000, 2010, pers. comm. 2009), these characters are apparently insufficient to determine phylogenetic relationships, particularly when high variation appears in each group species (Ch. 5: App. 1–2; Ch. 6: App. 1–2).

Monophyly of *Hibbertia* subg. *Hemistemma*/character evolution

The combined phylogenetic analysis tree is broadly congruent with the molecular analysis tree, and confirms evolutionary lineages and taxa relationship. Although a high number of homoplasy characters was revealed from the analysis, the reliable morphological synapomorphies within each Clade/taxon are illustrated in table 6.

Table 6. Characters and character states indicated as synapomorphies resulted from combined analysis (See table 1).

Clade	Character/character state	taxa
A	22/3	*H. reticulata*
B	Non	Non
C	22/2	*H. praemorsa*
D	65/1	*H. platyphylla subsp. platyphylla*
E	1/1	*H. decumbens*
F	46/2; 63/5	*H. exutiacies*
F	20/0	*H. obtusibracteata*
F	6/2	*H. woronarana*
G	67/2; 106/0	*H. cistiflora*
G	78/0	*H. riparia*
H	54/2; 79/3; 102/1	*H. acicularis*
H	25/2	*H. aspera subsp. aspera*
H	77/0&1	*H. hirsuta*
H	93/2	*H. glebosa subsp. oblongata*
H	128/2	*H. setifera*
outgroup	18/3; 50/3; 65/3	*H. ancistrophylla*
Basal Grade	37/3; 53/2	*H. hypsophylloides*

Variation appears clearly in characters such as surface indumentum (simple, hooked and stellate hair1s) (Ch. 5: App. 1 and 2) and floral features (stamen number, filament fusion, anther dehiscence, style base and attachment to ovary) (Ch. 6: App. 1, 2). Several infrataxa are not part of monophyletic, for example: *H. humifusa* (subsp. *debilis* and subsp. *erigens*) are paired with different species, and thus, reclassification of this group of taxa is needed (*H. humifusa* subsp. *debilis* + *H. acicularis* endemic VIC) (96% JK) and (*H. humifusa* subsp. *erigens* + *H. platyphylla* subsp. *platyphylla*) (69% JK) based on the characters and character states illustrated in Tab. 7 below.

Table 7. Characters and character states used to define *H. humifusa* infrataxa (see Tab. 1)

H. humifusa subsp. *debilis* + *H. acicularis*	*H. humifusa* subsp. *erigens* + *H. platyphylla* subsp. *platyphylla*
10/3; 43/0; 74/0; 76/0; 110/1; 116/0; 129/1; 134/0; 142/1; 146/1	80/2; 92/1; 96/1; 128/1; 129/1; 141/2

Congruent with the molecular data, *H. truncata* was associated with *H. boweniensis* with strong support (100% JK), *H. humifusa* subsp. *debilis* + *H. acicularis* (96% JK) (Fig. 2). However, the above relationship is not applied on other infrataxa such as *H. hermaniifolia* and *H. sericea* infrataxa, because of the geographic location (NSW) for the members of this group. In contrast, all *H. sericea* varieties are placed within one monophyletic group (Clade C) based on indumentum, floral and leaf cuticular characters (see Ch. 5; Ch. 6; Ch. 7).

Conclusions

Phylogenetic analysis of combined morphological and molecular data sets revealed eight distinct clades, mostly congruent the molecular analysis. It is concluded that indumentum and reproductive features are the most variable features within *Hibbertia* subg. *Hemistemma*. Surface indumentum types (simple, stellate and/or hooked hairs) are homoplasious and have changed multiple times within the tree. This was not unexpected, due to the wide range of geographical distribution of these taxa within SE Australia, and suggests of evolution due to environmental factors. From this study, it is also concluded that the informal species groups proposed by Toelken are not supported. Only four of Toelken's groups were recovered to some degree by both molecular and morphological datasets, but none was monophyletic, as originally differ.

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Chapter 5

The taxonomic importance of vegetative characters in south-eastern Australia *Hibbertia* subg. *Hemistemma* (Dilleniaceae)

Abstract

The vegetative morphology and surface indumentum patterns in the south-eastern Australian clade of *Hibbertia* subgenus *Hemistemma* are defined and their phylogenetic relationships are assessed. The study focuses on 87 of the ~93 species subspecies and varieties within this clade and there is variation in many character states, specifically the presence of simple hairs (in most species), stellate and hooked hairs together on the stems and both leaf surfaces; hair length and branching (branch number and length are correlated significantly). Hair base form is also important, with tuberculate bases present in many species (Toelken 1998, 2000). Character mapping of these features onto a previously generated molecular phylogeny showed that villous hairs, leaf blade width, entire leaf margins, glabrous abaxial sepal and stem surfaces, bracts and ovaries appear to be important. The vegetative and indumentum data support species relationships within the clades, but some of the infrataxa within more variable species complexes were found not to form monophyletic lineages.

Introduction

The genus *Hibbertia* Andrews (Dilleniaceae: Hibbertioideae) is considered to have four subgenera: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn; defined largely on features such as stamen number, fertility, fusion and arrangement, carpel and ovule number (Horn 2005, 2007). *Hibbertia* is the most variable genus in the family and possesses complex and taxonomically useful indumentum (vestiture) patterns (Horn 2005). Previous studies on *Hibbertia* variation included wood anatomy(Dickison 1967a), pollen morphology (Dickison 1967b) , carpels (Dickison 1968), leaf anatomy (Dickison 1970), leaf venation (Rury and Dickison 1977) and all showed taxonomically variation between *Hibbertia* species. However, the phylogenetic relationships of the SE Australian clade within *Hibbertia* subg. *Hemistemma* of Horn (2005) are a matter of debate and require further study.

Subgenus *Hemistemma* contains ~160 species, representing all taxa with monosymmetric androecia (Horn 2005), but within it there are 3–5 distinct molecular clades (Horn 2005). One of these clades consists almost entirely of *Hemistemma* species from south-eastern Australia (hereafter the SE clade; Fig. 1) and a series of 16 informal species groups within the SE clade (Table 1) are recognised by Toelken (1998, 2000, 2010, unpubl.)

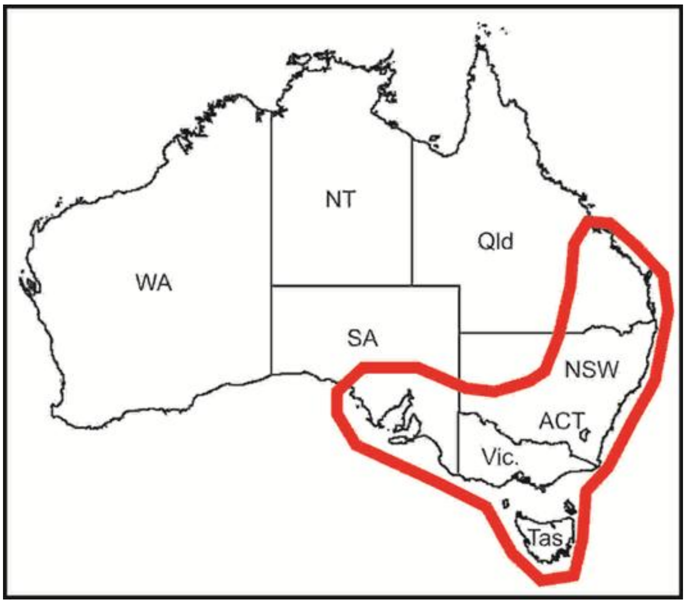

Figure 1. The distribution of south-eastern Australian clade of *Hibbertia* subgenus *Hemistemma.*

Traditionally, defining species and taxa within *Hibbertia* subgenus *Hemistemma* has been problematic (Toelken 1998, 2000, 2010). Several studies have incorporated indumentum features, Black (1919, 1952) distinguished *H. sericea* var. *major* and var. *scabrifolia* in part on hair characters, he also included *H. cinerea* as a variety of *H. sericea,* based on surface indumentum similarities. Hair types and shapes were also used by Wakefield (1955, 1957) to help to define *H. australis* and *H. stricta*. Wheeler (2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b) made significant taxonomic studies on 126 *Hibbertia* in Western Australia, recognising 19 new endemic species and six varieties, all based on morphological characters. Toelken (1998, 2000) revised two groups species: *Aspera* and *Sericea* and proposed 16 informal groups species based largely on floral and indumentum characters (see Ch. 2: Tab. 1)

Indumentum characteristics of *Hibbertia* species such as simple and/or stellate hairs have been considered to be the plesiomorphic condition for the Dilleniaceae (Horn 2007). Considerable variation in hair form within the genus has been found, specifically: simple unicellular hairs with a tuberculate base (aggregation of epidermal cells on the base of a hair); and a range of different fasciculate of simple hairs to form the stellate (Toelken 1998; Horn 2005). Such hair characters exhibit a range of taxonomically useful variations, especially when combined with other morphological features, such as androecial structure and disposition (Toelken 1998). The aims of this study are:

a) To understand the variation of indumentum morphology of the SE Australian species of *Hibbertia* subg. *Hemistemma* and their phylogenetic significance.
b) To determine any distinct lineages and explore character evolution.

Materials and Methods

Eighty-seven of the ~93 *Hibbertia* taxa (species, subspecies and varieties were sampled) from the SE Clade of Horn (2005) (Ch. 2: Tab. 1), based mainly on herbarium specimens (Ch.2: Tab. 2). *Hibbertia ancistrophylla* J.Wheeler from Western Australia was used as the outgroup, based on its position in a sister clade, following Horn (2005). Measurements for 64 vegetative characters were coded into discrete states (Table 3); some binary, but most were multistate and/or polymorphic due to intra-specific variation.

Herbarium specimens were reconstituted using a few drops of detergent applied for 10–15 minutes to dry leaves and flowers, with the specimens examined under a Zeiss dissecting light microscope at 10–30× magnification and photographed using a digital camera (Panasonic/Lumix model DMC-FS42). Surface indumentum characters for dry samples were also observed SEM (NeoScope JCM-5000) in the herbarium. The specimens examined are listed in Chapter 2: Table 2, and the characters and states recorded are listed in Table 1.

Table 1. List of characters with their character states used for character mapping derived from Ch. 2: Tab. 3 and following the numbering used there

1	Habit: erect (0); procumbent (1); decumbent (2)
2	Plant height (m): 0.1–0.5 (0); 0.6–1 (1); ≥1.1 (2)
3	Simple hairs on stem: present (0); absent (1)
4	Hooked hairs on stem: present (0); absent (1)
5	Stellate hairs on stem: present (0); absent (1)
6	Indumentum on the stem surface: sparsely pubescent (0); densely pubescent (1); absent (2)
7	Basal hair cells: unspecialised (0); tuberculate (1)
8	Hair length (mm): 0.1–0.5 (0); 0.6–1 (1); 1.1–1.5 (2); >1.5 (3)
9	Hair branch number: unbranched (0); 1–2 (1); 3–4 (2); 5–7 (3); >7 (4)
10	Hair branch length: equal (0); semi-equal (1); unequal (2); absent (3)
11	Petiole indumentum: present (0); absent (1)
12	Simple hairs on petiole: present (0); absent (1)
13	Hooked hairs on petiole: present (0); absent (1)
14	Stellate hairs on petiole: present (0); absent (1)
15	Indumentum on petiole surface: sparsely pubescent (0); densely pubescent (1); absent (2)
16	Axillary hair tufts: present (0); absent (1)
17	Leaf blade shape: broadly obovate (0); obovate (1); narrowly obovate (2); oblanceolate(2); narrowly oblanceolate (3); linear-oblanceolate (4)
18	Leaf base: cuneate (0); acute (1); obtuse (2); flanged (3)
19	Leaf apex: acute (0), rounded (1); acuminate (2); obtuse (3); truncate-apiculate (4)
20	Leaf margin: entire (0); recurved (1); revolute (2)
21	Leaf lamina length (mm): <5–10 (0); 10.1–15 (1); 15.1–20 (2); >20.1 (3)
22	Leaf blade width: (mm): <1–5 (0); 5.1–10 (1); 10.1–15 (2); >15 (3)
23	Leaf mucro length (mm): absent (0); <0.5–0.8 (1); >0.9 (2)
24	Leaf mid-vein width (mm): <0.1–0.2 (0); 0.3 – 0.4 (1); > 0.4 (2)
25	Leaf adaxial midrib indumentum: present (0); absent (1)
26	Leaf abaxial midrib indumentum: present (0); absent (1)
27	Leaf adaxial surface indumentum: present (0); absent (1)
28	Simple hairs on leaf adaxial surface: present (0); absent (1)
29	Hooked hairs on leaf adaxial surface: present (0); absent (1)
30	Stellate hairs on leaf adaxial surface: present (0); absent (1)
31	Indumentum on the leaf adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
32	Leaf abaxial surface indumentum: present (0); absent (1)
33	Simple hairs on leaf abaxial surface: present (0); absent (1)
34	Hooked hairs on leaf abaxial surface: present (0); absent (1)
35	Stellate hairs on leaf abaxial surface: present (0); absent (1)
36	Indumentum on the leaf abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
39	Bract surface indumentum: present (0); absent (1)
40	Simple hairs on bract surface: present (0); absent (1)
41	Hooked hairs on bract surface: present (0); absent (1)
42	Stellate hairs on bract surface: present (0); absent (1)
43	Indumentum on bract surface: sparsely pubescent (0); densely pubescent (1); absent (2)
54	Outer sepal margin cilia: absent (0); present (1)
55	Simple hairs on outer sepal adaxial surface: present (0); absent (1)
56	Hooked hairs on outer sepal adaxial surface: present (0); absent (1)
57	Stellate hairs on outer sepal adaxial surface : present (0); absent (1)
58	Indumentum on outer sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
59	Simple hairs on outer sepal abaxial surface: present (0); absent (1)
60	Hooked hairs on outer sepal abaxial surface: present (0); absent (1)
61	Stellate hairs on outer sepal abaxial surface : present (0); absent (1)
62	Indumentum on outer sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)

69	Inner sepal margin cilia: absent (0); present (1)
70	Simple hairs on inner sepal adaxial surface: present (0); absent (1)
71	Hooked hairs on inner sepal adaxial surface: present (0); absent (1)
72	Stellate hairs on inner sepal adaxial surface: present (0); absent (1)
73	Indumentum on inner sepal adaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
74	Simple hairs on inner sepal abaxial surface: present (0); absent (1)
75	Hooked hairs on inner sepal abaxial surface: present (0); absent (1)
76	Stellate hairs on inner sepal abaxial surface: present (0); absent (1)
77	Indumentum on inner sepal abaxial surface: sparsely pubescent (0); densely pubescent (1); absent (2)
89	Hairs type between anthers and petals: simple hairs (0); stellate hairs + simple hairs (1), mixture short and long simple hairs (2); glabrous (3)
90	Indumentum between anthers and petals: sparsely pubescent (0); densely pubescent (1); absent (2)
99	Simple hair on ovary: present (0); absent (1)
100	Stellate hairs on ovary: present (0); absent (1)
101	Indumentum on ovary surface: sparsely pubescent (0); densely pubescent (1); absent (2)

Data analysis

The vegetative and indumentum characters were coded (Tab. 1) for species of *Hibbertia*, subspecies and varieties from the SE clade and mapped onto the combined matrix from Ch. 4 produced by using parsimonious ratchet analysis with the program WinClada version 1.00.08 (Nixon 2002).

Results

After mapping the vegetative and indumentum characters onto the combined tree provided in Ch. 4, to explore character evolution, Clade A (61% JK) with 5 taxa was defined by 1–2 hair branch number (9/1) and absence of axillary hair tufts (16/1) (Fig. 4). Leaf blade width >15 mm (22/3) was the only autapomorphy for *H. reticulata* which was strongly supported (100% JK) as sister to a terminal *H. cinerea* and *H. hirta* pair (81% JK), circumscribed by a stamen length of 1–3 mm (84/0) (Fig. 1).

Clade B was circumscribed by the characters: densely pubescent petiole surface (15/1) and linear-oblanceolate leaf blade shape (17/4). Within this clade, a terminal pair comprised of *H. spathulata* + *H. hermaniifolia* subsp. *recondita* was poorly supported (53% JK), but defined by the characters: 0.6–1 m plant height (2/1), absence of simple hairs on petiole (12/1), absence of abaxial indumentum on inner sepals (77/2), >three clusters stamen clusters (82/3) and stamens clustered around the carpel (83/0) (Fig. 1).

Clade C represented all taxa characterised by linear-lanceolate bracts (37/2), strongly recurved bract margins (38/2), and ovate or ovate–elliptic inner sepals (63/0&1). All *H.*

sericea subspecies were located in this Clade, and *H. crinita* was a sister to an *H. praemorsa* + *H. sericea* var. *sericea* pair, united by the unique synapomorphy of leaf blade width 10.1–15 mm (22/2) and homoplasious synapomorphies such as: sparsely pubescent stems and adaxial leaves (6/0; 31/0), hairs 1.1–1.5 mm long (8/2), unequal hair branches (10/2), and simple hairs between the anthers and petals (89/0) (Fig. 1). The last four clades were located as unresolved polytomy (Fig. 1); however, only Clade D had moderate support (69% JK) for the species pair *H. platyphylla* subsp. *platyphylla* + *H. humifusa* subsp. *erigens*; this relationship supported by the synapomorphy of acuminate inner sepal base (65/1).

The absence of stellate adaxial outer sepal hairs (57/1) was a homoplasious synapomorphy for Clade E. Within this clade, there was one well-supported group (94% JK): *H. empetrifolia* subsp. *empetrifolia* (SA) + *H. aspera* subsp. *pilosifolia* (QLD); circumscribed by: acute leaf bases (18/1), stellate adaxial outer sepal hairs (57/0) and a rounded to cuspidate inner sepal apex (64/3).

Clade F was defined by the characters: linear-oblanceolate leaf blade shape (17/4), acuminate inner sepal apex (64/1), absence of adaxial stellate hairs on the inner sepals (72/1) and simple hairs between the anthers and petals (89/0). The absence of inner sepal (63/5) was a unique synapomorphy for *H. exutiacies*, while, revolute leaf margins (20/2) defined *H. obtusibracteata* (Fig. 1).

Clade G was defined by the character 5.1–10 mm inner sepal length (66/1); however, the character ≥10.1 mm inner sepal width (67/2) was a unique synapomorphy for the pair *H. cistiflora* + *H. oxycraspedotus*, which also shared: 1–2.5 mm bract length (44/1) and absence of simple hairs on the outer sepal adaxial surface (55/1). Clade H was the largest in the tree, but was not defined by any of the morphological characters mapped here. Nevertheless, relationships between some taxa within the clade were strongly supported (100% JK), such as *H. sessiliflora* + *H. empetrifolia* subsp. *uncinata*, *H. boweniensis* + *H. strigosa* (Fig. 1).

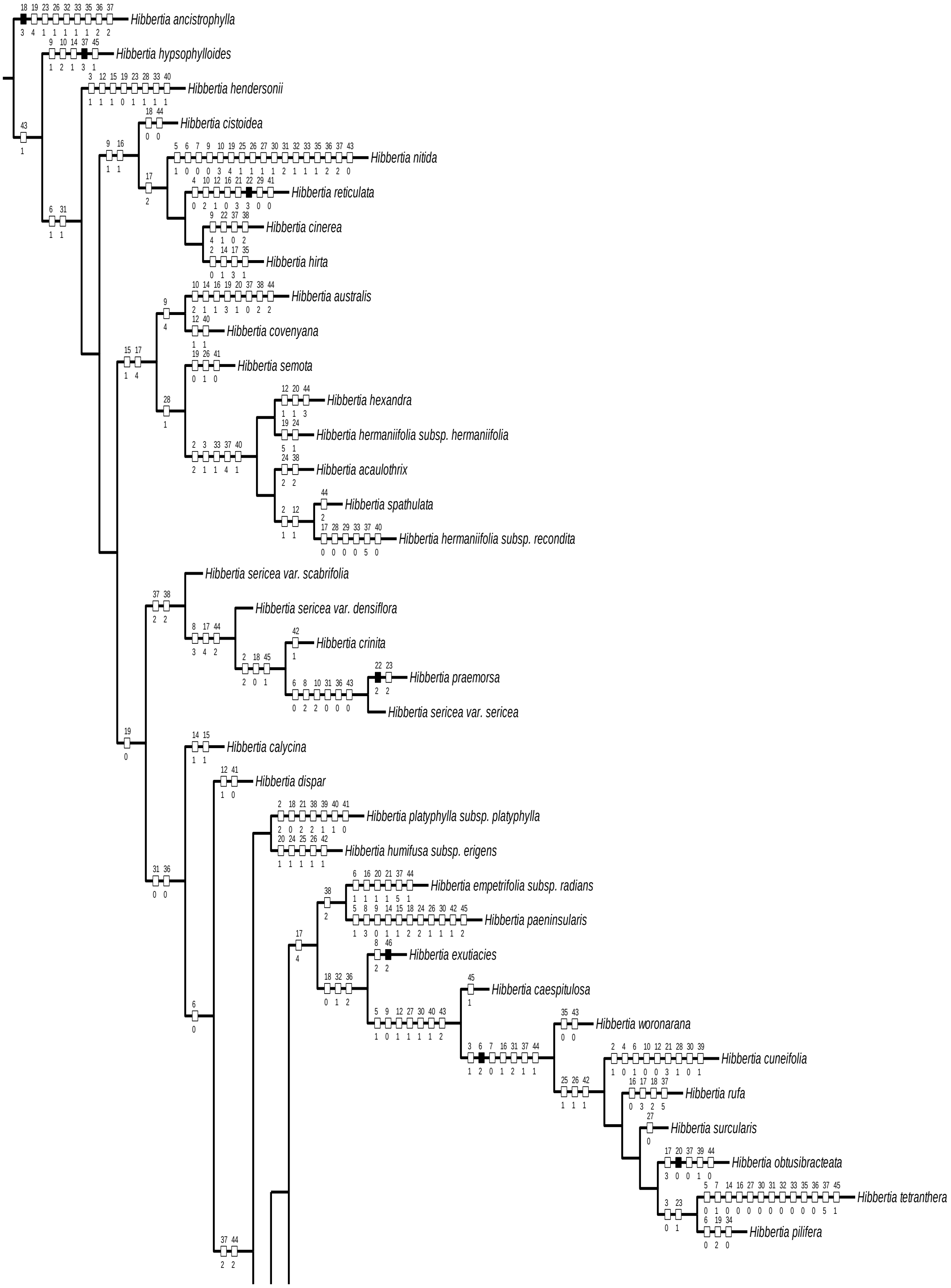

Figure 1. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for vegetation and indumentum characters (characters 1–46). Open squares indicate homoplasy.

