CHAPTER IV

CHARACTER EVOLUTION IN THE GENUS *GONIOTHALAMUS*

4.1 INTRODUCTION

Evolution is a difficult phenomenon to study. It is rarely fast enough to be observed directly and only in exceptional cases is it possible to find physical evidence, such as fossils or ancient DNA, of past states and events. Fortunately, evolution leaves its footprint in the distribution of traits among living organisms. By studying this footprint, we can infer how organisms originated through the successive splitting of ancestral lineages, a process depicted in phylogenetic trees. Given a phylogenetic tree, the evolutionary history of individual traits of interest can be reconstructed. The evolution of characters among taxa can be studied both to infer history and interpret processes of change.

Saunders (2002) proposed that there are many characters of the flowers that are taxonomically important in *Goniothalamus*, i.e. size, shape, color and indument. The relative size of the outer and inner petals is often used as a taxonomic character at the generic level. In addition, the staminal connective shape is diagnostically important at the species level. In interspecific classification, the structure and indument of the carpel are also taxonomically important. Besides the structure of the seeds, i.e. size, shape, indument and color as well as the presence of exotestal hairs, with differences in length, color and density are also particularly important in infrageneric classification.

Scharaschkin and Doyle (2006) studied character evolution in *Anaxagorea* using a combined morphological and molecular phylogenetic analysis. A high level of homoplasy in stamen and leaf venation characters was observed. The distributions of characters on the tree confirm assumptions that several distinctive similarities between *Anaxagorea* and other Magnoliales are primitive retentions. A number of morphological synapomorphies were identified for a clade containing most Central American species and another comprising all Asian species.

The objective of this study is to assess the evolution of selected morphological characters using the phylogenetic reconstruction generated from combination of *trnL-F* and ITS data set as a framework. The combined molecular tree from Figure 3.3 in Chapter III was the most parsimonious tree with moderate to high bootstrap support. Therefore this study will use original tree without collapsing branches to avoid polytomy condition.

4.2 MATERIALS AND METHODS

4.2.1 Phylogenetic framework

The phylogenetic tree derived from combined ITS-*trnL-F* data (this study; Chapter III) is employed. Due to the limited number of taxa in this tree, the taxa sampled for evaluating morphological evolution will also be restricted. Only 23 species of *Goniothalamus* were included in this study (Table 3.1). *Annona squamosa* was selected as the outgroup based on molecular phylogenetic analyses in this study (Chapter III).

4.2.2 Morphological characters

A total of 43 characters (1 habit, 1 shoot, 3 leaf, 26 flower and 12 fruit and seed traits) were investigated. The list of morphological characters and the assigned character states is given in Appendix B. The character states for each taxon were scored from herbarium and descriptions from various journal articles and flora accounts (Sinclair, 1955; Keßler, 1996; Saunders, 2002, 2003, unpublished data).

4.2.3 Character evolution

Evaluation of morphological character evolution was performed in MacClade version 3.08 (Maddison and Maddison, 2001). Tracing of discrete-valued characters indicated nodes fixed in traced character, with unordered type and equal weight. The root was placed at the *Anona squamosa* clade.

4.3 RESULTS AND DISCUSSION

Although definitions of characters and their states are presented in the Appendix C, most of the practical and conceptual problems encountered and the arguments used in defining analysis of morphological characters were treated in this section because we viewed our analysis of morphological characters as one of the main results of this study.

4.3.1 Habit and vegetative characters

In general, the plesiomorphic character state (small tree habit) dominates in the genus, with only one taxon evolving to the derived state (larger trees) namely *G. giganteus* (character 1, Figure 4.1A). Likewise, most of the members of the genus are not too hairy (plesiomorphic) except in three unrelated lineages. Densely hairy or velutinous primary shoots is partially synapomorphic in the *G. undulatus-G. sawtehii-G. sp.* (sroi) clade (character 2, Figure 4.1B). In contrast, although that nitid adaxial leaf surface is the apomorphic, this state is very homoplastic (character 3, Figure 4.1C). This character serves as partial synapomorphic in the *G. malayanus-G. cheliensis* clade.

Prominence of secondary veins on the adaxial leaf surface is also homoplastic (character 4, Figure 4.2A). The ancestral node is equivocal, and consequently the plesiomorphic and apomorphic character states within the genus could not be ascertained. Nonetheless, it seems that there are three (possibly four) clades in which distinctly prominent secondary veins have been derived from impressed or slightly prominent veins. Also, the distinctly prominent secondary vein on the adaxial leaf surface may be synapomorphic for clade I and II, in which the subclade of one taxon has reversal condition, i.e. *G. tapis*.

The last character in this category, tertiary vein arrangement (character 5, Figure 4.2B), is inconclusive due to the fact that the various taxa with percurrent venation are linked by nodes that equivocal. Reticulate venation appears certain for two clades, namely the *G. tamirensis-G. elegans* clade and clade I, of which one contains two taxa, namely *G. malayanus* and *G. cheliensis*, with reversed condition. However, as outgroup node is equivocal, character state polarity could not be determined.