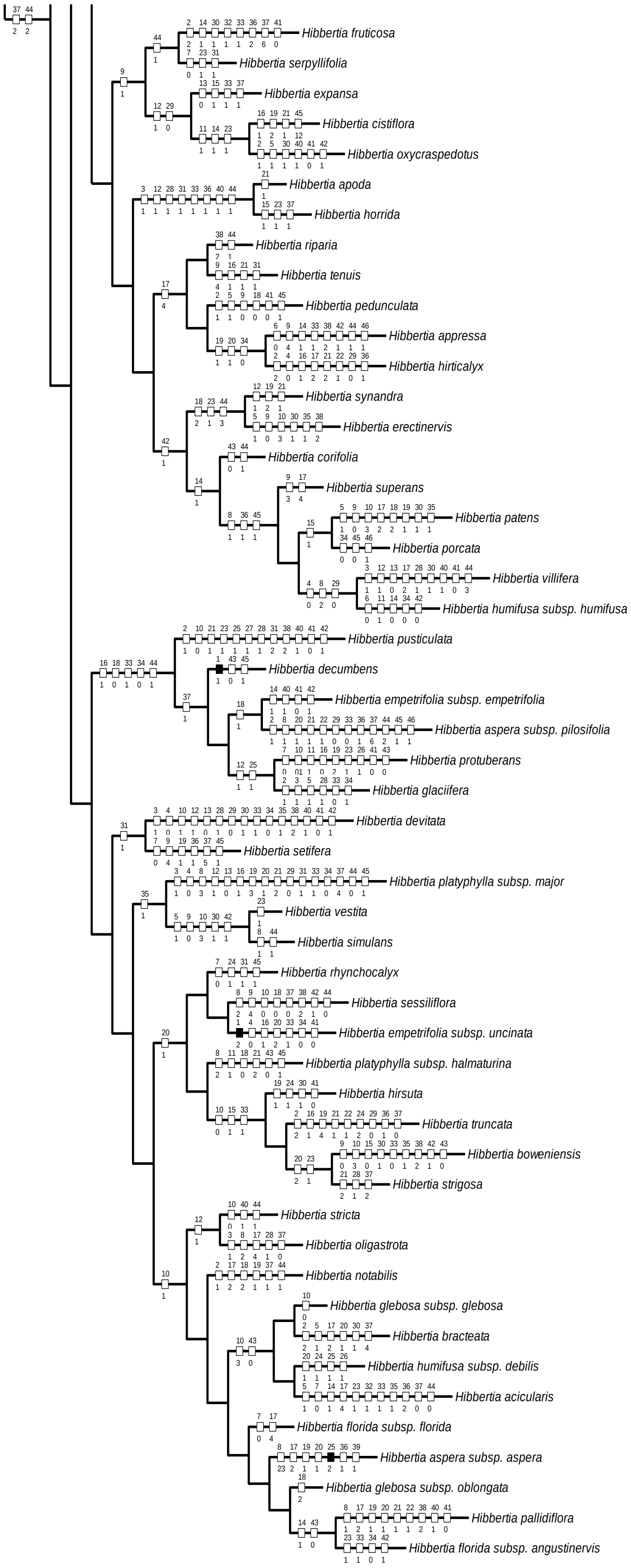

Fig. 1. continued

Discussion

Most of species of *Hibbertia* in the SE Clade produce woody, branched, wiry stems 0.1–1 m long, generally with a dense indumentum on leaf and stem, elongate internodes, and a decumbent habit. The plants often scramble over other vegetation, although some species are erect, while others are procumbent. The variations in growth form are homoplasious and appear to represent convergence rather than phylogenetic history.

Leaf shape varied from linear to linear-elliptic and/or linear-lanceolate in most taxa (*c.* 60%), oblanceolate (*c.* 30%) and less commonly oblong-oblanceolate (*c.* 10%) in taxa such as *H. aspera* subsp. *pilosifolia*, *H. decumbens* and *H. hermaniifolia* subsp *recondita* (App. 1; Fig. 2). Examination of leaf shape also revealed the close morphological relationship between several taxa, for example, a lineage within Clade A comprised the species: *H. nitida*, *H. reticulata*, *H. cinerea*, and *H. hirta*, with one sister relationship *H. cistoidea* shares narrowly obovate leaf shape (App. 1; Fig. 2).

Four main types of leaf base were observed within the SE Clade (App. 1): acute (50 taxa), cuneate (24 taxa) and the remainder obtuse (13 taxa), with the outgroup (*H. ancistrophylla*) possessing flanged leaf bases. Phylogenetically, leaf bases were informative and used to define various groups, such as: a group within Clade C consisting of: *H. crinita*, *H. praemorsa* and *H. sericea* var. *sericea* based on cuneate leaf base; and a species pair: *H. synandra* and *H. erectinervis* (Fig. 2E).

Although leaf length and width showed considerable variation, leaf size of most members of the SE Clade were in the <5–10 × <1–5 mm size class. Species were very variable e.g.: (<5–10) 15.1–20 × <1–5 mm in *H. astrotrichota* and (15.1–20) >20.1 × (<1–5) 10.1–15 mm in *H. hermaniifolia* subsp. *hermaniifolia* (App. 1). Larger leaf sizes were observed consistently in *H. pallidiflora* and *H. cinerea* (10.1–15 × 5.1–10 mm); and in all *H. platyphylla* subspecies, *H. hendersonii* and *H. strigosa* (15.1–20 × 10.1–15 mm); while *H. reticulata* had the largest leaves (>20.1 × >15 mm) (App. 1). Although leaf dimensions were useful at the species level, no large groups or clades were defined based on these characters, suggesting a good taxonomic, but not strong phylogenetic significance (App. 1; Fig. 2; 3).

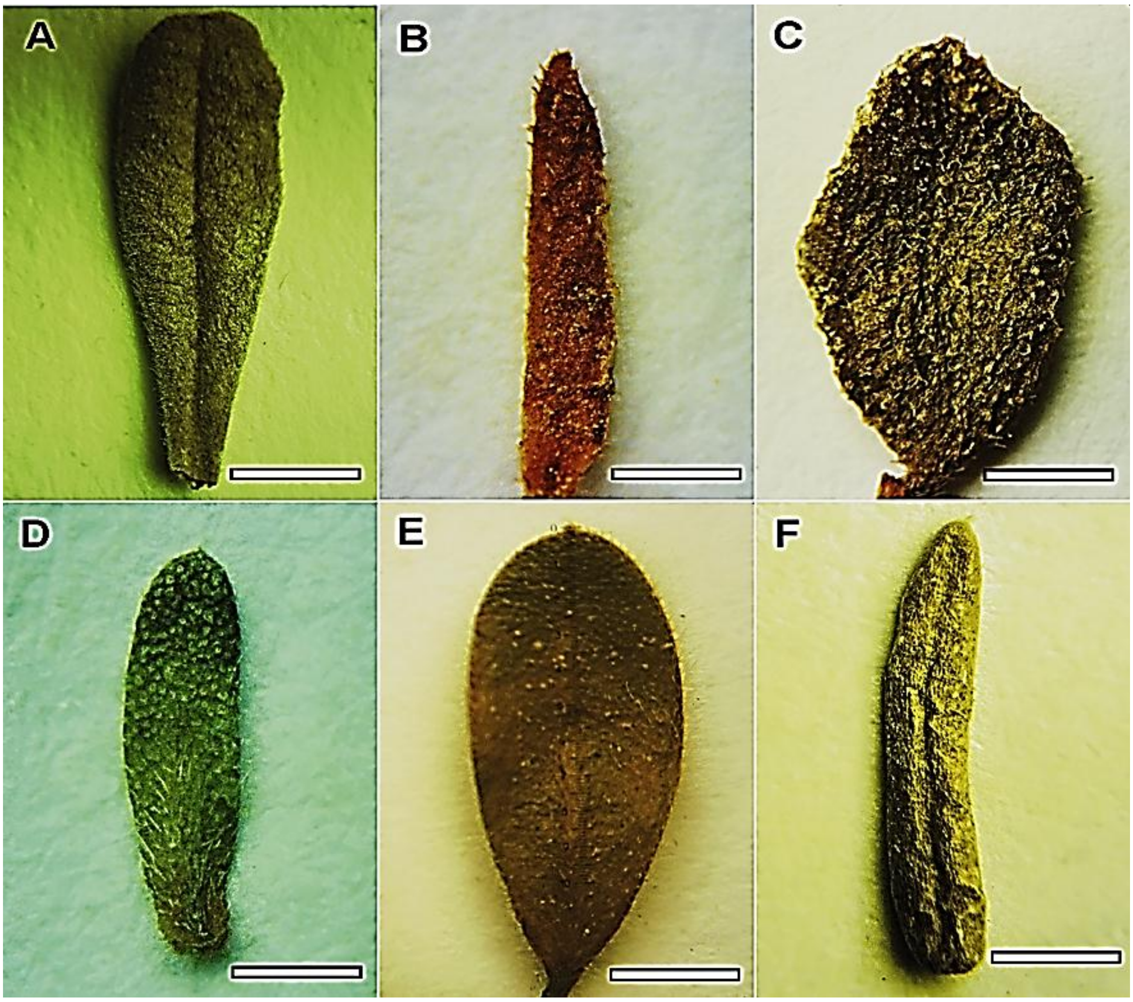

Figure 2. Leaf shape of species from *Hibbertia* subg. *Hemistemma.* A) *H. acaulothrix* (NSW, 386); B) *H. corifolia* (NSW 496); C) *H. decumbens* (CANB 036200); D) *H. hypsophylloides* (BRI 430130); E*) H. praemorsa* (MEL 119768); F) *H. rufa* (MEL 580142). Scale bars: A, C & E = 7 mm, B, D & F = 2.5 mm.

The indumentum features were the most variable characters examined, but these features are generally constant within taxa and appear to show some phylogenetic signal. The distribution patterns of hair characters in the lineages within the SE Clade are as follows:

Stellate hairs are represented by a cluster of simple unicellular hairs with a connate but non-tuberculate base (App. 2). The branches of stellate hairs are either equal, semi–equal or unequal and branch number varied in the SE Clade from 1–2 to >7 branches. Stellate hairs are considered to be highly important in the taxonomy of *Hibbertia* species (Toelken 1998, 2000, 2010). This appears clearly from the resulted clades, for example, presence of stellate outer sepal adaxial and abaxial hairs and between anthers and petals are used to define the basal grade species *H. hendersonii*; but absence of stellate hairs on leaf abaxial surface (36/1) and presence on outer sepal abaxial surface (61/0) are used to define lineages rather than clades, such as a lineage within Clad G consisting: *H. apoda* + *H. horrida*, and a lineage within Clade H that formed a monophyletic group: *H. platyphylla* subsp. *major*, *H. vestita* and *H. simulans* (Fig. 1).

Simple hairs in *Hibbertia* consist of unicellular epidermal cells with (Fig. 3B, 3D) or without a tuberculate base (an aggregation of epidermal cells at the bases of the hair) (Fig. 3C). However, all observed simple hairs were longer than stellate hairs with two main types recorded (App. 2): straight and apically hooked (Fig. 3B). These hair types occur variously on stem, leaf and reproductive parts, except for the seeds (App. 2; Fig. 3). Both simple and stellate hairs could be present on the same surface, therefore, hair type patterns may differ between species, e.g. hooked simple hairs, stellate hairs + simple hairs; stellate hairs + hooked simple hairs; mixed short and long simple hairs; stellate hairs only; or glabrous. However, as expected, glabrous surfaces were uncommon (App. 2).

Hair length mostly varied from 0.1–0.5 mm (52 taxa), but 8 taxa bore hairs or medium length (0.6–1 mm), including *H. cinerea, H. pallidiflora, H. superans, H. simulans* and *H. patens*, while the remainder had at least some long hairs (1–>1.5 mm), such as *H. crinita, H. sessiliflora* and *H. platyphylla* subsp. *major*. Nevertheless, most taxa possessed a mixture of hair lengths.

Hair branch number was 3–4 in most of the SE Clade taxa, or 5–>7 branches in *H. cinerea, H. sessiliflora* and *H. platyphylla* subsp. *platyphylla*. Unbranched hairs were present in 20 taxa, including *H. paeninsularis, H. bracteata* and *H. villifera*, but most taxa showed a mixed number of hair branches.

Morphologically, hair length mostly varied from 0.1–0.5 mm (52 taxa), but 8 taxa hairs with medium length (0.6–1 mm), including *H. cinerea, H. pallidiflora, H. superans, H. simulans* and *H. patens*, while the remainder had at least some long hairs (1–>1.5 mm), such as *H. crinita, H. sessiliflora* and *H. platyphylla* subsp. *major*. Nevertheless, most taxa possessed a mixture of hair lengths.

The character hair branch number is recorded 3–4 in most taxa, or 5–>7 branches in *H. cinerea, H. sessiliflora* and *H. platyphylla* subsp. *platyphylla*. However, unbranched hairs were present in 20 taxa, including *H. paeninsularis, H. bracteata* and *H. villifera*, but most taxa showed a mixed number of hair branches (App. 2; Fig. 3).

The character equal hair branch length were confirmed in *H. superans*, *H. glebosa* subsp. *glebosa* and *H. humifusa* subsp. *humifusa*; the remainder with semi-equal or unequal branch length such as in *H. australis*, *H. patens* and *H. setifera*.

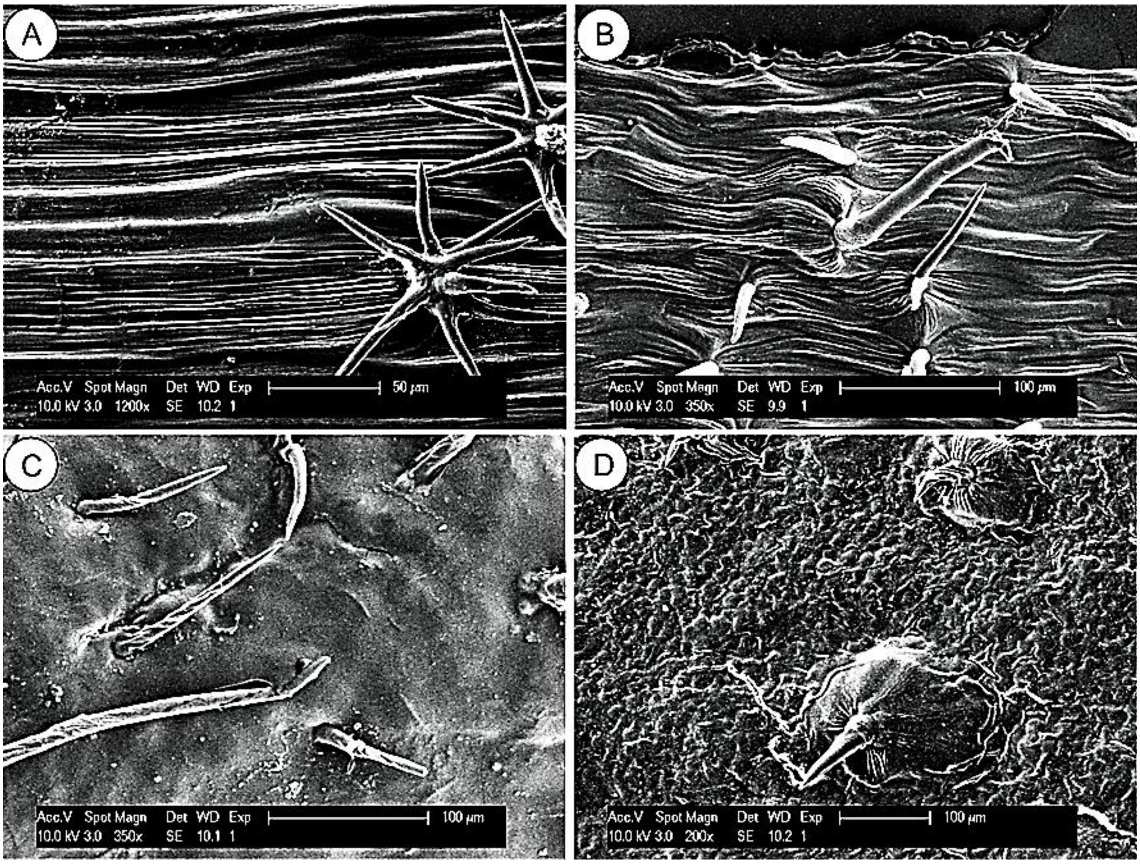

Figure 3. Hair bases for species in the south-eastern Australian clade of *Hibbertia* subgen. *Hemistemma*. A) *H. oligastrota* showing abaxial leaf surface displaying stellate hairs without tuberculate base (BRI359875); B) *H. expansa* showing stem hooked hairs with tuberculate base (CANB037098); C) *H. hirsuta* stem showing simple hairs without tuberculate base (CANB200389); D) *H. platyphylla* subsp. *platyphylla* stem showing simple hairs with tuberculate base (AD98631006).

Character evolution in the SE clade of *Hibbertia* subg. *Hemistemma*

Vegetative and surface indumentum characteristics have often been the most significant taxonomic features used to identify species of *Hibbertia* (Toelken 1995, 1998, 2000; Wheeler 2002b, 2003, 2004a), but have rarely been used phylogenetically in the genera.

Although 45 vegetative and surface indumentum characters were included in the combined analysis, only a few characters (such as leaf shape, apex, base and surface indumentum) appear to be useful to define phylogenetic relationships within the SE Clade. For example, the change of leaf shape from lanceolate and/or oblong-oblanceolate to linear-elliptic and/or linear-lanceolate of most studied taxa is probably subject of rapid evolution, and most linear–elliptic or linear-lanceolate leaves are reduced in size from (15.1–)–>20 × (5–) 10–15 mm in *H. hermaniifolia* subsp. *hermaniifolia* to 1–5 × 1–5 mm in *H. acicularis* (see App. 1). However, the wide homoplasy in most characters reduces their overall usefulness for phylogenetic study of the subgenus.

Toelken (1995, 1998, 2000) and Horn (2005) both noted these reductions in shape and sizes of the leaves. While, clades possessing the characters: mixture short and long simple hairs on stem are also present on bract and inner sepal adaxial surface, hairs between stamens and petals are also present. The hair density (sparsely pubescent) between stamens and petals- as noted in few taxa by Horn (2005) are considered the base of distinction between *Hibbertia* species by Toelken (Toelken 1998, 2000, 2010), thus, this character is a homoplasy among different *Hibbertia* taxa.

Horn (2005) states that surface indumentum (vestiture) of *Hibbertia* species have fasciculate hairs (trichomes) present in most *Hemistemma*, including Wheeler (2002a) and Gilg and Werdermann (1925) *Hibbertia* species. Also, surface indumentum density is also varying from sparsely, densely pubescent and pubescent to glabrescent or/and rarely glabrous are the most homoplasious characters recorded in this study.

Unfortunately, indumentum on leaf adaxial and abaxial surfaces (simple, stellate and hooked hairs) as illustrated in figure 3 also variable, even among the same taxa (App.2; Figs. 3, 5). This problem applies to all studied taxa within this study, and confirms the observation of Toelken (1998, 2000).

Examination of surface indumentum density reveals differences from villous, densely pubescent and pubescent to glabrescent and/or rarely glabrous (App. 2). These characters are unique in *Hibbertia* species, particularly on leaf and stem surfaces vary in: a) length as Horn (2005, 2007) confirmed, b) branch number and c) mixed hairs. Most *Hibbertia* species have simple and stellate hairs as considered by (Toelken 1998, 2000) to form a base of identifying *Hibbertia* species(Fig. 4), but from present study, hooked hairs are presenting in 13 taxa within different clades: *H. cuneifolia*, *H. empetrifolia* subsp. *empetrifolia*, *H. empetrifolia* subsp. *uncinata*, *H. nitida*, *H. oxycraspedotus*, *H. tetranthera*, *H. reticulata*, *H. humifusa*, *H. platyphylla* ssp. *halmaturina*, *H. platyphylla* ssp. *major*, *H. villifera*, *H. hirsuta* and *H. devitata* (App. 2).

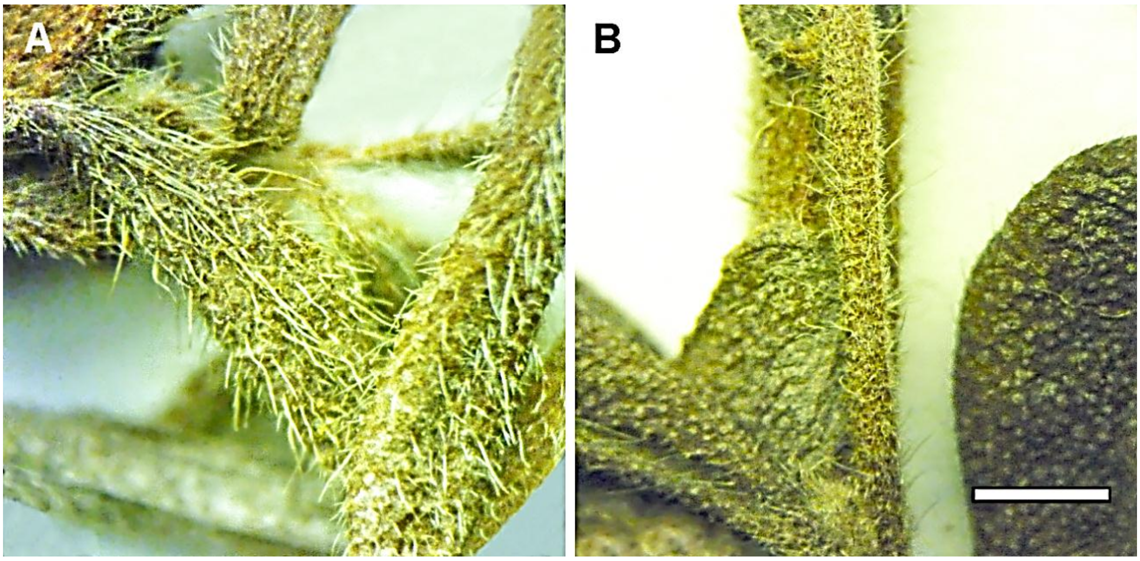

Figure 4. Leaf and stem surface of *H. aspera* subsp. *aspera* (BRI *253673*), A) dense leaf indumentum; B) dense stem indumentum. Scale bar = 3 mm

Toelken (2000) in his study on *H. sericea* species stated that this species and its varieties were ‘isolated geographically and/or ecologically, and might have developed local forms’. Nevertheless, many species are described for the first time in this study such as *H. oligastrota*, *H. posticulata*, *H. protuberans* and *H. semota*, assemble the features of stellate hairs and/or simple hairs on stem, bract and on sepal surfaces (Fig. 5) on both adaxial and abaxial leaf surfaces, leaf blade >10 mm. These characters are supporting the monophyly of the clades they belong to.

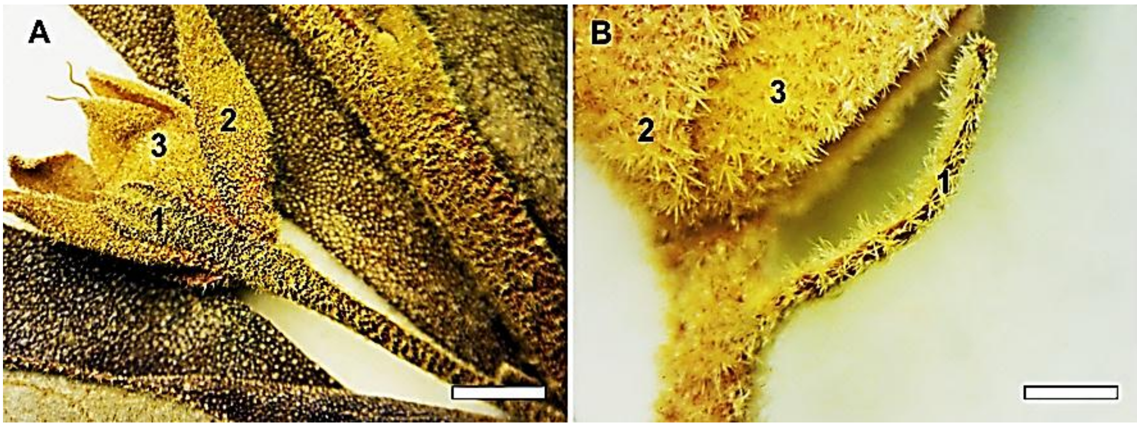

Figure 5. Dense surface indumentum of A) *H. hexandra* (CANB 205127) showing 1) bract, 2) outer sepal and 3) inner sepal; B) *H. covenyana* (CANB 00404379) showing 1) bract, 2) outer sepal and 3) inner sepal. (Scale bars: A=2 mm; B=1.5 mm)

Several vegetative and surface indumentum synapomorphies are listed in Tab. 2.

Table 2. Vegetative-indumentum synapomorphies within the resulted clades (See table 1).

Clade	Character/character state	taxa
A	22/3	*H. reticulata*
B	22/2	*H. praemorsa*
F	46/2	*H. exutiacies*
E	1/1	*H/ decumbens*
F	6/2	*H. woronarana*
F	20/0	*H. obtusibracteata*
H	1/2	*H. empetrifolia* subsp. *uncinata*
H	25/2	*H. aspera* subsp. *aspera*
outgroup	/18/3	*H. ancistrophylla*

Taxa in many clades have >1.5 mm hair length and branches number 5–7 branches, but reduction in length and branches number are observed as illustrated in Table 3. However, other characters such as absence of cilia from outer sepals margin, pubescent to glabrescent outer sepal abaxial surface are useful and supports a group of taxa: *H. florida* subsp. *florida*, *H. serpyllifolia*, *H. expansa* and *H. florida* subsp. *angustinervis*, and thus, these characters are the basis for the phylogenetic assumptions.

Table 3. Hair length and hair branch number in taxa within different clades.

Clade	Taxa	hair length mm	Clade	Taxa	hair branch number
A	*H. nitida*	1.1–1.5	A	*H. nitida*	unbranched
F	*H. exutiacies*	1.1–1.5	G	*H. pedunculata*	unbranched
G	*H. superans*	0.6–1	G	*H. corifolia*	1–2
H	*H. empetrifolia* subsp. *uncinata*	0.1–0.5	G	*H. superans*	5–7
H	*H. glebosa* subsp. *oblongata*	0.6–1	H	*H. truncata*	1–2
H	*H. sessiliflora*	1.1–1.5	H	*H. glebosa* subsp. *glebosa*	3–4

Conclusions

Vegetative and surface indumentum characters are among the characters that defining the clades. Hair characteristics such as simple hairs, hooked hairs, mixture short and/or long simple hairs and stellate hairs on stem are the most significant features of species of *Hibbertia* subg. *Hemistemma*. Leaves of most studied taxa are linear to linear-lanceolate, with recurved or revolute margins. It is now clear that the informal groups of Toelken are not supported. The vegetative and indumentum characters did not reflect the phylogenetic relationships among *Hibbertia* subg. *Hemistemma* and instead are probably more useful to define individual species rather than the lineages. The analysis produced eight clades, three of them are considered small in compare to the reminder clades. Most vegetative and indumentum characters are considered homoplasious, except few synapomorphies such as absence of petiole, glabrous leaf surfaces, leaf blade width, entire leaf margin, flat bract margin but of limited value phylogenetically.

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Appendix 1. Summary of leaf morphological characters for each of the *Hibbertia* taxa within the SE Australian clade of subgenus *Hemistemma*

Taxon	Leaf character/ shape, apex, margin, length and width in mm
H. acaulothrix	Leaves oblanceolate-elliptic, apex rounded, revolute margin, 10.1–15 × 10.1–15 mm
H. acicularis	Leaves linear-lanceolate, apex acuminate, revolute margin, 1–5 × 1–5 mm
H. apoda	Leaves linear-elliptic, apex obtuse, revolute margin, (5–) 10–15 × 1–5 mm
H. appressa	Leaves lanceolate-elliptic, apex rounded, recurved margin, 1–5 ×1–5 mm
H. aspera subsp. *aspera*	Leaves oblanceolate, apex rounded, recurved margin, 10.1–15 × 1–5 mm
H. aspera subsp. *pilosifolia*	Leaves broadly obovate, apex rounded, recurved margin, 10.1–15 × 10.1–15 mm
H. australis	Leaves oblong-lanceolate, apex obtuse, recurved margin, 5–10 × 1–5 mm
H. boweniensis	Leaves linear- elliptic, apex obtuse, revolute margin, 1–5 × 1–5 mm
H. bracteata	Leaves oblanceolate-elliptic, apex acuminate, recurved margin, (5–) 10–15 × 1–5 mm
H. caespitulosa	Leaves linear- elliptic to oblong-oblanceolate, apex acute, revolute margin, 5–10 × 1–5 mm
H. cinerea	Leaves oblanceolate-elliptic, apex rounded, revolute margin, 10.1–15 × 10.1–15 mm
H. cistiflora	Leaves linear-elliptic to linear-lanceolate, apex acuminate, revolute margin, 10.1–15 × 1–5 mm
H. cistoidea	Leaves oblanceolate-elliptic, apex rounded, revolute margin, (5–) 10–15 × 1–5 mm
H. corifolia	Leaves linear-elliptic to linear-lanceolate, apex acute, revolute margin, 1–5 × 1–5 mm
H. covenyana	Leaves oblanceolate-elliptic, apex rounded, revolute margin, 1–5 × 1–5 mm
H. crinita	Leaves linear-lanceolate, apex rounded, revolute margin, 10.1–15 × 1–5 mm
H. cuneifolia	Leaves linear, apex acuminate, revolute margin, >20.1 × 1–5 mm
H. decumbens	Leaves oblong-elliptic to broadly obovate, apex rounded, revolute & recurved margin, 5–10 × 1–5 mm
H. devitata	Leaves linear-elliptic to linear-lanceolate, apex acute, revolute margin, 1–5 ×1–5 mm
H. dispar	Leaves linear-elliptic, apex acute, revolute margin, 10.1–15 × 1–5 mm
H. empetrifolia subsp. *radians*	Leaves lanceolate-elliptic, apex acute, recurved margin, 5–10 × 1–5 mm
H. empetrifolia subsp. *uncinata*	Leaves oblong-elliptic, apex acute, recurved margin, 5–10 × 1–5 mm
H. erectinervis	Leaves linear- elliptic to lanceolate-elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. expansa	Leaves linear-lanceolate to lanceolate-elliptic, apex acute to acuminate, revolute margin, 10.1–15 × 1–5 mm
H. exutiacies	Leaves linear-lanceolate, apex acuminate, recurved margin, 1–5 × 1–5 mm
H. florida subsp. *angustinervis*	Leaves linear-lanceolate, apex acute, revolute margin, 1–5 × 1–5 mm
H. florida subsp. *florida*	Leaves linear-lanceolate, apex acute, revolute margin, 1–5 × 1–5 mm
H. fruticosa	Leaves linear to linear-elliptic, apex acute, revolute margin, (5–) 10–15 × 1–5 mm
H. glaciifera	Leaves linear-elliptic, apex rounded, revolute margin, (5–) 10–15 × 1–5 mm
H. glebosa subsp. *glebosa*	Leaves oblong-lanceolate, apex obtuse, recurved margin, 5–10 × 1–5 mm
H. glebosa subsp. *oblongata*	Leaves linear- elliptic to linear-lanceolate, apex acute, revolute margin (5–) 10–15 × 1–5 mm
H. hendersonii	Leaves linear- elliptic, apex rounded, revolute margin, 10.1–15 × 10.1–15 mm

H. hermaniifolia subsp. *hermaniifolia*	Leaves oblanceolate, apex emarginate, revolute margin, (15.1–)–>20 × (5–) 10–15 mm
H. hermaniifolia subsp. *recondita*	Leaves broadly obovate, apex marginate, revolute margin, 1–5 × 1–5 mm
H. hexandra	Leaves oblanceolate-elliptic, apex rounded to obtuse, recurved margin, (5–) 10–15 × 1–5 (–10) mm
H. hirsuta	Leaves oblong-oblanceolate, apex rounded, recurved margin, (5–) 10–15×1–5 mm
H. hirta	Leaves linear-oblanceolate, apex rounded & obtuse, revolute margin, 5–10 × 1–5 mm
H. hirticalyx	Leaves oblanceolate-elliptic, apex rounded, recurved margin, 10.1–15 × 10.1–15 mm
H. horrida	Leaves linear- elliptic, apex rounded, revolute margin, 1–5 × 1–5 mm
H. humifusa subsp. *debilis*	Leaves linear-elliptic, apex acute, recurved margin, (5–) 10–15 × 1–5 mm
H. humifusa subsp. *erigens*	Leaves linear-elliptic, apex acute, recurved margin, (5–) 10–15 × 1–5 mm
H. humifusa subsp. *humifusa*	Leaves linear-elliptic, apex acute, recurved margin, (5–) 10–15 × 1–5 mm
H. hypsophylloides	Leaves linear- elliptic, apex rounded, revolute margin, (5–) 10–15 × 1–5 mm
H. nitida	Leaves oblanceolate-elliptic, apex truncate–apiculate, entire & recurved margin (5–) 10–15 × 1–5 mm
H. notabilis	Leaves oblanceolate-elliptic, apex rounded, revolute margin, 5–10 × 1–5 mm
H. obtusibracteata	Leaves linear-elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. oligastrota	Leaves linear, apex acute, revolute margin, (5–) 10–15 × 1–5 mm
H. oxycraspedotus	Leaves linear-elliptic to linear-lanceolate, apex acute, revolute margin, 1–5 × 1–5 mm
H. paeninsularis	Leaves lanceolate-elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. pallidiflora	Leaves oblanceolate-elliptic, apex rounded, recurved margin, 10.1–15 × 10.1–15 mm
H. patens	Leaves oblanceolate-elliptic, apex rounded, recurved margin, 10.1–15 × <1–5 mm
H. pedunculata	Leaves linear-lanceolate, apex acute, revolute margin, (5–) 10–15 × 1–5 mm
H. pilifera	Leaves linear-lanceolate, apex acuminate, revolute margin, 1–5 × 1–5 mm
H. platyphylla subsp. *halmaturina*	Leaves linear-elliptic, apex obtuse, recurved margin, 10.1–15 × 1–5 mm
H. platyphylla subsp. *major*	Leaves linear-elliptic, apex obtuse, recurved margin, 10.1–15 × 1–5 mm
H. platyphylla subsp. *platyphylla*	Leaves linear-elliptic, apex rounded, revolute margin, 10.1–15 × 1–5 mm
H. porcata	Leaves linear-elliptic, apex acute, revolute margin, 10.1–15 × 1–5 mm
H. praemorsa	Leaves oblong-elliptic, apex rounded or obtuse or emarginate, recurved margin, (5.1–) 6.5–9.5 (–13.2) × (2.4–) 3–4.4 (–7.6) mm
H. protuberans	Leaves linear, apex acuminate, revolute margin, (5–) 10–15 × 1–5 mm
H. pusticulata	Leaves linear to oblong-lanceolate, apex acuminate, revolute margin, 10.1–15 × 1–5 mm
H. reticulata	Leaves oblanceolate-elliptic, apex rounded, recurved margin, >20.1 × >15 mm
H. rhynchocalyx	Leaves oblong-elliptic to oblong-lanceolate, apex obtuse to truncate–apiculate, recurved margin (5–) 10–20 × 1–5 (–10) mm
H. riparia	Leaves linear-lanceolate to lanceolate-elliptic, apex acute, revolute margin, 1–5 ×1–5 mm
H. rufa	Leaves linear-elliptic, apex acute, revolute margin, 1–5 ×1–5 mm
H. semota	Leaves linear to linear-lanceolate, apex acute, revolute margin, (5–) 10–15 × 1–5 mm
H. sericea var. *densiflora*	Leaves linear-lanceolate, apex acute, revolute margin, (5–) 10–15×1–5 mm

H. sericea var. *scabrifolia*	Leaves linear-lanceolate to oblong-oblanceolate, apex acute, revolute margin, 1–5 ×1–5 mm
H. sericea var. *sericea*	Leaves linear-oblanceolate, rarely linear, apex obtuse to round, revolute margin, (6–) 10.1–15 × 1–5 mm
H. serpyllifolia	Leaves linear-elliptic to oblong-elliptic, apex acute, revolute margin, 1–5 ×1–5 mm
H. sessiliflora	Leaves linear-elliptic, apex acute, recurved margin, 1–5 × 1–5 mm
H. setifera	Leaves linear-oblanceolate, apex rounded, revolute margin, 1–5 × 1–5 mm
H. simulans	Leaves linear, apex acute, revolute margin, 1–5 × 1–5 mm
H. spathulata	Leaves oblong-oblanceolate, apex obtuse, revolute margin, (5–) 10–15 × 1–5 mm
H. stricta	Leaves linear- elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. strigosa	Leaves linear- elliptic, apex acute, revolute margin, (5–) 10–15 × 1–5 mm
H. superans	Leaves linear, apex acute, revolute margin, 10.1–15 × 1–5 mm
H. surcularis	Leaves linear-lanceolate, apex acute, revolute margin, 1–5 ×1–5 mm
H. synandra	Leaves linear- elliptic, apex acuminate, revolute margin, 10–15 ×1–5mm
H. tenuis	Leaves linear- elliptic to linear-lanceolate, apex acute, revolute margin, 10.1–15 × 1–5 mm
H. tetranthera	Leaves linear-oblanceolate to lanceolate-elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. truncata	Leaves oblanceolate, apex obtuse to truncate–apiculate, recurved margin, 10.1–15 × 10.1–15 mm
H. vestita	Leaves linear- elliptic to lanceolate-elliptic, apex acute, revolute margin, 1–5 × 1–5 mm
H. villifera	Leaves oblong-elliptic, apex acute, recurved margin, 1–5 × 1–5 mm
H. woronarana	Leaves linear to linear -lanceolate, apex acuminate, revolute margin, (5–) 10–15 × 1–5 mm
H. calycina	Leaves linear- elliptic, apex acute, revolute margin, (5–) 10–15 × 1–5 mm

Appendix 2. Summary of leaf indumentum characters for each of the *Hibbertia* taxa within the SE Australian clade of subgenus *Hemistemma*

Taxon	Simple hair length	Hooked hairs	Axillary hair tufts	Tuberculate hair bases	Stellate hairs
H. acaulothrix	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, inner sepal, ovary
H. acicularis	long & short	outer & inner sepal	absent	absent	absent
H. apoda	short	outer & inner sepal	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, ovary
H. appressa	short	abaxial leaf surface	present	present	stem
H. aspera subsp. *aspera*	short	absent	absent	present	stem., adaxial & abaxial leaf surfaces, bract, ovary
H. aspera subsp. *pilosifolia*	short	stem	absent	present	stem, adaxial leaf surface, bract
H. australis	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces, bract, outer sepal& inner sepal
H. boweniensis	short & long	absent	present	present	outer & inner sepal
H. bracteata	short	absent	absent	absent	outer sepal

H. caespitulosa	long & short	inner sepal	present	present	bract
H. calycina	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. cinerea	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces
H. cistiflora	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces, bract
H. cistoidea	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, outer& inner sepal, ovary
H. corifolia	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, inner sepal, ovary
H. covenyana	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, ovary
H. crinita	long	absent	present	present	stem, adaxial & abaxial leaf surfaces, outer & inner sepal
H. cuneifolia	long & short	stem,	absent	absent	absent
H. decumbens	short	abaxial leaf surface	absent	present	stem, adaxial & abaxial leaf surfaces, bract
H. devitata	short	stem, adaxial & abaxial leaf surfaces, bract, inner sepal	present	present	stem, outer sepal, between anthers and petals
H. dispar	short	bract	present	present	stem, adaxial & abaxial leaf surfaces, outer & inner sepal
H. empetrifolia subsp. *radians*	short	absent	absent	present	stem, bract
H. empetrifolia subsp. *uncinata*	short	stem, adaxial & abaxial leaf surfaces, bract, outer sepal	absent	present	stem, adaxial & abaxial leaf surfaces, bract, outer& inner sepal
H. erectinervis	short & long	absent	present	present	absent
H. expansa	short & long	adaxial leaf surface, outer sepal	present	present	stem, abaxial leaf surface, bract, outer & inner sepal, ovary
H. exutiacies	long	absent	present	present	stem, adaxial leaf surface, bract, outer sepal
H. florida ssp. *angustinervis*	short	outer sepal	present	present	stem, adaxial & abaxial leaf surfaces, inner sepal, ovary
H. florida ssp. *florida*	short& long	outer sepal	present	present	stem, adaxial & abaxial leaf surfaces, inner sepal, ovary
H. fruticosa	short	bract, inner sepal	present	present	stem
H. glaciifera	short	outer & inner sepal	absent	present	stem, adaxial & abaxial leaf surfaces, bract, inner sepal

H. glebosa subsp. *glebosa*	long	outer sepal& inner sepal	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, between anthers and petals, ovary
H. glebosa subsp. *oblongata*	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer sepal& inner sepal
H. hendersonii	short	absent	present	present	stem, between anthers and petals, adaxial & abaxial leaf surfaces, bract, outer & inner sepal , between anthers and petals, ovary
H. hermaniifolia subsp. *hermaniifolia*	short	absent		present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, ovary
H. hermaniifolia subsp. *recondita*	short	adaxial & abaxial leaf surfaces, bract surface	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, ovary
H. hexandra	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, inner sepal, ovary
H. hirsuta	short	stem, abaxial leaf surface, bract	present	present	stem, abaxial leaf surface, bract, inner sepal
H. hirta	short& long	absent	absent	present& absent	stem, adaxial leaf surface
H. hirticalyx	sort	inner sepal	absent	present	stem, adaxial & abaxial leaf surfaces, bract, inner sepal
H. horrida	short	outer & inner sepal	present	present	stem, , adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. humifusa ssp. *debilis*	short & long	absent	present	present	stem, abaxial leaf surface, outer & inner sepal
H. humifusa ssp. *erigens*	short & long	absent	present	present	stem, adaxial & abaxial leaf surfaces, outer & inner sepal
H. humifusa ssp. *humifusa*	short & long	adaxial & abaxial leaf surfaces	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. hypsophylloides	short & long	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract
H. nitida	long	stem	absent	absent	outer sepal
H. notabilis	short	absent	absent	present	adaxial& abaxial leaf surfaces, bract, ovary
H. obtusibracteata	short	absent	absent	absent	outer sepal
H. oligastrota	long	outer& inner	present	present	abaxial leaf surface, bract, outer& inner sepal, between anthers and petals

H. oxycraspedotus	short	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal	present	present	outer & inner sepal
H. paeninsularis	long	absent	present	present	absent
H. pallidiflora	short	absent	absent	present	stem, adaxial leaf surface
H. patens	short & long	absent	present	present	absent
H. pedunculata	short	bract, outer sepal	present	present	outer & inner sepal, ovary
H. pilifera	short	absent	absent	absent	abaxial leaf surface, outer sepal
H. platyphylla ssp. *halmaturina*	long	stem	present	present	adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. platyphylla ssp. *major*	long	stem, abaxial leaf surface, bract, outer & inner sepal	absent	present	stem, adaxial leaf surface,
H. platyphylla ssp. *platyphylla*	short	outer sepal	absent	present	stem,
H. porcata	long	absent	present	present	stem, adaxial & abaxial leaf surfaces, outer & inner sepal, ovary
H. protuberans	short	abaxial leaf surface, bract, outer& inner sepal	present	absent	stem, adaxial surface, abaxial leaf surface, bract
H. pusticulata	short	bract, outer sepal	absent	present	stem, adaxial & abaxial leaf surfaces, inner sepal
H. reticulata	long	stem, adaxial, bract, outer & inner sepal	present	present	stem adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. rhynchocalyx	short & long	absent	present	absent	stem, abaxial leaf surface, bract, outer & inner sepal, ovary
H. riparia	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, inner sepal
H. rufa	short	absent	present	absent	absent
H. semota	short	bract	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, between anthers and petals, ovary
H. sericea var. *densiflora*	long	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, between anthers and petals
H. sericea var. *scabrifolia*	short	inner sepal	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal , ovary

H. sericea var. *sericea*	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, between anthers and petals
H. serpyllifolia	short	absent	present	absent	stem, adaxial & abaxial leaf surfaces, bract
H. sessiliflora	long	absent	present	present	stem, adaxial & abaxial leaf surfaces, outer & inner sepal
H. setifera	short	absent	present	absent	stem, adaxial & abaxial leaf surfaces, bract, outer sepal & inner sepal
H. simulans	long	absent	present	present	absent
H. spathulata	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal, ovary
H. stricta	short	outer sepal	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer sepal, ovary
H. strigosa	short	absent	present	present	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal
H. superans	long	absent	present	present	stem, adaxial & abaxial leaf surfaces
H. surcularis	short	absent	absent	absent	outer sepal
H. synandra	short	absent	present	present	stem, abaxial leaf surface, inner sepal
H. tenuis	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces, bract, outer sepal& inner sepal
H. tetranthera	short	stem, adaxial & abaxial leaf surfaces	present	present	stem
H. truncata	short	absent	absent	present	stem, adaxial & abaxial leaf surfaces, bract
H. vestita	short & long	absent	present	present	stem
H. villifera	long	stem, adaxial & abaxial leaf surfaces, bract, outer & inner sepal	present	present	stem
H. woronarana	short	absent	absent	present	± stem, abaxial leaf surface, inner sepal

Chapter 6

The taxonomic importance of floral characters in the south-estern Australian species of *Hibbertia* subg. *Hemistemma* (Dilleniaceae)

Abstract

Floral characteristics of the south-eastern Australian clade of Hibbertia subgenus Hemistemma are described for 93 taxa. The morphological data show high degree of variation between the species in characters such as: stamen number, stamen arrangement in clusters, anther dehiscence, filament fusion, ovary shape, style position and attachment to ovary. From the results, using stamen number and anther dehiscence characters in grouping taxa of species of *Hibbertia* subg. *Hemmistemma* is critical due to high variations between the studied taxa. The characters absence of inner sepal, ≥10.1mm inner sepal width, ≥15.1 mm petal width and rounded outer and inner sepal basewere found to be important synapomorphies for clades within the subgenus.

Introduction

Hibbertia (Dilleniaceae: Hibbertioideae) is currently includes ~380 species (many new and undescribed), most endemic to Australia and some extending to Madagascar, Malaysia, and Fiji (CHAH 2008). The genus was delimited by Horn (2005) on the basis of morphology and molecular analysis of Dilleniaceae, and features such as androecium characteristics, carpel and ovules numbers were used to define the phylogenetic relationships of the four subgenera: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn within the Dilleniaceae (Horn 2005, 2007). However, definitive identification of *Hibbertia* species is one of the major problem, due to high levels of diverse among same species (Horn 2007).

Toelken proposed 16 informal groups species in the SE Australian clade of *Hibbertia* subgenus *Hemistemma* Horn (2005) (Ch. 2: Tab. 1). There have been numerous previous systematics studies on Hibbertia morphology. Bentham (1863) described 41 species from Australia *in Flora Australiensis* and morphological studies, include those Dickison (1968) on *Hibbertia* carpels, Harden (1990) on SA Australian *Hibbertia*, Toelken (1995, 1998, 2000, 2010) on numerous *Hibbertia* species, Tucker (2000; 2003) on floral ontogeny and stamen number reduction, Wheeler (2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b) on WA Australian species. There have also been studies at the genus and family levels, such as Gilg (1925) on Dilleniaceae family, Endress (1997) on gynoecium diversity of Laurales and Horn (2005, 2007) on the Dilleniaceae family and its genera. However, no detailed studies on floral characteristics have been carried out for the SE Australian *Hemistemma* clade species. The aims of this study are:

a) To understand the floral characters, determine any variation and assess the phylogenetic relationships among the studied taxa.
b) To determine any distinct lineages within the SE Australian clade of *Hibbertia* subg. *Hemistemma*, and explore character evolution.

Materials and methods

Data were scored from eighty-seven of the ~93 *Hibbertia* taxa in the clade (species, subspecies and varieties) within all 16 of Toelken's informal groups (Ch. 2: Tab. 1), based mainly on herbarium specimens (Ch. 2: Tab. 2). *Hibbertia* ancistrophylla J.Wheeler from Western Australia was used as the outgroup, based on its position in a sister clade, following Horn (Horn 2005). Measurements for 41 floral morphological characters were coded into discrete states, table 1 show only floral characters derived from Ch. 2: Tab. 3.

Herbarium specimens were reconstituted using few drops of detergent applied for 10–15 minutes to dry leaves and flowers, with the specimens examined under a dissecting light microscope (Zeiss model 1968) at × 10–30 magnification and photographed using a digital camera (Panasonic/Lumix model DMC-FS42 2005). The specimens examined are listed in table 2 (Chapter 2).

Table 1. List of characters with their character states used in the analysis derived from Ch. 2: Tab. 3.

44	Bract length (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)
45	Bract width (mm): <0.5–0.9 (0); 1–2.5 (1); 2.6–5.5 (2); ≥5.6 (3)
46	Flower position: terminal (0); lateral (1)
47	Flower pedicel: present (0);absent (1)
48	Outer sepal shape: ovate (0); ovate-elliptic (1); ovate-lanceolate (2); lanceolate (3); lanceolate-ovate (4); oblong-ovate (5); oblong-elliptic (6)

49 Outer sepal apex: acute (0); acuminate (1); rounded(2); mucronate (3); obtuse (4)
50 Outer sepal base : acute (0), acuminate (1), cuneate (2), rounded (3)
51 Outer sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
52 Outer sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2)
53 Outer sepal margin disposition: revolute (0); recurved (1); incurve (2)
54 Outer sepal margin cilia: absent (0); present (1)
63 Inner sepal shape: ovate (0); ovate-elliptic (1); lanceolate-ovate (2); oblong-ovate (3); oblong-obovate (4); absent (5)
64 Inner sepal apex: acute (0); acuminate (1); rounded (2); rounded-cuspidate (3); rounded-retuse (4); absent (5)
65 Inner sepal base: acute (0); acuminate (1); cuneate (2); rounded (3); absent (4)
66 Inner sepal length (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
67 Inner sepal width (mm): 1–5 (0); 5.1–10 (1); ≥10.1 (2); absent (3)
68 Inner sepal margin disposition: revolute (0); recurved (1); incurve (2); absent (3)
69 Inner sepal margin cilia: absent (0); present (1)
78 Petal shape: ovate (0); obovate (1); spathulate (2); oblong- obovate (3); oblanceolate (4); narrowly obtriangular (5)
79 Petal length (mm): 1–5 (0), 5.1–10 (1), 10.1–15 (2), ≥15.1 (3)
80 Petal width (mm): 1–3 (0); 3–6 (1); 6.1–10 (2); >10.1 (3)
81 Stamen number: 3–5 (0); 5–10 (1); 10–15 (2); ≥15 (3)
82 Stamen arrangement: in one cluster (0); in two cluster (1); in three cluster (2); >three clusters (3)
83 Stamens cluster position: stamens cluster around carpel (0); stamens cluster around carpel and with another cluster (1); stamens cluster on one side of carpel (2); stamens cluster between the carpels (3).
84 Stamen length (mm): 1–3 (0); 3.1–6 (1); >6.1 (2)
85 Anther length (mm): 1–1.5 (0); 1.6–2 (1); 2.1–3 (2); >3.1 (3)
86 Anther width (mm): 0.2–0.5 (0); 0.6–0.8 (1); >0.9 (2)
87 Anther apex: acute (0); round (1); truncate (2); retuse (3)
88 Anther dehiscence: lateral slits (0); terminal pores (1); terminal pores and lateral slits (2); terminal pores and introrse slits (3); introrse slits (4)
91 Filament fusion: free (0); basally connate (1); half connate (2); all connate (3)
92 Anther shape: linear (0); oblong (1); narrowly oblong (2); broadly oblong (3)
93 Pistil shape: uncompressed (0); compressed (1)
94 Style colour: yellow (0); red (1); greenish-yellow (2)
95 Style base: straight (0); recurved (1)
96 Style attachment to ovary: apical (0); lateral (1); semi lateral (2)
97 Stigma shape: capitate (0); discoid (1); linear (2)
98 Ovary shape: spherical (0); compressed (1); ovoid (2)
99 Simple hair on ovary: present (0); absent (1)
100 Stellate hairs on ovary: present (0); absent (1)
102 Seed shape: spherical (0); compressed (1); ovoid (2)
103 Seed colour: black (0) brown (1); brown-black (2); shiny black (3)
104 Seed dimension (mm): 1–1.5 (0); 1.6–2 (1); 2.1–2.5 (2); >2.5 (3)
105 Aril length: >1/3 of the seed (0); 1/3–1/2 of the seed (1)

Results

When the floral features were mapped onto the combined tree produced in Ch 4, the outgroup *H. ancistrophylla* was defined by two floral synapomorphies: rounded outer sepal (50/3) and rounded inner sepal base (65/3); as well as the homoplasious characters: absence of simple hairs on inner sepal abaxial surface (74/1); absence of indumentum on inner sepal abaxial surface (77/2); basally connate filament fusion

(91/3); and lateral style attachment (96/1) (Fig. 1). Two species segregated next forming basal grade (*H. hypsophylloides* and *H. hendersonii*) each species defined by several homoplasious features as illustrated in Tab. 2.

Table 2. Characters and their states used to define each basal grade (see Tab. 1).

H. hypsophylloides	*H. hendersonii*
62/0; 64/2&4' 72/1; 78/3; 82/3; 83/0; 88/2; 98/99; 100/1	49/1; 51/2; 55/1; 59/1; 66/2; 70/1; 74/1; 89/1; 91/0; 102/2

Unexpectedly, five different taxa included within Clade A (Ch. 4: Fig. 2–6) with low support (61% JK), but the Clade was not defined by any floral features. As expected, a close relationship of 4 species revealed from the analysis comprised *H. nitida*, *H. reticulata*, *H. cinerea* and H. hirta based on floral features: acuminate outer sepal apex (49/1); not compressed pistil shape (93/0); presence of simple hairs on ovary (99/0); and absence of satellite hairs on ovary (100/1).

Clade B was defined by two floral features: uncompressed pistil shape (93/0) and yellow and red style colour (94/0&1). This Clade comprised 8 taxa, most arranged within three groups, the first one (47% JK) comprised *H. australis* and *H. covenyana*; the second group with two close related taxa (*H. hexandra* and *H. hermaniifolia* subsp. *hermaniifolia*) had strong support (95% JK) and was defined by the floral feature 1–1.5 mm anther length (85/0); the third group (53% JK) consisted of *H. spathulata* and *H. hermaniifolia* subsp. *recondita* and could be in part defined by the floral features: >three clusters stamen arrangement (82/3) and terminal pores and lateral slits anther dehiscence (88/2) (Fig. 1).

Clade C had no support, but defined by the floral features: ovate and lanceolate-ovate inner sepal shape (63/0&2), terminal pores and lateral slits anther dehiscence (88/2); half connate filament fusion (91/2), and broadly oblong anther shape (92/3). As expected, all *H. sericea* subspecies were located in this Clade with one sister relationship species *H. crinita*.

The last five clades were located as an unresolved polytomy (Fig. 1) most low supported (Fig. 1). However, only Clade D was moderately supported (69% JK), included a close related taxa *H. platyphylla* subsp. *platyphylla* + *H. humifusa* subsp. *erigens* supported by the synapomorphy of acuminate inner sepal base (65/1) and sharing the homoplasious floral features: 6.1–10 mm petal width (80/2), oblong anther shape (92/1), and lateral style attachment (96/1) (Fig. 1).

Although Clade G was an unresolved polytomy, two strong supported subgroups were formed within it. The first comprised *H. riparia* and *H. tenuis* (94% JK), defined by the synapomorphy ovate petal shape (78/0), sharing the homoplasious: recurved inner sepal margin disposition (68/1) and terminal pores and lateral slits anther dehiscence (88/2). The second group comprised *H. appressa* and *H. hirticalyx* with strong support (96% JK), sharing several floral features: 1–5 mm outer sepal length (51/0); sparsely pubescent indumentum on outer sepal adaxial surface (58/0), ovate inner sepal (63/3), 1–5 mm inner sepal length (66/0), 1–3 mm stamen length (84/0), 0.2–0.5 mm anther width (86/0), round anther apex (87/1), yellow style colour (94/0), straight style base (95/0), and apical style attachment to ovary (96/0) (Fig. 1).

Clade H formed an unresolved polytomy, but contained several supported groups. The first group with moderate support (66% JK) was the species pair *H. devitata* and *H. setifera*, sharing two floral features: 1–5 mm outer sepal length (51/0) and revolute inner sepal margin disposition (68/0). The second group was strongly supported (100% JK) and comprised *H. sessiliflora* and *H. empetrifolia* subsp. *uncinata*, sharing the floral features three clusters stamen arrangement (82/2), with a sister relationship to *H. rhynchocalyx*.

The third group comprised a serious of species arranged within a monophyletic group: *H. boweniensis* and *H. strigosa* that shared two floral features: terminal pores and lateral slits anther dehiscence (88/2) and free filaments (91/0), with three sister relationships species *H. platyphylla* subsp. *halmaturina, H. hirsuta* and *H. truncata* (72%; 77%; 100% JK) supported respectively.

Within Clade G, the basal Clade species were strong supported (Fig. 1) as follows: 1) *H. humifusa* subsp. *debilis* + *H. acicularis* (96% JK), defined by the synapomorphies: ≥15.1 mm petal length (79/3) and compressed seed shape (102/1). Two taxa were not defined by any floral features. A close relationship of *H. pallidiflora* and *H. florida* subsp. *angustinervis* (92% JK) was also undefined by floral features (Fig. 1).

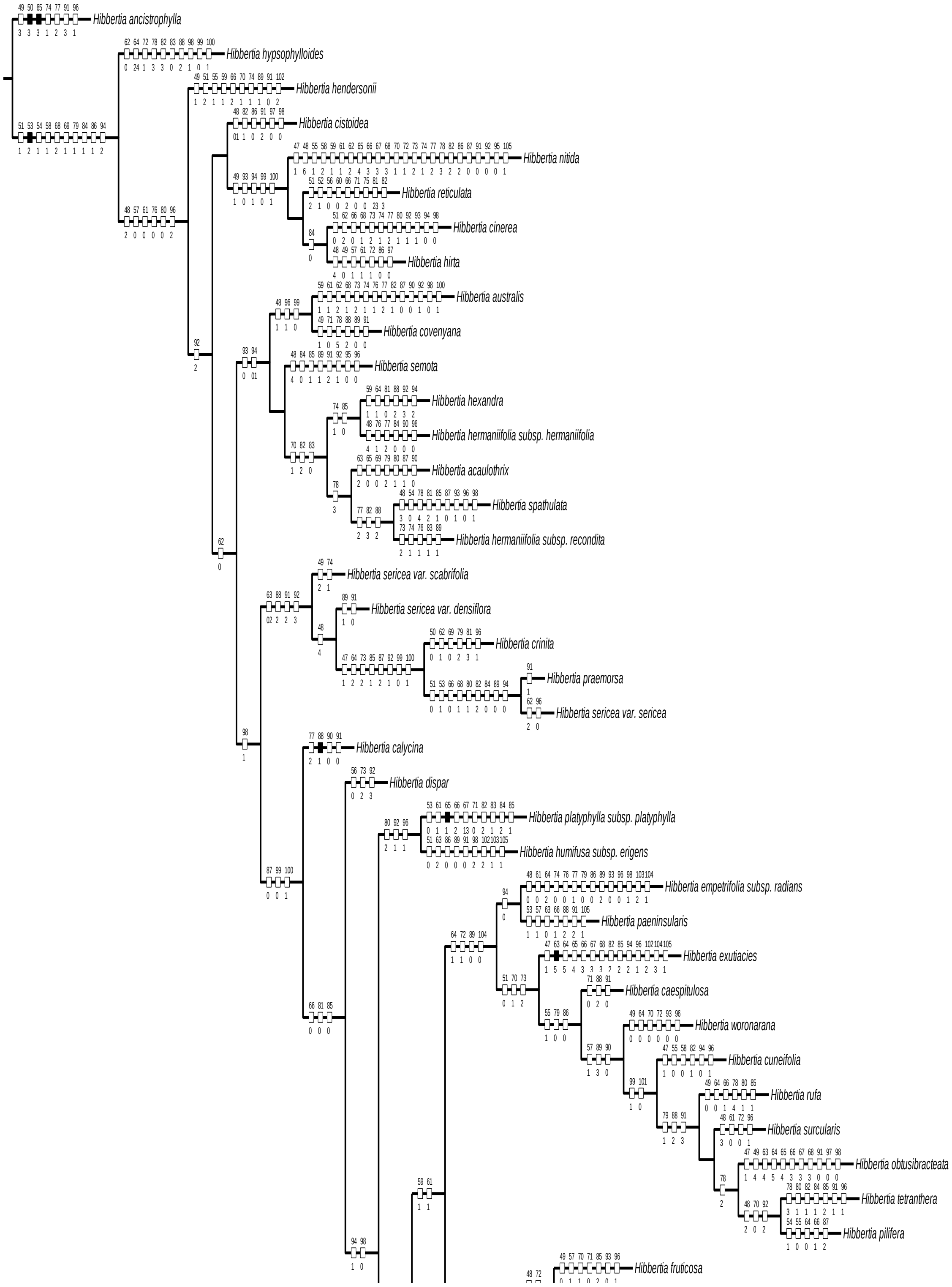

Figure 1. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for floral and seed characters (characters 47–105). Open squares indicate homoplasy.

Fig. 1. Floral and seed characters continued

Discussion

The 16 informal groups species produced by Toelken (unpubl.) were not supported and dispread instead within 8 clads produced from the combined molecular and morphological phylogenetic analysis (Ch. 4: Tab. 4).

The combined analysis revealed several homoplasious floral characters such as outer and inner sepal characteristics (shape and base) (see Fig. 2). However, the analysis highlighted strong diversification in characters such as androecium and gynoecium characteristic (Dickison 1970; Tucker and Bernhardt 2000).

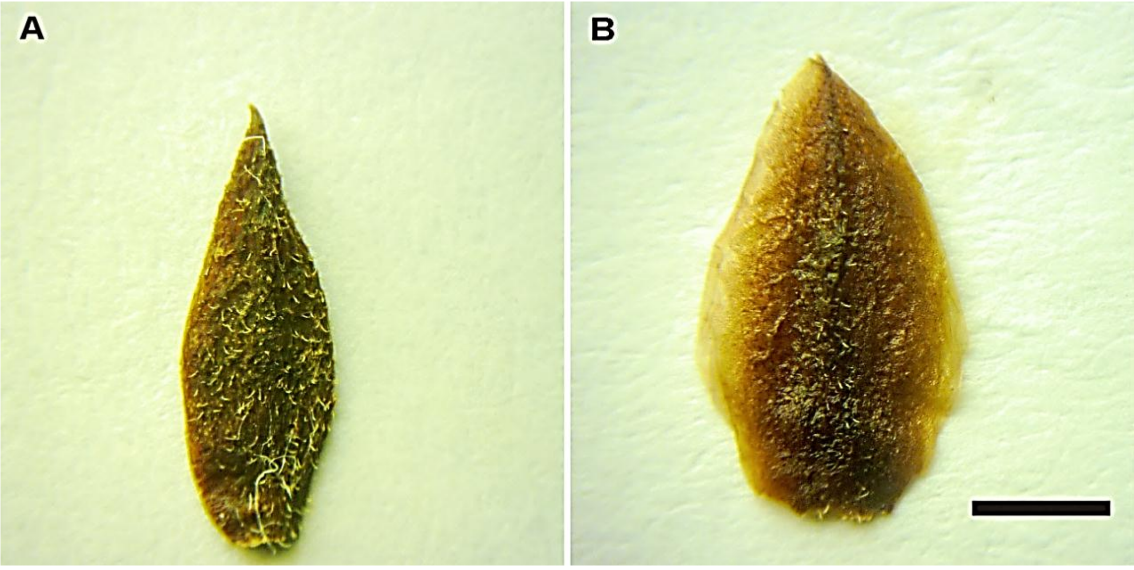

Figure 2. Sepals of *H. protuberans* (BRI*010716*) showing: A) outer sepal lanceolate, B) inner sepal lanceolate-ovate. Scale bar A & B= 2 mm

After expanding characters and character states that was used in the analysis to get the best resolution of the species relationships. Floral diversity appears clearly in characters such as stamen number which is considered the most variable feature in *Hibbertia* species (Toelken 2000). This becomes obvious when examination of the specimens revealed differences in stamen number from 2 in *H. hirsuta* to 27– 30 in *H. vestita* (App. 1; Figures 3, 4). This character makes the correlation among the studied taxa more difficult, especially, to conclude whether these variations due to “pollination syndrome” convergence (Toelken 1998; Tucker and Bernhardt 2000; 2003; Horn 2005).

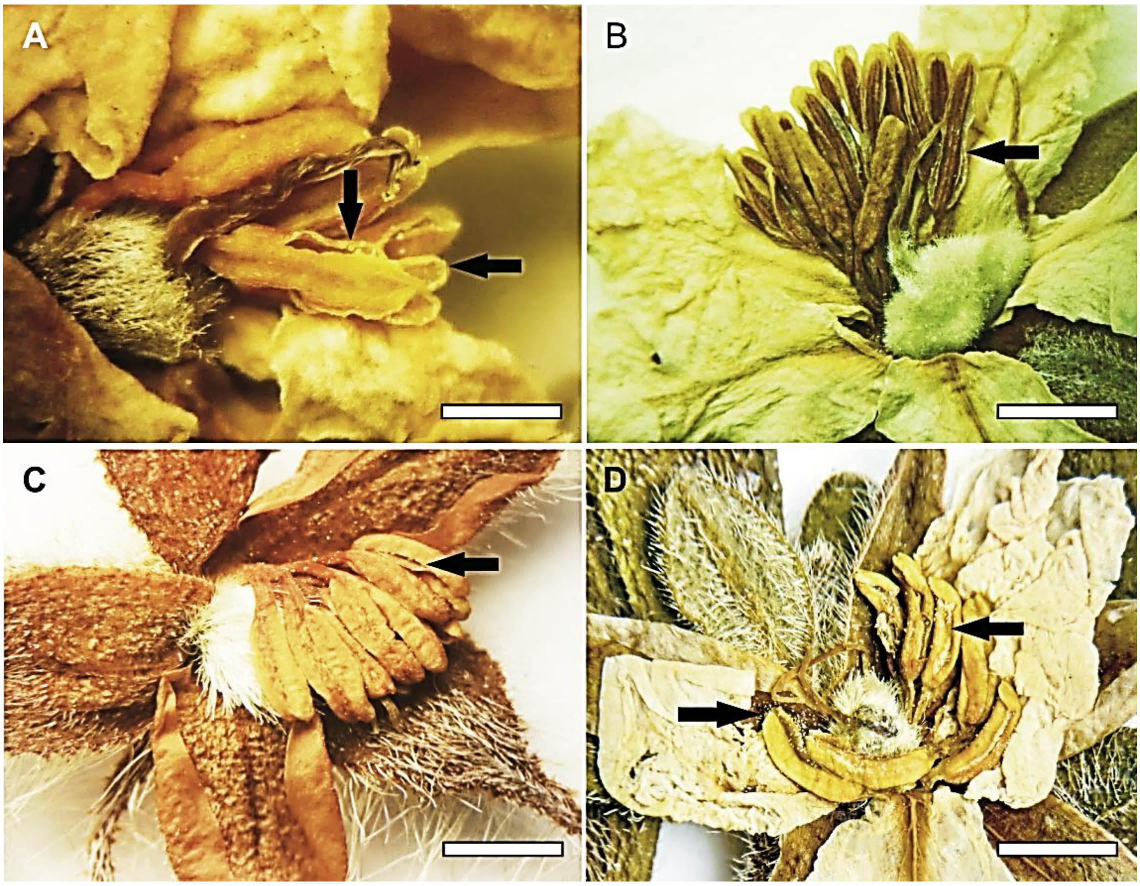

Figure 3. Anther dehiscence types of *Hibbertia* species, A) *H. caespitulosa* showing anther dehiscence by terminal pores and lateral slits (AD*106483*); B) *H. hendersonii* showing anther dehiscence by lateral slits only (BRI*182353*); C) *H. hirta* showing anther dehiscence by introrse slits (CANB0*056123*); D) *H. praemorsa* showing anther dehiscence by terminal pores and introrse slits (CANB*8504647*). (Scale bars A & B = 2 mm, C & D = 1.3mm)

Another significant finding is anther dehiscence, which was first noted by Toelken (1995, 1998, 2000). Five types of dehiscence are examined in this study: 1) lateral slits which is dominant in most studied taxa, 2) terminal pores and lateral slits in 13 taxa, 3) introrse slits only in *H. rhynchocalyx* from NSW (Gibraltar Rang National Park) and *H. hirta* from NSW (Budawang Rang), 4) terminal pores only recorded in one taxa *H. hermaniifolia* subsp. *recondita* from VIC (Gippsland Collins road), 5) terminal pores and introrse slits in only two species *H. cinerea* from SA (Southern Eyre Peninsula) and *H. praemorsa* (Clade D) from NSW (Morton National Park) (App. 1; Fig. 3D).

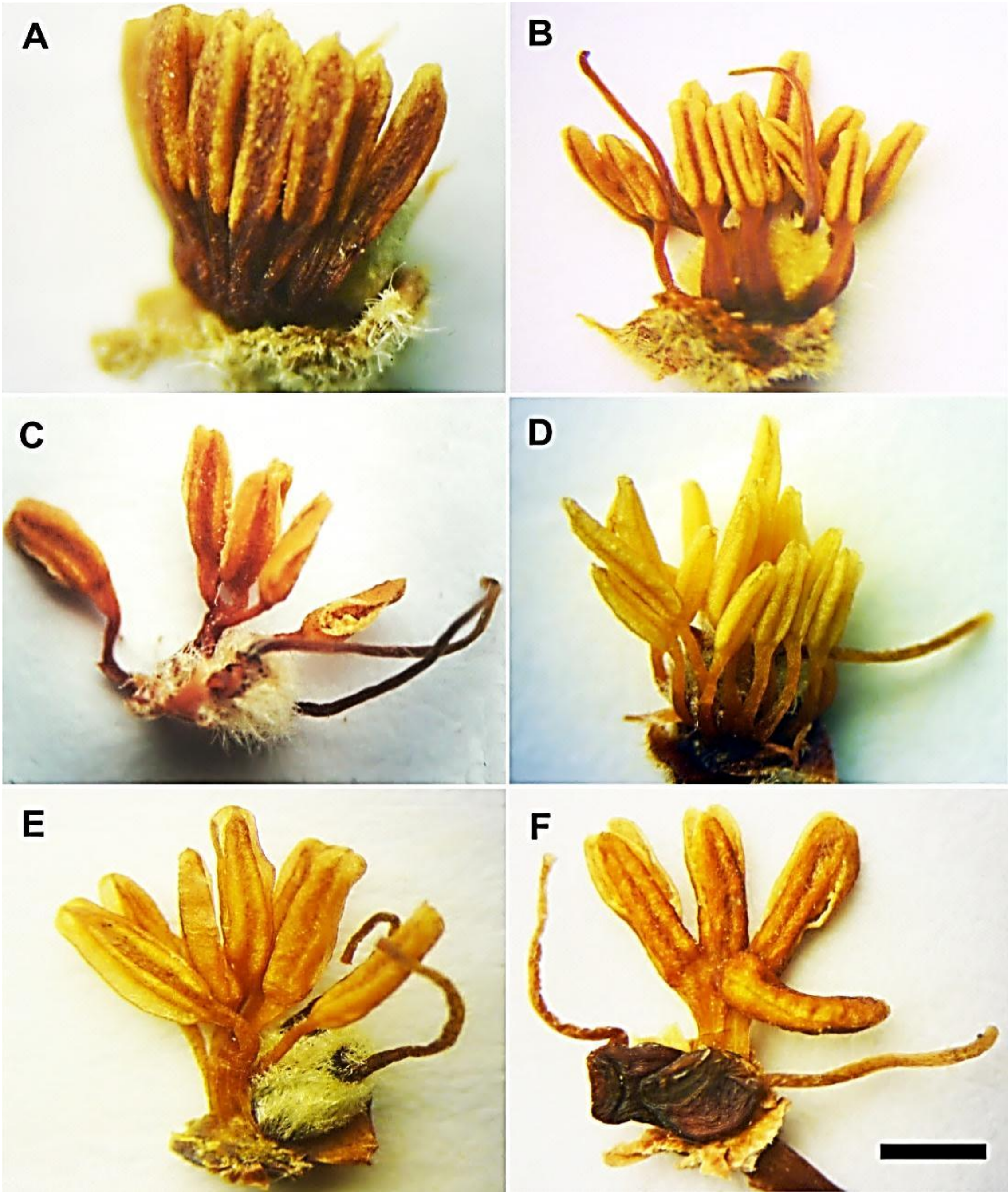

Figure 4 Stamen and filaments characteristics for species in the south-eastern Australian clade of *Hibbertia* subg. *Hemistemma*. A) *H. cistoidea* showing free filaments in one cluster (QSL*133172*); B) *H. acaulothrix* showing basally connate stamens in two clusters (NSW *No. unknown*); C) *H. exutiacies* showing basally connate stamens in three clusters (AD*7317115*); D) *H. spathulata* showing basally connate stamens in more than three clusters (MEL*119822*); E) *H. oxycraspedotus* showing half connate stamens in one cluster (CANB*381942*); F) *H. rufa* all connate stamens in one cluster (MEL*580142*). Scale bar= 1.5 mm.

Unfortunately, using stamen number and anther dehiscence as definitive characters in grouping taxa, especially between closely related species of *Hibbertia* subg. *Hemistemma* (Fig 1) makes the relationship among the species more complicated, and

Toelken (1995, 1998, 2000) mentioned these variations and the diversification of floral characters is significant in *Hibbertia* (Gilg and Werdermann 1925; Cronquist 1988; Tucker and Bernhardt 2000). The phylogenetic analysis utilized in this study reveals low consistency (CI: 27) and retention indexes (RI: 40), and thus, diversity among the perceive species might due to factors such as “ecological specialisation” (Stebbins and Hoogland 1976; Horn 2005).

Toelken (2010) compared *H. glebosa* styles with all other *Australis* group specimens and concluded that both subspecies *H. glebosa* subsp. *glebosa* (Clade H, 91% JK) from SA (Mt Lofty) and *H. glebosa* subsp. *oblongata* (=*H. oblonga*) (Clade H, 79% JK) from SA (Kangaroo Island) had the same style condition attached dorsally on the ovary. However, in this study, *H. glebosa* subsp. *glebosa* was seem to have semi-lateral style attachment with recurved base, and *H. glebosa* subsp. *oblongata* (=*H. oblonga*) has lateral style attachment to ovary without a recurved base (App. 2; Fig. 5A). In addition, *H. australis* (Clade B, 93% JK) from SA (Northern Lofty) has an apical style attachment and the base is recurved. In the same way, *H. cinerea* (Clade A, 81% JK) from SA (Eyre Peninsula) has apical style and the base is recurved (Fig. 4). Thus, although similarities in few morphological characters do exist, many taxa within the same clade are variable.

The gynoecium (Fig. 5) of most studied taxa consists of a compressed pistil; the style attachment varying as: 1) apical, 2) lateral and 3) semi-lateral, and these differences are considered valuable for pollination (Bernhardt 1984, 1986) . Style base also display two character states: 1) straight and 2) recurved.

There are three ovary shapes: 1) spherical, 2) compressed and 3) ovoid, each with 2–4 seeds. Seed of most studied taxa could not found, so only 37 taxa are recorded. In all taxa examined, seeds are brown to black, sometimes shiny black and seed varied from 1–1.5 mm for *H. riparia* (Clade G) from SA (Kangaroo Island), *H. paeninsularis* from SA (Kangaroo Island), *H. obtusibracteata* from SA (Kangaroo Island), *H. hirsuta* from CANB (Mount Nelson) are 1–1.5 mm to 2.1–2 or >2.5 mm for the remain taxa. The aril length for most examined taxa is >1/3 of the seed, and considered vestigial (Horn 2007). Therefore, seed characteristics are considered not significant in this study.

Character evolution

In our evaluation of phylogenetic relationships of *Hibbertia* subg. *Hemistemma*, most taxa are defined by homoplasious character states (Table 2).

Table 2. Floral and seed characters and character states used to define each Clade (See Ch. 2: Tab.3)

Clade	Character/character state
A	Non
B	93/0; 94/0&1
C	80/2; 92/1; 96/1
D	64/1; 72/1; 89/0; 104/0
E	57/1; 91/0; 95/0
F	63/0&2; 88/2; 91/2; 92/3
G	66/1; 87/3; 102/2; 103/1
H	Non
outgroup	49/3; 50/2; 65/3; 74/1; 77/2; 91/3; 96/1

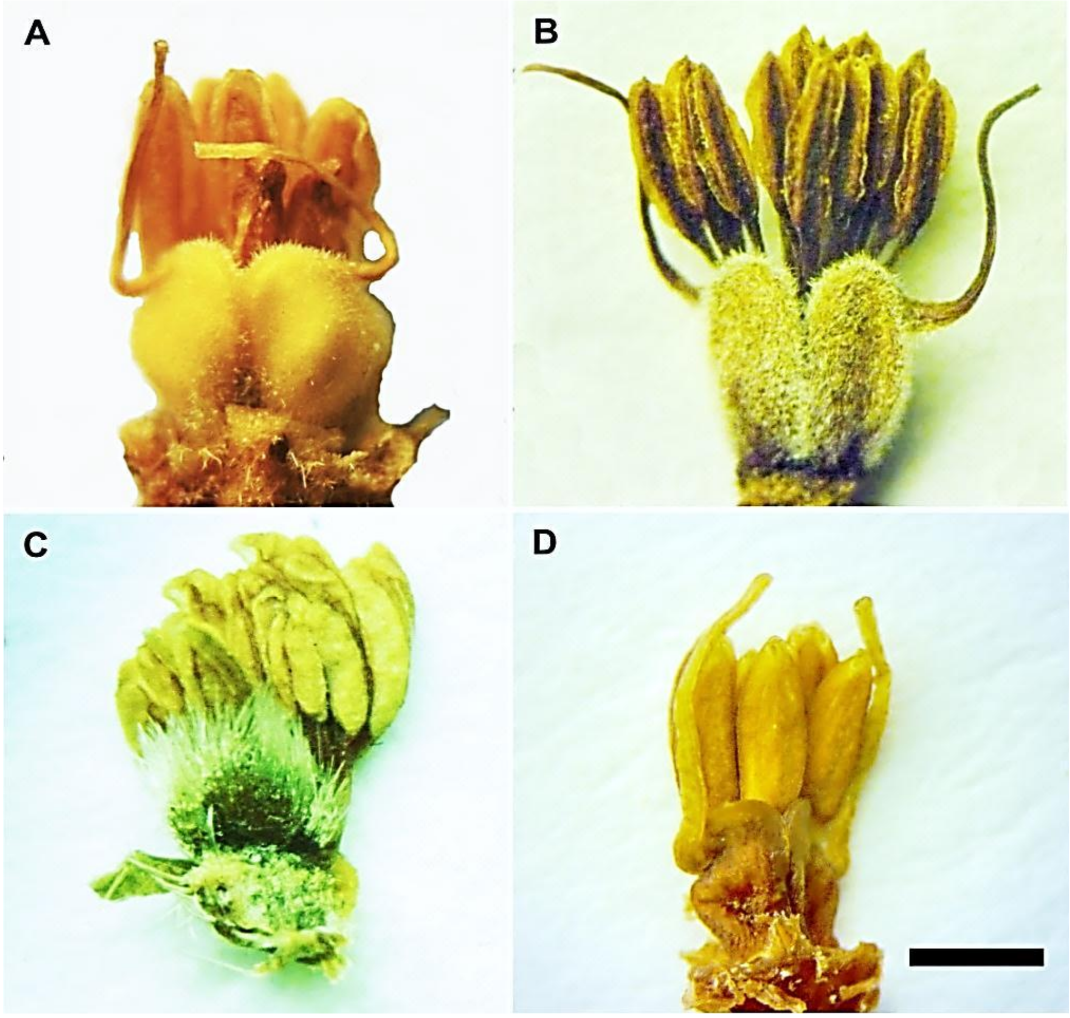

Figure 5. Gynoecium characteristic for species in the south-eastern Australian clade of *Hibbertia* subg. *Hemistemma*. A) *H. glebosa* subsp. *glebosa* showing compressed ovary and semi-lateral attachment style with recurved base (AD*98326221*); B) *H. oligastrota* showing compressed ovary and lateral attachment style with recurved base (QSL*359875*); C) *H. appressa* showing compressed ovary and apical attachments with straight base (TASHB*97456*); D) *H. cistiflora* showing compressed ovary and apical attachments style with recurved base (CANB*004753*). Scale bar= 1.5 mm

Flower position in most *Hibbertia* species is mostly terminal, but *H. nitida* (Clade A), *H. hermaniifolia* subsp. *recondita* (Clade B), *H. acaulothrix* (Clade B), *H. hermaniifolia* subsp. *hermaniifolia* (Clade B), *H. appressa* (Clade G) and *H. porcata* (Clade G) are lateral. In contrast with Toelken (2000), this study found that *H. exutiacies* (Clade F) and *H. platyphylla* subsp. *halmaturina* (Clade H) have both terminal and lateral flowers respectively (Ch. 4: 2–6).

The most reliable morphological synapomorphies revealed from the analysis are illustrated in Table 3 below:

Table 3. Synapomorphies of floral character in the clades produced from the analysis.

Clade	Character/character state	Taxa
A	Non	
B	Non	
C	Non	
D	65/1	*H. platyphylla* subsp. *platyphylla*
E	Non	
F	63/5	*H. exutiacies*
G	67/2; 78/0	*H. cistiflora; H. riparia*
H	77/0&1; 79/3	*H. hirsuta; H. acicularis*
outgroup	50/3; 65/3	*H. ancistrophylla*

In most species, a pedicel is present, but is absent in: *H. crinita*, *H. sericea* var. *sericea* (75% JK supported within Clade C), *H. exutiacies*, *H. platyphylla* subsp. *halmaturina*, *H. nitida*, *H. obtusibracteata* and *H. cuneifolia*. Inner sepal width in most species is 1–5 mm; but it is 5.1–10 mm in only three species: *H. expansa*, *H. porcata* and *H. platyphylla* subsp. *platyphylla*.

The analysis supports the conclusion of Tucker (2003) in that *Hibbertia* stamen number decreases according to flower size, which might due to pollination syndrome (Toelken 1998), and stamen number is considered to have high significance for character evolution, with a unique arrangement “clusters” (Horn 2005, 2007).

As a result, the diverse of stamen number occurs as follow: 30 stamens in *H. semota* within Clade F, 27– 30 stamens in *H. reticulata* and *H. vestita* and 26–28 stamens in *H. covenyana* within Clade H. However, several *Hibbertia* species possess low stamen number such as *H. hirsuta* has (2 stamens only) and *H. aspera* subsp. *pilosifolia*, *H. empetrifolia*, *H. empetrifolia* subsp. *uncinata* and *H. woronarana* with 4 stamens (App.1).

On the other hand, stamen arrangement and position are variable and it is difficult to constitute a phylogenetic conclusion based on this character; filaments fusion also varies (Fig. 3). In addition, anther dehiscence in most *Hibbertias* species by terminal pores & lateral slits, or merely by lateral slits, but other types of dehiscence might indicate evolution for pollination syndromes. Many *Hibbertia* species possess anther width between (0.2–) 0.5–0.6–(0.8) mm, but more than 0.9 mm is restricted to *H. nitida*, *H. empetrifolia* subsp. *empetrifolia* (Figures 5; 6), *H. platyphylla* subsp. *platyphylla*, *H. platyphylla* subsp. *major*, *H. sericea* var. *sericea* and *H. oxycraspedotus*.

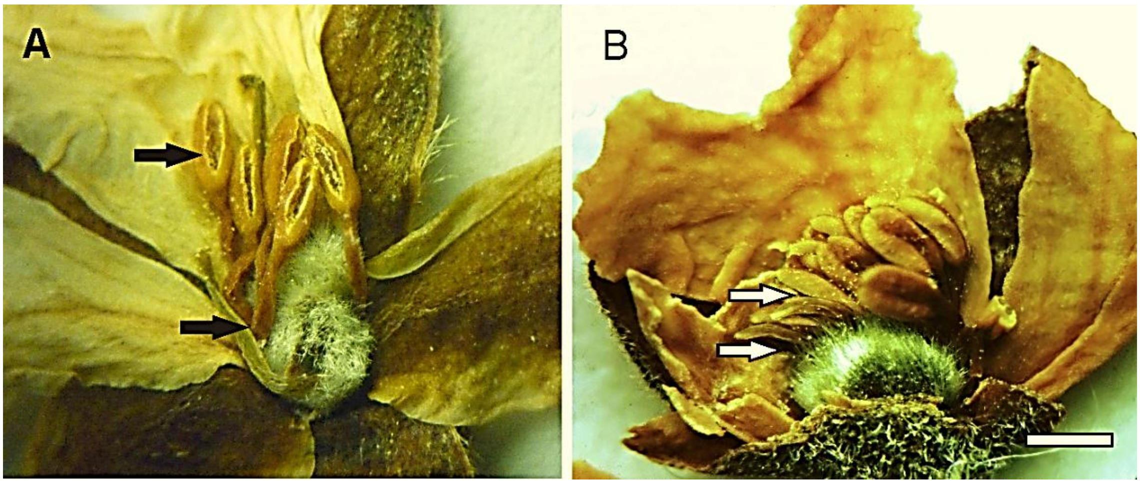

Figure 6. Androecium and gynoecium characters of A) *H. dispar* showing basally connate filaments, lateral slits anther dehiscence, compressed ovary, semi lateral style attachment and recurved style base *98801233* (AD); B) *H. empetrifolia* subsp. *empterifolia* displaying all connate fillaments, lateral slits anther dehiscence, ovary not compressed, style apical attachment to ovary and straight base 96620012 (AD). (Scale bar A & B= 1.5 mm)

Conclusions

When mapped onto the eight clades produced in this study, the floral characters: rounded outer sepal base, acuminate inner sepal shape, rounded inner sepal base, absence of inner sepal, ≥10.1 mm inner sepal width, ≥15.1 mm and petal length are the morphological synapomorphies. The analysis confirmed that rounded outer sepal base character is an ambiguous synapomorphy relative to the outgroup *H. ancistrophylla*. Most floral and seed characters are homoplasious. Clade A is a small with strongly supported sister group *H. reticulata*, but Clade D, E, F, G and H are unresolved polytomy. In contrast, phylogenetic diversity due to pollination syndrome and environmental factor occurs among *Hibbertia* species. Stamen number are varied from 2 stamens in *H. hirsuta*, 4 stamen *H. aspera* subsp. *pilosifolia*, *H. empetrifolia*, *H.*

empetrifolia subsp. *uncinata* and *H. woronarana*, 26–28 stamens in *H. covenyana*, 27–30 stamens in *H. reticulata* and *H. vestita* and 30 stamens in only *H. semota*. Phylogenetic relationships mainly based on stamen arrangement and position might not possible. The character filament fusion is also reveals variation among the species. None of the 16 informal species groups of Toelken (unpubl.) were supported on floral features.

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Appendix 1. Stamen characteristics: number, cluster, filament fusion, anther shape and anther dehiscence of species endemic southern-eastern Australian clade of *Hibbertia* subg. *Hemistemma*

Taxon	Stamens number	Stamen cluster number	filament fusion	Anther shape	Anther dehiscence
H. acaulothrix	9–12	two	basally connate	narrowly oblong	lateral slits
H. acicularis	5–6	one	basally connate	oblong	lateral slits
H. apoda	6	one	half connate	narrowly oblong	lateral slits
H. appressa	(7–) 9–2	three	half connate	narrowly oblong	terminal pores & lateral slits
H. aspera	(2–) 4–6 (–9)	one	half connate	broadly oblong	lateral slits
H. aspera subsp. *aspera*	12	one	half connate	broadly oblong	lateral slits
H. aspera subsp. *pilosifolia*	4–5	two	free	narrowly oblong	lateral slits
H. australis	5–6 (–12)	two	half connate	oblong	lateral slits
H. boweniensis	18–20	>three	free	narrowly oblong	terminal pores & lateral slits
H. bracteata	9–12 (–17)	two	half connate	narrowly oblong	lateral slits
H. caespitulosa	5	two	free	narrowly oblong	terminal pores & lateral slits
H. calycina	10	one	free	narrowly oblong	terminal pores
H. cinerea	9–12	one	basely connate	oblong	terminal pores & introrse slits
H. cistiflora	6	one	basally connate	broadly oblong	lateral slits
H. cistoidea	12–18 (–20)	one	free	broadly oblong	lateral slits
H. corifolia	25	>three	basally connate	oblong	lateral slits
H. covenyana	26–28	one	free	narrowly oblong	lateral slits
H. crinita	(8–) 10–15 (–19)	one	half connate	oblong	terminal pores & lateral slits
H. cuneifolia	6	two	basally connate	broadly oblong	lateral slits
H. decumbens	8–12	one	free	linear	lateral slits
H. devitata	6–8	three	free	narrowly oblong	lateral slits
H. dispar	5	one	basally connate	broadly oblong	lateral slits
H. empetrifolia subsp. *empetrifolia*	6	three	all connate	narrowly oblong	lateral slits
H. empetrifolia subsp. *radians*	3–6	one	basely connate	oblong	lateral slits
H. empetrifolia subsp. *uncinata*	4	two and/or three	half connate	narrowly oblong	lateral slits
H. erectinervis	25	>three	free	narrowly oblong	terminal pores & lateral slits
H. expansa	8–12	>three	basally connate	narrowly oblong	lateral slits
H. exutiacies	3–6	three	basally connate	oblong	lateral slits
H. florida subsp. *angustinervis*	14	>three	basally connate	broadly oblong	lateral slits

H. florida subsp. *florida*	12	>three	free& basally connate& all connate	narrowly oblong	lateral slits
H. fruticosa	22	>three	basally connate	narrowly oblong	lateral slits
H. glaciifera	11	one	free	broadly oblong	lateral slits
H. glebosa subsp. *glebosa*	6	one	basely connate	oblong	terminal pores & lateral slits
H. glebosa subsp. *oblongata*	7	one	all connate	linear	terminal pores & lateral slits
H. hendersonii	24	one	free	broadly oblong	lateral slits
H. hermanniifolia subsp. *hermaniifolia*	11	two	basally connate	narrowly oblong	lateral slits
H. hermanniifolia subsp. *recondite*	12	> three	free	narrowly oblong	terminal pores
H. hexandra	6	two	basally connate	broadly oblong	terminal pores & lateral slits
H. hirsuta	2	one	basally connate	narrowly oblong	lateral slits
H. hirta	8	one	basely connate	narrowly oblong	introrse slits
H. hirticalyx	(7–) 8–12	one	half connate	narrowly oblong	lateral slits
H. horrida	7	>three	half connate	broadly oblong	terminal pores & lateral slits
H. humifusa subsp. *debilis*	3–11	one	free	oblong	lateral slits
H. humifusa subsp. *erigens*	(6–) 7–12	one	free	oblong	lateral slits
H. humifusa subsp. *humifusa*	(6–) 7–10 (–11)	in three & > three	basally connate	oblong	terminal pores & lateral slits
H. hypsophylloides	24	>three	basally connate	broadly oblong	terminal pores & lateral slits
H. nitida	7–8 (–9)	three	free	linear	lateral slits
H. notabilis	10	one	half connate	narrowly oblong	lateral slits
H. obtusibracteata	6	one	free	broadly oblong	terminal pores & lateral slits
H. oligastrota	(2–) 15–17	two	free	narrowly oblong	lateral slits
H. oxycraspedotus	7	one	half connate	narrowly oblong	terminal pores & lateral slits
H. paeninsularis	6–8	one	half connate	oblong & broadly oblong	terminal pores & lateral slits
H. pallidiflora	6–13	one	basely connate	linear	lateral slits
H. patens	8–12	three	all connate	narrowly oblong	lateral slits
H. pedunculata	18	three	basally connate	narrowly oblong	lateral slits
H. pilifera	5	one	all connate	narrowly oblong	terminal pores & lateral slits
H. platyphylla subsp. *halmaturina*	9–11 (–14)	two	basally connate	oblong	lateral slits
H. platyphylla subsp. *major*	12–17 (–18)	two	basally connate	oblong	lateral slits
H. platyphylla subsp. *platyphylla*	8–10 (–13)	one &/or three	basally connate	oblong	terminal pores & lateral slits

H. porcata	12	three	free & half connate	narrowly oblong	lateral slits
H. praemorsa	7–9	one	basally connate	narrowly oblong	terminal pores & introrse slits
H. protuberans	6	two	basally connate	narrowly oblong	lateral slits
H. pusticulata	8	two	free	narrowly oblong	lateral slits
H. reticulata	25–31	> three	basally connate	narrowly oblong	terminal pores & lateral slits
H. rhynchocalyx	12	one	all connate	narrowly oblong	introrse slits
H. riparia	6–7 (–8)	two	all connate	broadly oblong	terminal pores & lateral slits
H. rufa	4	one	all connate	broadly oblong	terminal pores & lateral slits
H. semota	9–10 (–30)	one	half connate	broadly oblong	lateral slits
H. sericea var. *densiflora*	10	one	free	broadly oblong	terminal pores & lateral slits
H. sericea var. *scabrifolia*	10	one	half connate	broadly oblong	terminal pores & lateral slits
H. sericea var. *sericea*	8–10	three	basally connate	oblong	terminal pores & lateral slits
H. serpyllifolia	12	>three	free	broadly oblong	lateral slits
H. sessiliflora	4–8 (–10)	three	all connate	oblong	terminal pores & lateral slits
H. setifera	9	three	free	narrowly oblong	lateral slits
H. simulans	8–12	three	all connate	narrowly oblong	lateral slits
H. spathulata	16	> three	basally connate	narrowly oblong	terminal pores & lateral slits
H. stricta	3–8	one	basally connate	broadly oblong	terminal pores & lateral slits
H. strigosa	19	one	free	narrowly oblong	terminal pores & lateral slits
H. superans	6–10	three	half connate	oblong	lateral slits
H. surcularis	4	one	all connate	broadly oblong	terminal pores & lateral slits
H. synandra	17	one	half connate	broadly oblong	terminal pores & lateral slits
H. tenuis	6	one	free	oblong	terminal pores & lateral slits
H. tetranthera	4	two	basally connate	narrowly oblong	terminal pores & lateral slits
H. truncata	9–12	one	basely connate	narrowly oblong	lateral slits
H. vestita	27–30	>three	basally connate	narrowly oblong	terminal pores & lateral slits
H. villifera	9	one	free	narrowly oblong	lateral slits
H. woronarana	4	two	basally connate & half connate	broadly oblong	lateral slits

Appendix 2. Ovary shape, style base and style position of *Hibbertia* species endemic south-eastern Australian clade of *Hibbertia* subg. *Hemistemma*

Taxon	Ovary shape	Style base	Style position
H. acaulothrix	not compressed	recurved	semi lateral
H. acicularis	compressed	recurved	semi lateral
H. apoda	compressed	not recurved	semi lateral
H. appressa	compressed	straight	apical
H. aspera	not compressed	recurved	semi lateral
H. aspera subsp. *aspera*	not compressed	recurved	semi lateral
H. aspera subsp. *pilosifolia*	not compressed	straight	apical
H. australis	not compressed	recurved	lateral
H. boweniensis	compressed	recurved	semi lateral
H. bracteata	compressed & not compressed	recurved	semi lateral
H. caespitulosa	compressed	recurved	semi lateral
H. calycina	compressed	recurved	semi lateral
H. cinerea	compressed	recurved	apical
H. cistiflora	compressed	recurved	apical
H. cistoidea	not compressed	straight	apical
H. corifolia	compressed	recurved	semi lateral
H. covenyana	compressed & not compressed	recurved	lateral
H. crinita	compressed	recurved	lateral
H. cuneifolia	compressed & not compressed	recurved	lateral
H. decumbens	not compressed	straight	apical
H. devitata	not compressed	recurved	semi lateral
H. dispar	compressed	recurved	semi lateral
H. empetrifolia subsp. *empetrifolia*	not compressed	straight	apical
H. empetrifolia subsp. *radians*	not compressed	recurved	apical
H. empetrifolia subsp. *uncinata*	compressed & not compressed	straight	apical
H. erectinervis	compressed	recurved	apical
H. expansa	compressed	recurved	semi lateral
H. exutiacies	compressed	recurved	lateral
H. florida subsp. *angustinervis*	compressed	recurved	semi lateral
H. florida subsp. *florida*	compressed	recurved	semi lateral
H. fruticosa	not compressed	recurved	lateral
H. glaciifera	not compressed	straight	apical
H. glebosa subsp. *glebosa*	compressed	recurved	semi lateral
H. glebosa subsp. *oblongata*	compressed	not recurved	lateral
H. hendersonii	compressed	recurved	semi lateral
H. hermaniifolia subsp. *hermaniifolia*	not compressed	recurved	apical
H. hermaniifolia subsp. *recondite*	not compressed	recurved	semi lateral
H. hexandra	not compressed	recurved	semi lateral
H. hirsuta	compressed	recurved	semi lateral
H. hirta	not compressed	recurved	apical
H. hirticalyx	not compressed	straight	apical
H. horrida	compressed	recurved	lateral
H. humifusa subsp. *debilis*	compressed	recurved	semi lateral
H. humifusa subsp. *erigens*	compressed	recurved	lateral
H. humifusa subsp. *humifusa*	compressed	recurved	semi lateral
H. hypsophylloides	compressed	recurved	apical
H. nitida	not compressed	straight	semi lateral
H. notabilis	compressed	straight	apical

H. obtusibracteata	compressed	recurved	semi lateral
H. oligastrota	compressed	recurved	lateral
H. oxycraspedotus	compressed	recurved	semi lateral
H. paeninsularis	compressed	recurved	semi lateral
H. pallidiflora	not compressed	recurved	apical
H. patens	spherical	recurved	apical
H. pedunculata	compressed	recurved	semi lateral
H. pilifera	compressed	recurved	semi lateral
H. platyphylla subsp. *halmaturina*	compressed	recurved	lateral
H. platyphylla subsp. *major*	compressed	recurved	lateral
H. platyphylla subsp. *platyphylla*	compressed	recurved	lateral
H. porcata	spherical	recurved	semi lateral
H. praemorsa	spherical	recurved	semi lateral
H. protuberans	compressed	recurved	apical
H. pusticulata	compressed	straight	semi lateral
H. reticulata	ovoid	recurved	lateral
H. rhynchocalyx	compressed	straight	apical
H. riparia	compressed	recurved	semi lateral
H. rufa	compressed	recurved	semi lateral
H. semota	not compressed	straight	apical
H. sericea var. *densiflora*	compressed	recurved	semi lateral
H. sericea var. *scabrifolia*	compressed	recurved	semi lateral
H. sericea var. *sericea*	compressed	recurved	semi lateral
H. serpyllifolia	compressed	recurved	semi lateral
H. sessiliflora	not compressed	recurved	apical
H. setifera	compressed	recurved	apical
H. simulans	compressed	recurved	apical
H. spathulata	compressed	recurved	apical
H. stricta	compressed	recurved	apical
H. strigosa	compressed	recurved	semi lateral
H. superans	compressed	recurved	lateral
H. surcularis	compressed	recurved	lateral
H. synandra	compressed	recurved	semi lateral
H. tenuis	compressed	recurved	lateral
H. tetranthera	compressed	recurved	lateral
H. truncata	not compressed	straight	apical
H. vestita	not compressed	recurved	semi lateral
H. villifera	compressed	recurved	lateral
H. woronarana	not compressed	recurved	apical

Chapter 7

The taxonomic importance of leaf cuticle characters in *Hibbertia* subg. *Hemistemma* (Dilleniaceae) from south-eastern Australia

Abstract

Detailed phylogenetic analyses for 87 taxa of *Hibbertia* subgenus *Hemistemma* endemic south-eastern Australia incorporating 43 leaf cuticle characteristics is presented. These characters are recorded for the first time from adaxial and abaxial leaf surfaces, with phylogenetic relationships summarized, and taxon delimitation and character evolution discussed. From the results, leaf cuticles of all studied taxa are undulate and sinuous cell outline, rounded and undulate are common on leaf abaxial surfaces with two main sizes (length) of epidermal anticlinal cells 30– >55 µm. The positions and types of trichome basesfound to be taxonomically important on both adaxial and abaxial leaf surfaces, but stellate trichomes and branched hairs are more complicated.

Introduction

Hibbertia Andrews (Dilleniaceae: Hibbertioideae) is an Australian native genus with high diversity of species (~380 species), mainly in eastern Australia, SW Western Australia and in the Northern Territory (CHAH 2008), extending to the mainland of Fiji, Madagascar, New Caledonia and New Guinea (Tucker and Bernhardt 2003). Recent morphological and molecular detailed studies of Dilleniaceae revealed four subgenera: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn (Horn 2005). Subgenus *Hemistemma* includes ~160 species from south-eastern Australia (Horn 2007), and possesses high variability, particularly in indumentum (vestiture) patterns and floral characteristics (Toelken 1995, 1998, 2000; Horn 2005) and the 16 informal species groups within SE Aust. clade of subgenus *Hemistemma* (see Ch. 2: Table 1) proposed by Toelken (unpubl.) required further study. No attempts have been made previously to use leaf cuticular characters in their phylogenetic relationships.

There have been many previous studies on *Hibbertia* species including inflorescence characteristics of thirty-seven species (de Candolle 1824), morphology and geographical distribution of sixty-seven species (Bentham 1863), leaf venation patterns (Rury and Dickison 1977), wood anatomy (Dickison 1967), pollen morphology (Dickison *et al.* 1982), carpels morphology (Dickison 1968), leaf anatomy (Dickison 1970), general morphological description of species from Western Australia (Wheeler 2002a, 2002c, 2002d, 2002b, 2003, 2004a, 2004b) , leaf mesophyll characteristics for all genera of Dilleniaceae (Horn 2005), and morphological and surface indumentum features in *Hibbertia* (Toelken 1995, 1998, 2000, 2010).

This study was designed to 1) understand leaf cuticular characters and their significance of the 16 informal groups of Toelken (unpubl.) of species of *Hibbertia* subg. *Hemistemma*, 2) explore character evolution, 3) recognise any intra-specific variations and species boundaries, 4) determine any distinct phylogenetic lineages within the SE Australian *Hibbertia* subgenus *Hemistemma*.

Materials and methods

Taxon sampling

Eighty-seven of ~93 *Hibbertia* taxa representing species, subspecies and varieties within all 16 informal groups were included in this study (Ch. 2: Table 1). Samples were obtained from SA herbarium specimens (Ch.2: Table 2) from different locations within SE Australia. In total 43 characters of leaf cuticle were coded into discrete states (Table 1), some binary, but most were multistate and/or polymorphic due to intra-specific polymorphic variation.

Preparation of material for anatomical studies

Following the methods of Christophel and Lys (1986), mature leaf samples placed into test tubes and soaked overnight in 80% ethanol then placed into 1 ml of 30% H_2O_2 and 0.5 ml of fresh 80% ethanol and heated in a boiling water bath for 3–18 hours. When the sample turns light yellow to white, it transferred to a Petri dish with water for examination. If internal cellular materials remain, the sample returned to the ethanol peroxide solution reheated until clear of cellular contents. The cuticles rinsed in water to remove reagents and the samples stained with 1.0% Crystal Violet for 10–40 seconds, rinsed and mounted in phenol glycerine jelly. The coverslips were ringed with clear nail polish to retard dehydration and the prepared slides observed by light microscopy and photographed using digital camera. SEM photos were also conducted in Adelaide Microscopy by using both XL20 and XL30 type/ Philips (manufactured in 1994 by Philips in Eindhoven, The Netherlands).

version 1.00.08 (Nixon 2002).

Character mapping

The characters and character states were mapped using WinClada version 1.00.08 (Nixon 2002) onto the results of the combined analysis tree from Ch. 4 to explore character evolution (Fig. 1). Mapped charters are indicated in asterisks.

Table 1. List of leaf cuticular characters with their character states used in the analysis derived from Ch. 2: Tab. 3.

106 Stomatal distribution: amphistomatic (0); hyperstomatic (1); hypostomatic (2)
107 Adaxial epidermal anticlinal cells small (<30 μm): absent (0); present (1)
108 Adaxial epidermal anticlinal cells small medium sized (30–50 μm): absent (0); present (1)
109 Adaxial epidermal anticlinal cells small large (>55 μm): absent (0); present (1)
110 Adaxial epidermal cell length to width ratio: 1–2 (0); 2.1–3 (1); 3.1–4 (2); >4.1 (3)
111 Adaxial epidermal anticlinal cell walls rounded: absent (0); present (1)
112 Adaxial epidermal anticlinal cell walls angular: absent (0); present (1)
113 Adaxial epidermal anticlinal cell walls undulate: absent (0); present (1)
114 Adaxial epidermal anticlinal cell walls sinuous: absent (0); present (1)
115 Adaxial epidermal anticlinal cell walls : thin (0); thick (1)
116 Adaxial epidermal anticlinal cell walls smooth: absent (0); present (1)
117 Adaxial epidermal anticlinal cell walls beaded: absent (0); present (1)
118 Adaxial epidermal anticlinal cell walls buttressed: absent (0); present (1)
119 Adaxial epidermal periclinal cell walls smooth: absent (0); present (1)
120 Adaxial epidermal periclinal cell walls granular: absent (0); present (1)
121 Adaxial epidermal periclinal cell walls papillate: absent (0); fine (1); prominent (2)
122 Adaxial epidermal periclinal cell walls striate: absent (0); present (1)
123 Adaxial epidermal trichome bases: absent (0); rare 1 trichome base /400 × (1); common 2–7 trichome bases/400 × (2); very common >10 trichome basses/400 × (3)
124 Adaxial epidermal vein cells forming areoles: absent (0); present (1)
125 Abaxial epidermal anticlinal cells small (<30 μm): absent (0); present (1)
126 Abaxial epidermal anticlinal cells small medium sized (30–50 μm): absent (0); present (1)
127 Abaxial epidermal anticlinal cells small large (>55 μm): absent (0); present (1)
128 Abaxial epidermal cell length to width ratio: 1–2 (0); 2.1–3 (1); 3.1–4 (2); >4.1 (3)
129 Abaxial epidermal anticlinal cell walls rounded: absent (0), present (1)
130 Abaxial epidermal anticlinal cell walls angular: absent (0), present (1)
131 Abaxial epidermal anticlinal cell walls undulate: absent (0); present (1)
132 Abaxial epidermal anticlinal cell walls sinuous: absent (0); present (1)
133 Abaxial epidermal anticlinal cell walls : thin (0); thick (1)
134 Abaxial epidermal anticlinal cell walls smooth: absent (0); present (1)
135 Abaxial epidermal anticlinal cell walls beaded: absent (0); present (1)

136 Abaxial epidermal anticlinal cell walls buttressed: absent (0); present (1)
137 Abaxial epidermal periclinal cell walls smooth: absent (0); present (1)
138 Abaxial epidermal periclinal cell walls granular: absent (0); present (1)
139 Abaxial epidermal periclinal cell walls papillate: absent (0); fine (1); prominent (2)
140 Abaxial epidermal periclinal cell walls striate: absent (0); present (1)
141 Abaxial epidermal trichome bases: absent (0); rare 1 trichome base /400 × (1); common 2–7 trichome bases/400 × (2); very common >10 trichome basses/400 × (3)
142 Abaxial epidermal vein cells forming areoles: absent (0); present (1)
143 Stomata small (<10 μm long): absent (0); present (1);
144 Stomata medium (10–20 μm long): absent (0); present (1);
145 Stomata large (>20 μm long): absent (0); present (1)
146 Stomata sunken: absent (0); present (1)
147 Elliptic stomata: absent (0); present (1)
148 Circled stomata: absent (0); present (1)

Results

When mapped onto the combined molecular and morphological tree produced in Ch 4 (Fig. 1), two leaf cuticular characters used to define the outgroup *H. ancistrophylla*: presence of adaxial epidermal anticlinal cell walls sinuous (114/1) and presence of abaxial epidermal anticlinal cell walls sinuous (132/1). Clade A (61% JK) was not defined by any leaf cuticular characters; however, 4 closely related taxa within this Clade: *H. nitida*, *H. reticulata*, *H. cinerea* and *H. hirta*, that (81% JK) based on presence of stomata medium (10–20 μm long) (Fig. 1).Clade B was also not defined by leaf cuticular characters; however, three groups in this Clade, the first one (47% JK) consisted of *H. australis* and *H. covenyana*; the second group comprised (*H. hexandra* + *H. hermanniifolia* subsp. *hermaniifolia*; 95% JK) that was not defined by leaf cuticular characters; the third group (*H. spathulata* + *H. hermaniifolia* subsp. *recondita*; 53% JK) that was also not defined by leaf cuticular characters.

Clade C represented all taxa characterised by presence of abaxial epidermal anticlinal cell walls undulate (131/1). All *H. sericea* subspecies were located in this Clade, and *H. crinita* was a sister to *H. praemorsa* + *H. sericea* var. *sericea* pair, sharing the characters 2.1–3 abaxial epidermal cell length to width ratio (128/1) and presence of abaxial epidermal vein cells forming areoles (142/1).

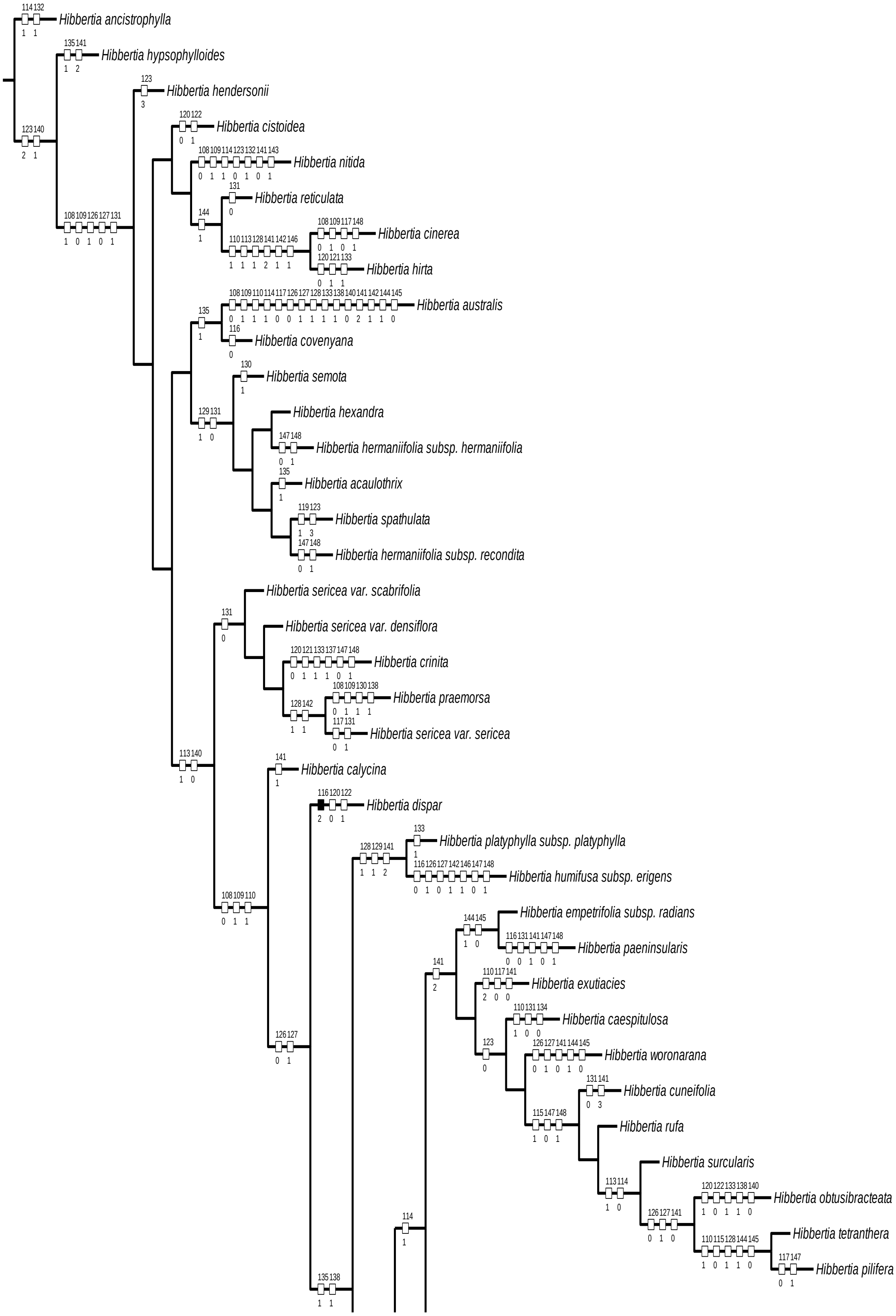

Figure 1. Randomly selected tree from 4 of the equally most parsimonious trees (L 8448; CI 27; RI 40; unif. 255) produced by a WinClada ratchet analysis of the combined molecular and morphological data (1000 reps, 10 trees held per rep) showing ACCTRAN character state reconstructions for anatomical characters (characters 106–148). Open squares indicate homoplasy.

Fig. 1. Anatomical characters continued

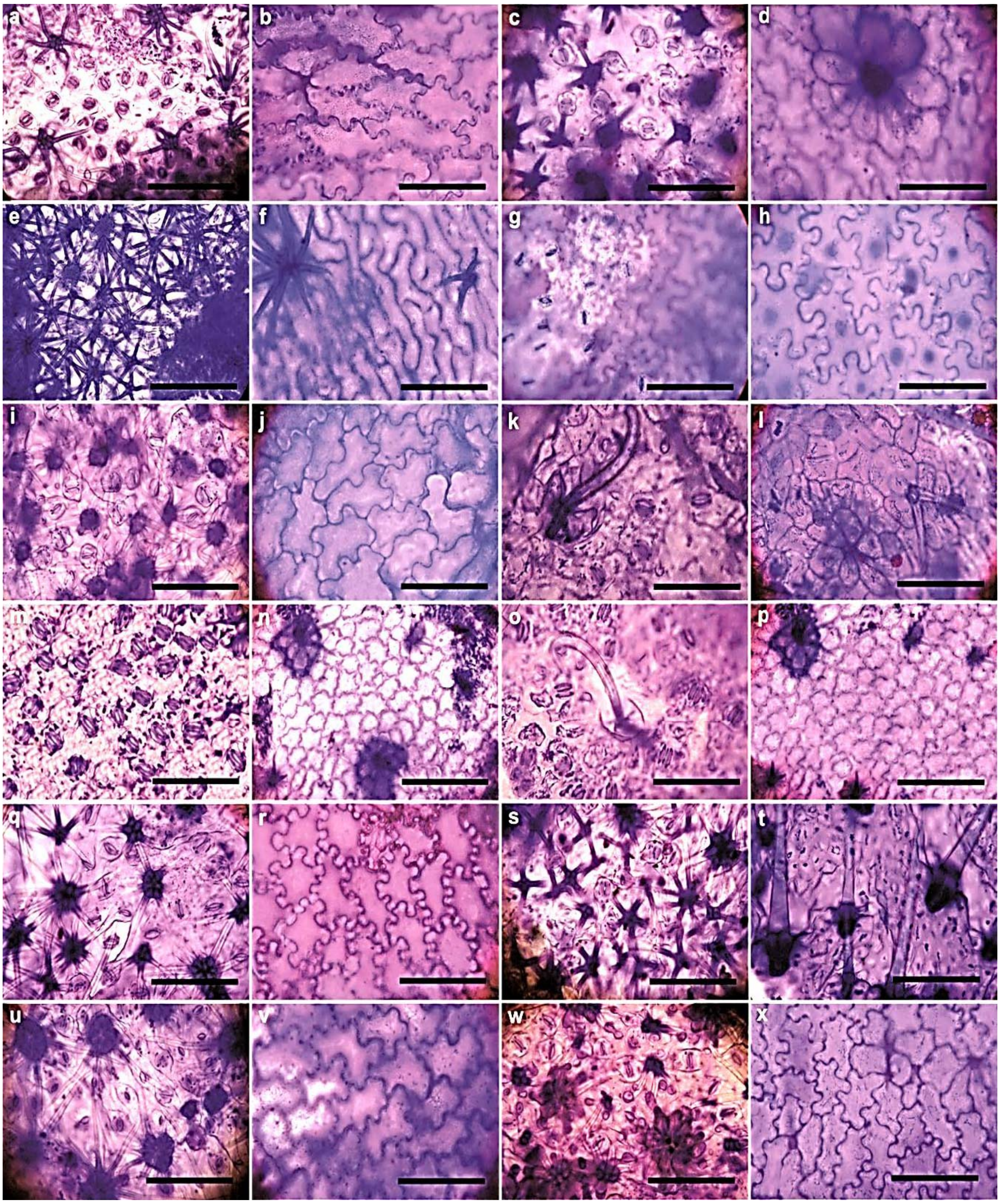

Figure 2. Leaf cuticle characters for species in the south-eastern Australian *Hibbertia* subg. *Hemistemma* using light microscopy: abaxial on the left, adaxial on the right. a, b *H. oligastrota* (QSL359875); c, d *H. aspera* subsp. *aspera* (BRI253673); e, f *H. australis* (AD8824292); g, h *H. bracteata* NSW131); i, j *H. notabilis* (MEL673624); k, l *H. aspera* subsp. *pilosifolia* (BRI182059); m, n *H. empetrifolia* subsp. *radians* (AD9843585); o, p *H. empetrifolia* subsp. *uncinata* (NSW218); q, r *H. covenyana* (CANB00404379); s, t *H. hermaniifolia* subsp. *recondita* (CANB237920); u, v *H. humifusa* subsp. *erigens* (MEL2019490); w, x *H. expansa* (CANB037098). Scale bars: 100 µm.

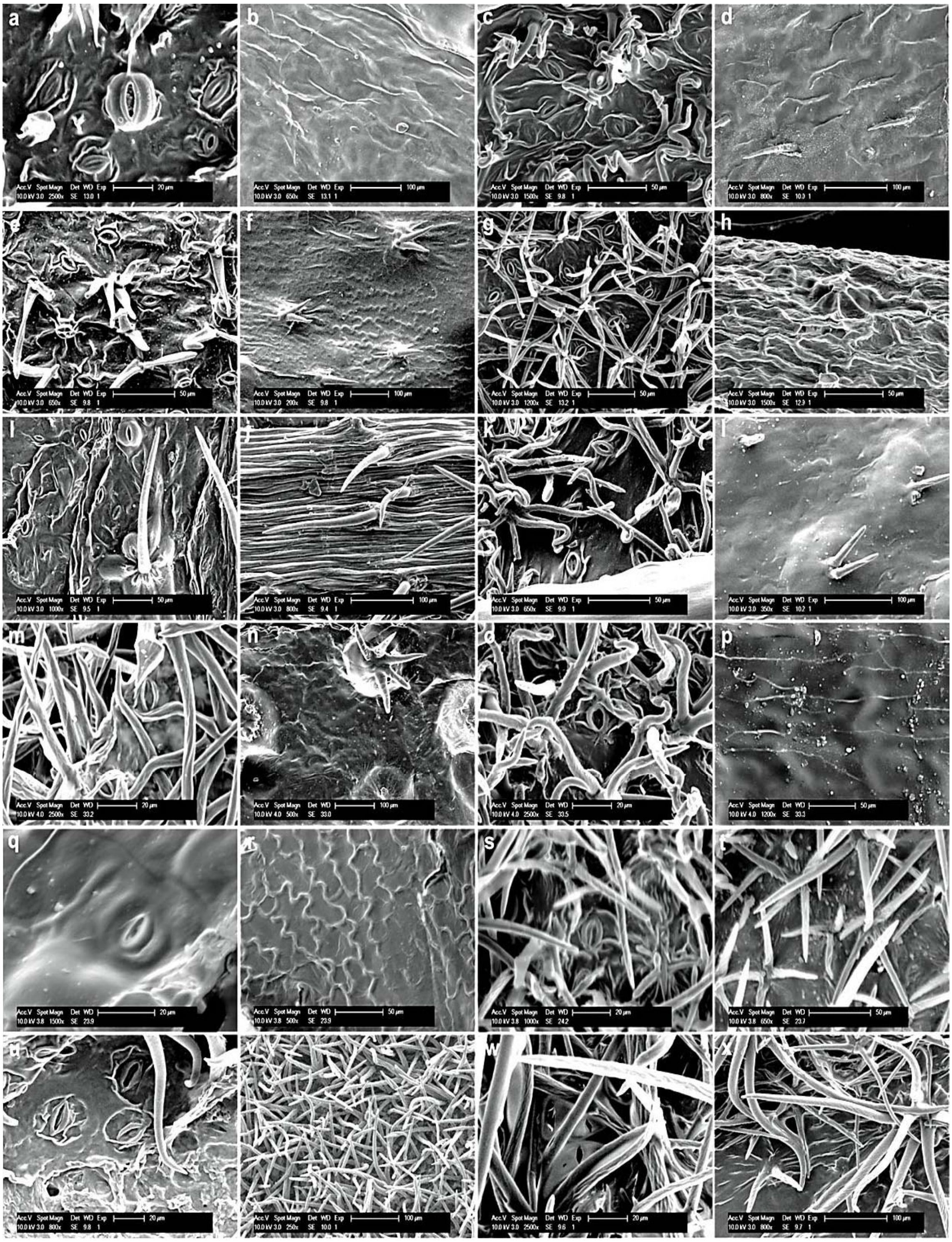

Figure 3. Leaf cuticle characters for species in the south-eastern Australian *Hibbertia* subg. *Hemistemma* using SEM: abaxial on the left, adaxial on the right. a, b *H. cuneifolia* (NSW224462); c, d *H. caespitulosa* (AD106483); e, f *H. synandra* (BRI235206); g, h *H. apoda* (CANB168185); i, j *H. boweniensis* (NSW254914); k, l *H. strigosa* (MEL695584); m, n *H. horrid* (CANB037408); o, p *H. serpyllifolia* (BRI405505); q, r *H. nitida* (NSW263); s, t *H. exutiacies* (AD7317115); u, v *H. oxycraspedotus* (CANB381942); w, x *H. hermaniifolia* subsp. *hermaniifolia* (NSW255007).

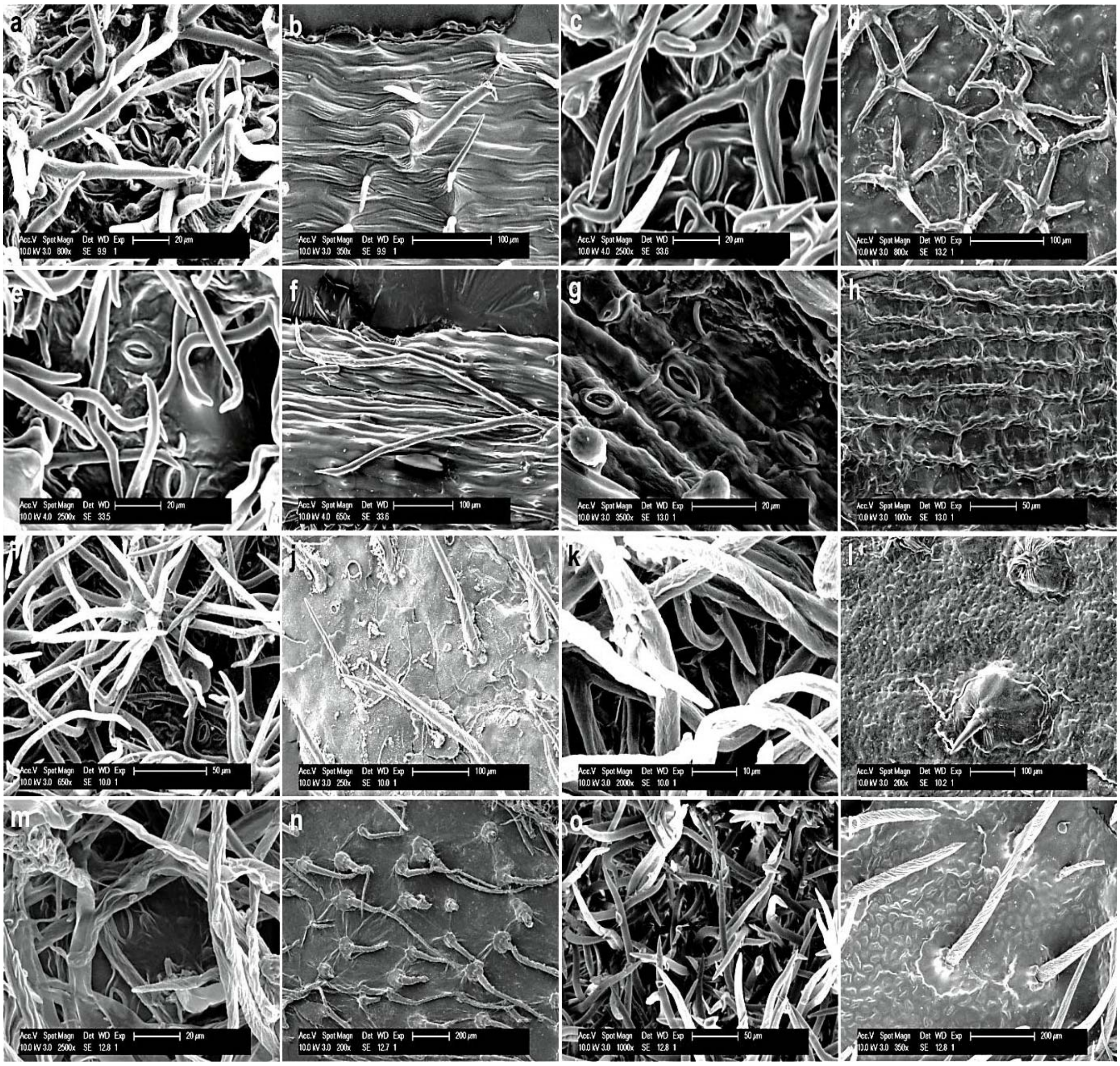

Figure 4. Leaf cuticle characters for species in the south-eastern Australian *Hibbertia* subg. *Hemistemma* using SEM: abaxial on the left, adaxial on the right. a, b *H. expansa* (CANB037098); c, d *H. dispar* (AD98801233); e, f *H. florida* subsp. *florida* (CANB00406335); g, h, *H. pilifera* (CANB161485); i, j *H. hirsuta* (CANB200389); k, l *H. platyphylla* subsp. *platyphylla* (AD98631006); m, n, *H. sericea* subsp. *densiflora* (MEL572143); o, p *H. sericea* subsp. *scabrifolia* (NSW403).

The last five clades were located as unresolved polytomy (Fig. 1); however, only Clade D had moderately support (Ch 4: 69% JK) for the close related *H. platyphylla* subsp. *platyphylla* + *H. humifusa* subsp. *erigens*, sharing the characters: 2.1–3 abaxial epidermal cell length to width ratio (128/1), presence of abaxial epidermal anticlinal cell walls rounded (129/1) and common 2–7 trichome bases/400× on leaf abaxial epidermal surface (141.2) (Fig. 1).

Clade E could be defined by the character rare 1 trichome base / 400× on leaf abaxial epidermal surface (141/1), the character presence of abaxial epidermal periclinal cell walls striate (140/1) defined the terminal pair *H. protuberans* + *H. glaciifera*.

Clade F. could be defined by the character presence of abaxial epidermal periclinal cell walls striate (140/1). However, all taxa within this Clade were poorly supported.

Clade G could be defined by the character absence of abaxial epidermal periclinal cell walls granular (138/0) and presence of abaxial epidermal periclinal cell walls striate (140/1). However, amphistomatic stomata distribution (106/0) was a unique synapomorphy for the pair (*H. cistiflora* + *H. oxycraspedotus*).

Clade H was the largest in the tree, and was defined by the character presence of adaxial epidermal anticlinal cell walls beaded (117/1). The character 3.1–4 abaxial epidermal cell length to width ratio (128/1) was a unique synapomorphy that defined *H. devitata* + *H. setifera*, which share also the character presence of adaxial epidermal anticlinal cell walls sinuous (114/1) (Figs 1).

Discussion

Leaf cuticular character of *Hibbertia* subg. *Hemistemma*

Leaf cuticles of species of *Hibbertia* subg. *Hemistemma* have undulate and sinuous cell outline adaxial surface, meanwhile leaf epidermal cells of all *Hibbertia* taxa were defined irregular or isodiametric, rounded and undulate are common on leaf abaxial surfaces (App. 1), but no buttressed anticlinal cell walls were found in all examined taxa. Two main sizes (length) of epidermal anticlinal cells were found, medium-sized (30–50 μm) in 19 taxa with the remainder (>55 μm) (App. 1). Cell wall thickness of all studied taxa was thick, but (33 taxa) with thin walls; cell walls of (AB) surface mostly thin, but 11 taxa were thick.

All studied species with trichome base (Figs. 2–4), three categories of trichome bases are recognised: 1) simple (Fig. 2t), 2) stellate (Fig. 2a, c, q), and 3) hooked (Fig. 2k, o). Taxa with simple trichomes with tuberculate bases (Fig. 2d; Fig. 3: i; Fig. 4: l, n, p). These structures are distributed on both (AD and AB) leaf surfaces and are coded as rare, common and very common (Fig. 2: k, q, u). Leaf surface with stellate trichomes (branched hairs) are more complicated or nearly impossible to examine, especially when the taxa with dense trichome layer that prevents examining leaf surfaces (Fig. 4i), namely *H. australis*, *H. hirsuta*, *H. notabilis*, *H. strigosa*, *H. oxycraspedotus*, *H. sericea* var. *sericea*. Hooked trichomes are also present on leaf surfaces (Fig. 3o).

Stomata are hypostomatic for all studied taxa agreeing with Horn (2005). Two types of stomata shape are found: 1) elliptic (Fig. 1m; 3a) which was common (*c*.80%), and 2) circular (Fig. 2i; 4g). However, many species had both shapes in the same species (App. 1). Stomata sizes are varied from small (<10 µm long), 2) medium (10–20 µm long) and large (>20 µm long). Most studied taxa lacked sunken stomata, except for 8 taxa *H. empetrifolia* subsp. *empetrifolia*, *H. cinerea*, *H. hirta*, *H. notabilis*, *H. rhynchocalyx*; *H. humifusa* subsp. *debilis*, *H. humifusa* subsp. *erigens*, *H. acicularis* (App. 1). Amphistomatic stomata of the define endemic NSW monophyletic pair species *H. cistiflora* + *H. oxycraspedotus.* Both species belong to the informal *Exutiacies* group.

Character evolution

The distribution of stomata on abaxial leaf surfaces are considered plesiomorphic, but stomata shape and size are the most variable (App. 1). Nevertheless, different anatomical characters are used to describe the phylogenetic relationships between the studied taxa, such as papillate periclinal cell walls on (AB) surfaces in the species: *H. glebosa* subsp. *glebosa*, *H. humifusa* subsp. *humifusa*, *H. humifusa* subsp. *debilis* and *H. humifusa subsp. erigens* (Fig. 2v); meanwhile, thin anticlinal cell walls on (AD) surfaces and thick anticlinal cell walls on (AB) surfaces for species: *H. serpyllifolia*, *H. expansa* and *H. florida* subsp. *angustinervis*, *H. hypsophylloides*, *H. synandra*, *H. erectinervis* and *H. vestita* (Fig. 2x). Anatomical characters that defined each Clade are illustrated in Table 2.

Table 2. Anatomical characters and character states used to define each Clade.

Clade	Character and character state
A	Non
B	Non
C	131/0
D	128/1; 129/1; 141/2
E	141/1
F	141/1
G	138/0; 140/1
H	117/1
outgroup	1144/1; 132/1

The presence of trichome bases is homoplasious, but the length and distribution of trichomes are highly variable, thus, these variations have been considered taxonomically significant for identifying *Hibbertia* species (Dickison 1970; Horn 2005). Some trichome bases consist of many epidermal cells (tuberculate base), such as in long simple hairs (Fig. 4b, j, p), but some species lack this, and the character is an important diagnostic feature for *Hibbertia* subg. *Hemistemma* (see Ch. 5). However, difficulties in examining some species with dense trichomes have been noticed and delimiting species features for taxa such as *H. sericea* var. *densiflora* are not apparent (Fig. 4m, n).

Table 3. Homoplasious characters and their states in the current study (see Ch. 4: Tab. 1).

No.	Character	Character state	Character Number
1	Cell length to width ratio (AD) surfaces	3.1–4	5
2	Anticlinal cell walls buttressed (AD) surfaces	Absence	13
3	Periclinal cell walls papillate (AD) surfaces	Fine	16
4	Vein cells forming areoles (AD) surfaces	Absence	19
5	Anticlinal cells small (<30 µm) (AD) surfaces	Absence	20
6	Cell length to width ratio on (AB) surfaces	2.1–3	23
7	Cell walls beaded(AB) surfaces	Presence	30
8	Anticlinal cell walls buttressed (AB) surfaces	Absence	31
9	Stomata large	Absence	40

Conclusions

Leaf cuticle characters were useful for better understanding of the phylogenetic relationships of the Australian endemic species of *Hibbertia* subgenus *Hemistemma*. The studied species arranged within eight clades, most in monophyletic groups, with strong supports for species pairs and sister relationships, with a high number of homoplasy. Trichome bases are extensively present in most studied taxa; with three types of trichome bases are recognised: simple, stellate and hooked hairs. Only taxa with simple trichomes are combined with tuberculate bases on both adaxial and abaxial leaf surfaces. Stomata features such as shape and size, anticlinal and periclinal cell walls as well as trichome bases are considered important characters, and thus, leaf cuticular characteristics are helpful in identifying *Hibbertia* species, but of limited value phylogenetically.

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App. 2 Characterization of leaf cuticles of the SE Aust. *Hibbertia* subg. *Hemistemma*

	Adaxial epidermis					Abaxial epidermis					Stomata		
Taxa	AW	Asz (µm)	WT	PW	TB	AW	Asz (µm)	WT	PW	TB	SP	SS	Ssz (µm)
H. acaulothrix	Ro-An	30–50	Th, #	St	++	Ro-An	30–50	#	fpa-St	++	hyp	ell	>20
H. acicularis	Sin	>55	Th	fpa	–	Ro-Un	>55	#	gr	+	hyp	cir	>20
H. apoda	Sin	>55	Th	gr	+	An-Un	>55	#	St	+++	hyp	ell	>20
H. appressa	An-Un	>55	#	St	+	Ro-Un	30–50	#	fpa	++	hyp	ell	>20
H. aspera subsp. *aspera*	An-Un	>55	Th	An	++	Ro-An	>55	#	fpa	++	hyp	cir	>20
H. aspera subsp. *pilosifolia*	An-Un	>55	Th	gr	++	Un	30–50	#	gr	+++	hyp	ell	10–20
H. australis	Sin	>55	#	gr	++	Un	>55	Th	gr	++	hyp	ell, cir	10–20
H. boweniensis	Un-Sin	>55	Th	St	–	Ro-Un, Sin	>55	#	fpa-St	+	hyp	ell	10–>20
H. bracteata	Sin	>55	#	gr	+	Sin	>55	#	gr	+	hyp	ell, cir	10–20, >20
H. caespitulosa	Sin	>55	#	gr-St	–	Ro-An	30–50	#	gr-St	++	hyp	ell	>20
H. calycina	Un	>55	#	gr-St	–	Un	30–50	#	fpa	+	hyp	ell	10–20, >20
H. cinerea	Ro-Un	>55	#	gr	+++	Ro-An	30–50	#	gr	++	hyp	ell, cir	10–20, >20
H. cistiflora	Un-Sin	>55	Th	St	+	An-Un	>55	Th	gr-St	+	hyp	ell	>20
H. cistoidea	Ro-An	30–50	#	St	++	Ro-Un	30–50	#	St	+++	hyp	ell	>20
H. corifolia	Un-Sin	>55	#	fpa-St	++	Un	>55	#	St	+++	hyp	ell	>20
H. covenyana	Ro-Un	30–50	Th	gr-St	++	Ro-Un	30–50	#	St	+++	hyp	cir	>20

H. crinita	Ro-An	30–50	Th	fpa	+++	Ro-An	30–50	Th	Sm, fpa	+++	hyp	cir	10–20
H. cuneifolia	Un-Sin	>55	Th	gr-St	–	Ro-An	30–50	#	fpa-St	+++	hyp	cir	>20
H. decumbens	Ro-An	30–50	Th	gr-Pa	++	Ro-Un	30–50	Th	fpa-St	+	hyp	ell, cir	10–20
H. devitata	Sin	>55	#	gr	++	An-Un	30–50	Th	gr	++	hyp	ell	>20
H. dispar	Un-Sin	>55	#	St	+	Ro-Un	>55	#	fpa-St	+++	hyp	ell, cir	>20
H. empetrifolia subsp. *empetrifolia*	An-Un	>55	Th	gr	++	Ro-An	>55	#	gr	++	hyp	ell	>20
H. empetrifolia subsp. *radians*	Sin	>55	Th	gr	+	Un	30–50	#	gr	++	hyp	ell	10–20
H. empetrifolia subsp. *uncinata*	Sin	30–50	#	Sm	++	Ro	30–50	Th	fpa	+	hyp	ell, cir	>20
H. erectinervis	Un-Sin	>55	Th	Pa-St	++	Ro-An	>55	Th	gr-St	++	hyp	ell, cir	10–20
H. expansa	Sin	>55	#	gr-St	++	Ro-An	>55	Th	St	+++	hyp	ell	>20
H. exutiacies	Un-Sin	>55	#	gr	+	An-Un	30–50	#	gr	–	hyp	ell	>20
H. florida subsp. *angustinervis*	Sin	>55	Th, #	St	–	Un	>55	Th	St	++	hyp	cir	>20
H. florida subsp. *florida*	Un-Sin	>55	Th	gr-St	++	Un	>55	#	gr-St	+++	hyp	cir	>20
H. fruticosa	Sin	>55	#	St	+	An-Un	>55	Th	St	–	hyp	cir	10–20
H. glaciifera	Ro-An	30–50	Th,	gr	+	An-Un	>55	#	Sm, St	++	hyp	?	?
H. glebosa subsp. *glebosa*	Un	>55	Th	fpa	++	Un	30–50	#	fpa	+++	hyp	ell, cir	10–20
H. glebosa subsp. *oblongata*	Un-Sin	>55	Th	fpa-St	+	Un	>55	Th	St	+	hyp	cir	>20
H. hendersonii	Ro-Un, Sin	30–50	Th	gr	+++	Un	30–50	Th	St	+++	hyp	ell, cir	>20
H. hermaniifolia subsp.	Ro-An	30–50	#	gr	+, ++	Ro-Un	30–50	#	St	++,	hyp	cir	>20

hermaniifolia										+++			
H. hermaniifolia subsp. *recondite*	Ro-An	30–50	#	gr-St	++	Ro-Un	30–50	#	St	+++	hyp	cir	>20
H. hexandra	Ro-An	30–50	#	gr-St	++	Ro	30–50	#	St	+++	hyp	ell	>20
H. hirsuta	Ro-Un, Sin	30–50	#	gr	++	Un	30–50	#	Sm-fPa, St	+++	hyp	cir	>20
H. hirta	Ro-Un	30–50	#	Sm-fPa	++, +++	Ro-Un	30–50	Th	gr	++	hyp	cir	10–20
H. hirticalyx	Ro-An	>55	Th	fPa	++	Ro-An	30–50	Th	fPa	+	hyp	cir	>20
H. horrida	Un-Sin	>55	#	gr	–, +	Ro-Un	30–50	#	St	+++	hyp	ell, cir	10–20
H. humifusa subsp. *debilis*	Un-Sin	>55	Th	gr	+	Ro-Un	30–50	#	gr	+++	hyp	cir	<10
H. humifusa subsp. *erigens*	Un-Sin	>55	Th	gr	+	Un	30–50	#	gr	++	hyp	cir	>20
H. humifusa subsp. *humifusa*	Un-Sin	>55	Th	gr	+	Ro-Un	30–50	#	fPa	++	hyp	ell	>20
H. hypsophylloides	Ro-Un, Sin	>55	Th	gr	++	Ro-An	>55	#	St	++	hyp	ell, cir	>20
H. nitida	Sin	>55	Th	gr	–	Un-Sin	30–50	#	gr	–	hyp	ell, cir	<10, >20
H. notabilis	Un-Sin	>55	#	gr	+	Ro	30–50	#	Sm-gr	+++	hyp	cir	>20
H. obtusibracteata	An-Un	>55	Th	St	–	An-Un	>55	Th	gr	–	hyp	cir	>20
H. oligastrota	Un-Sin	>55	Th	gr-St	+	Un-Sin	30–50	Th	gr-Pa	+++	hyp	cir	>20
H. oxycraspedotus	Un-Sin	>55	Th	gr-St	+	An-Un	>55	Th, #	fPa	+	hyp	ell, cir	>20
H. paeninsularis	Sin	>55	Th	gr	+	Ro-An	>55	#	gr	+	hyp	cir	10–20
H. pallidiflora	An-Un	>55	#	gr	++	Un	30–50	#	gr	+	hyp	ell	10–20

H. patens	Un-Sin	30–50	Th	fPa		Ro-An	30–50	#	fPa	++	hyp	ell, cir	>20
H. pedunculata	Sin	>55	Th	fPa	+	An-Sm	30–50	#	fPa	++	hyp	cir	>20
H. pilifera	Un-Sin	>55	#	St	–	An-Un	>55	#	St	–	hyp	ell	10–20
H. platyphylla subsp. *halmaturina*	An-Un	>55	Th	Sm-gr	++	Un	>55	#	gr	+++	hyp	ell	<10
H. platyphylla subsp. *major*	An-Un	>55	Th	gr	++	Un	>55	#	gr	+++	hyp	cir	>20
H. platyphylla subsp. *platyphylla*	Un	>55	Th	gr	+	Un	>55	Th	gr	++	hyp	ell	>20
H. porcata	Sin-Sm	>55	#	gr	++	An-Un	30–50	#	St	++	hyp	ell	>20
H. praemorsa	An-Un	>55	Th	gr	++	Ro-An	30–50	#	An-St	+++	hyp	ell, cir	>20
H. protuberans	Sin	>55	Th	gr-fPa, St	–	Un	>55	Th	St	–	hyp	ell, cit	>20
H. pusticulata	Un-Sin	>55	Th	gr	+	An-Un	>55	#	gr-fPa	+	hyp	ell	>20
H. reticulata	Ro-An	30–50	Th	gr-St	++	Ro-An	30–50	#	St	+++	hyp	ell, cir	10–20
H. rhynchocalyx	Ro-An	30–50	#	gr	++	Ro	30–50	#	gr	+++	hyp	ell	>20
H. riparia	Un-Sin	>55	#	gr	+	Ro-An	>55	#	gr	++	hyp	cir	>20
H. rufa	Sin	>55	Th	St	–	Ro	30–50	#	St	++	hyp	cir	>20
H. semota	Ro-An	30–50	Th	gr	++	Ro-An	30–50	#	St	+++	hyp	ell, cir	>20
H. sericea var. *densiflora*	An-Un	30–50	Th	gr-St	+	Ro-Un	30–50	#	fPa-St	+++	hyp	ell	>20
H. sericea var. *scabrifolia*	Un	30–50	Th	gr	++	Ro-An	30–50	#	fPa	+++	hyp	ell, cir	10–20
H. sericea var. *sericea*	Ro-Un	30–50	Th	gr	+++	Un	30–50	#	fPa	+++	hyp	ell	10–20
H. serpyllifolia	Sin	>55	#	gr-St	+	An-Un	>55	Th	gr	–	hyp	cir	>20

H. sessiliflora	Un-Sin	>55	Th	gr	+	Ro-Un	>55	#	gr	+++	hyp	ell	>20
H. setifera	Sin	>55	Th	gr	++	Un	>55	#	gr	+++	hyp	ell	>20
H. simulans	Un-Sin	>55	#	gr	+	gr	30–50	#	gr	++	hyp	ell, cir	>20
H. spathulata	Ro-An	30–50	#	St	+++	Ro-An	30–50	#	St	+++	hyp	ell	>20
H. stricta	Un	>55	Th	gr-St	++	Ro-Un	30–50	#	gr	+++	hyp	ell	>20
H. strigosa	Un-Sin	>55	Th	gr-fPa	+, ++	An-Un	>55	#	gr-fPa	+	hyp	ell	>20
H. superans	An-Un	>55	#	gr	++	Un	>55	#	St	+++	hyp	ell	>20
H. surcularis	Un	>55	Th	St	–	Ro-Un	30–50	#	St	++	hyp	cir	>20
H. synandra	Un-Sin	>55	Th	St	++	An-Un	>55	#	St	+	hyp	cir	>20
H. tenuis	Sin	>55	Th	gr	++	An-Un	>55	#	gr	++	hyp	cir	10–20
H. tetranthera	Un	>55	#	St	–	An-Un	>55	#	St	–	hyp	ell, cir	10–20
H. truncata	An-Un	>55	Th	gr	++	Un	30–50	#	gr	+++	hyp	ell	10–20
H. vestita	Un-Sin	>55	Th	gr	++	An-Un	>55	#	St	++	hyp	cir	>20
H. villifera	Sin	>55	#	gr	+	Ro-An	30–50	#	Sm	+++	hyp	ell	>20

Heading: AW, anticlinal wall shape; Asz, anticlinal wall size; WT, anticlinal wall thickness; PW, periclinal wall shape; SP, Stomata position; SS, stomata shape; Ssz, stomata size; TB, trichome base. Characters: absent (–); circled (cir); common (++); angular (an); elliptic (ell); fine papillate (fpa); granular (gr); hypostomatic (hyp); irregular (Irr); rare (+); rounded (Ro); sinuous (Sin); smooth (Sm); striate (St); thick (Th); thin (#); undulate (Un); very common (+++).

Chapter 8

General discussion

Hibbertia Andrews (Guinea flower) is one of 11 genera within the Dilleniaceae: *Acrotrema* Jac, *Curatella* Loefl., *Davilla* Vand., *Didesmandra* Stapf., *Dillenia* L., *Doliocarpus* Rol., *Hibbertia* Andrews, *Pachynema* R.Br.ex DC., *Pinzona* Mart.& Zucc., *Schumacheria* Vahl and *Tetracera* (Horn 2005, 2007). *Hibbertia* have four subgenera based on indumentum (vestiture) and floral features: *Hibbertia*, *Adrastaea* (R.Br. ex DC.) J.W.Horn, *Hemistemma* (Thouars) J.W.Horn and *Pachynema* (R.Br. ex DC.) J.W.Horn (Horn 2005, 2007). Subgenus *Hemistemma* consists of ~160 species, many endemic SE Australia; however, the 16 informal proposed groups by Toelken (unpubl.) are not described, and the phylogenetic relationships are poorly understood; thus the present study aimed to do a detailed phylogenetic study based on morphological and molecular data sets.

A detailed systematic study based on morphological and molecular data was the aim of this study. Although *Hibbertia* shows high diversity of morphological features, particularly of the indumentum (simple, hooked and/or stellate hairs) (Toelken 1995, 1998, 2000, 2010) and floral characteristics (Gilg and Werdermann 1925; Hoogland 1952; Endress and Igersheim 1997; Tucker and Bernhardt 2000), the phylogenetic relationships have not been examined and most taxa have not been explored using cpDNA.

A comprehensive morphological phylogenetic construction of *Hibbertia* subg. *Hemistemma* was conducted for ~93 taxa in present study to recognise any major lineage within the subgenus, determine any intra-specific variations, taxon boundaries and explore character evolution.

The morphological phylogenetic analyses produced seven distinct species clades which are poorly supported, and most defined by 1) indumentum-vegetative characters: hair type, hair density, presence of hairs between stamens and petals, presence and/or absence of cilia in outer sepal margin and seed dimension; 2) floral features such as: anther length, anther dehiscence, filament fusion, style base and attachment to ovary (see Ch. 2). Despite a very high number of homoplasious character states, as revealed by the morphological and combined analyses, several strongly supported groups are recognised such as *H. humifusa* subsp. *erigens* + *H. humifusa* subsp. *debilis* (99% JK), *H. platyphylla* subsp. *platyphylla* + *H. platyphylla* subsp. *major* (92% JK) and *H. praemorsa* + *H. sericea* var. *sericea* (90% JK). Another comparison between Toelken's informal species groups and the analysis results shows that none of the groups are supported.

We sequenced nuclear rDNA (ITS) and intergenic spacer regions of cpDNA (*trn*L–F) for most taxa (Chapter 3); however, amplification of the ITS region for *H. bracteata, H. corifolia, H. hendersonii, H. florida* subsp. *florida, H. obtusibracteata, H. reticulata, H. simulans, H. sericea* var. *densiflora, H. stricta, H. woronarana* and *H. sessiliflora* was not possible. The molecular analysis produced seven clades, and two subclades (G1 and G2). Although the clades have low support, strong supports (90%–100% JK) for several small assemblages of taxa were found. Most infrataxa have not placed together as monophyletic species.

Combined analysis of the nuclear ITS gene regions and cpDNA (*trn*L–F intergenic spacer) with the morphological analysis (Vegetative-indumentum, floral and anatomical data) (Ch. 4) was also undertaken. The combined analysis produced eight clades; the major clades A, B and C, with five clades in a terminal polytomy D, E, F, G and H, each defined by several morphological characters (Ch. 4). The characters glabrous stem, oblong-lanceolate leaf blade shape, elliptic bract, ≥15.1 mm petal length, absent of inner sepal, >15 mm leaf blade width, entire leaf margin, absent of petiole, ≥40 stamen number, 2.1–2.5 mm seed dimension and 3.1–4 abaxial epidermal cell length to width ratio are the most reliable synapomorphies (Ch. 4: Tab. 6). None of Toelken's informal proposed species groups is supported.

From morphological analysis based on vegetative and indumentum features (Ch. 5), *H. riparia* resolved as a sister to (*H. tenuis* + *H. australis*) endemic South Australia; *H. humifusa* subsp. *humifusa* placed as a sister to (*H. humifusa* subsp. *debilis* + *H. humifusa* subsp. *erigens*) endemic Victoria. Although 45 vegetative and surface indumentum characters were included in the combined analysis, the vegetative and indumentum characters did not reflect the phylogenetic relationships among *Hibbertia* subg. *Hemistemma* (Ch. 5: App. 1 and 2).

After mapping floral characters onto the combined resulted tree (Ch. 6), and found high variations between the species specifically in: stamen number, stamen arrangement (in clusters), anther dehiscence, filament fusion, ovary shape, style shape and position (see Ch. 6: App. 1 and 2). Sepal characteristics are the ancestral (in 2 whorls) excluding *H. exutiacies* which characterised by one sepal whorls; stamen number are varied from 2 in *H. hirsuta* to 27– 30 in *H. vestita,* five types of dehiscence: 1) lateral slits (most studied taxa), 2) terminal pores and lateral slits (13 taxa, 3) introrse slits only in *H. rhynchocalyx* from (Gibraltar Rang National Park) NSW and *H. hirta* from (Budawang Rang) NSW, 4) terminal pores only recorded in one taxa *H. hermaniifolia* subsp. *recondita* from (Gippsland Collins road) VIC, 5) terminal pores and introrse slits only in two species *H. cinerea* from (Southern Eyre Peninsula) SA and *H. praemorsa* (Clade D) from (Morton National Park) NSW. The floral characters confirmed that *H. glebosa* subsp. *glebosa* from SA (Mt Lofty) has semi-lateral style attachment with recurved base while *H. glebosa* subsp. *oblongata* (=*H. oblonga*) from SA (Kangaroo Island) has lateral style attachment and lacks a recurved base.

Anatomical investigation provided significant taxonomic information (Ch. 7). After mapping leaf cuticular characters onto the combined analysis tree, variations among the studied taxa are noticed based on leaf cuticular characters such as 1) trichome base, and 2) stomata shape and length. Some characters are considered homoplasious, especially: 1) anticlinal and periclinal epidermal cell walls shape and surface outline, 2) cell wall thickness.

Leaf cuticles of the studied taxa confirmed that all species have anomocytic stomata (Dickison 1970; Horn 2005). Sunken stomata are not common, but found only in 8 taxa: *H. empetrifolia* subsp. *empetrifolia, H. cinerea, H. hirta, H. notabilis, H. rhynchocalyx, H. humifusa* subsp. *debilis, H. humifusa* subsp. *erigens* and *H. acicularis*.

Anatomically, leaf cuticles of the SE Aust. clade of *Hibbertia* subg. *Hemistemma* display three main trichome bases: 1) rare, 2) common, and 3) very common. These bases are for simple, stellate and hooked hairs. As shown in Ch. 7: App. 1, 2–7 trichome bases/400× on (AB) surfaces is shared among: *H. tenuis, H. australis, H. empetrifolia* subsp. *radians, H. devitata, H. cinerea, H. pallidiflora, H. truncata, H. aspera* subsp. *pilosifolia, H. hirticalyx, H. aspera* subsp. *aspera, H. appressa, H. empetrifolia* subsp. *empetrifolia, H. empetrifolia* subsp. *uncinata, H. decumbens, H. notabilis, H. hirta, H. rhynchocalyx, H. cistoidea* and *H. glaciifera*.

It is notable that for the morphological results (Ch. 2), the two subspecies *H. glebosa* subsp. *glebosa* and *H. glebosa* subsp. *oblongata* are not resolved as monophyletic, instead, the first subspecies nested close to *H. stricta* within Clade G, meanwhile the second one placed as sister to Clade F, same as *H. empetrifolia* and *H. aspera* subspecies. The combined molecular and morphological analysis (Ch. 4: Fig. 2–6) placed all *Aspera* group species within five clades (A, D, E, G and H), and *Sericea* group species within two clades (C and H), suggesting that surface indumentum (simple, hooked and stellate) hairs are important in identifying their lineages (Ch. 4: Tab. 5). The most difficult species in this study are the undescribed taxa that have no documents or descriptions on it, and this made the study more challenging and exciting (see Ch. 2: Tab. 1).

Limitations of the study and future work

Although this study shows that there are useful taxonomica chracters to define species and phylogenetic relationships within the SE Ausatralian Clade of *Hibbertia* subg. *Hemistemma*, there are also a number of issues which it raises. The main problems in this study are related to the very high levels of character homoplasy seen particularly in hairs types, density, length and branching, as well as the high levels of diversification seen in androecium and gynoecium characters, often within species, suggesting that further species-level studies are required for many taxa as currently defined.

For the first problem, hair type, length and branches are useful characters and used to identify different studied species as well as for lineages (see Ch. 5), but with dense trichomes, it was difficult to examine leaf surfaces, particularly leaf cuticular surfaces under the compound microscope for measuring epidermal cell dimensions, and also to investigate in detail leaf cuticular surfaces such as *H. sericea* subspecies, and this was clearly happened when SEM images produced. The second problem is the variation in stamen number and dehiscence, when examine one specimen with particular number of stamen, other specimens for the same taxa revealed different stamen numbers and sometimes even anther dehiscence type, strongly suggesting that taxon boundaries are still unresolved on some cases. This also has implications for the specimens used in the molecular analyses, particularly in the light of most already formally described infrataxa not being places into monophyletic lineages in our study.

Futher study is therefore needed focusing on the south-eastern Australian species of subg. *Hemistemma*, but including more individual sand populations from different locations using phenetic and/or population genetic analyses in order to better resolve infra-specific variation and species limits. This will then allow more refined phylogenetic analyses for the study of character evolution and determination informative morphological features to be undertaken.

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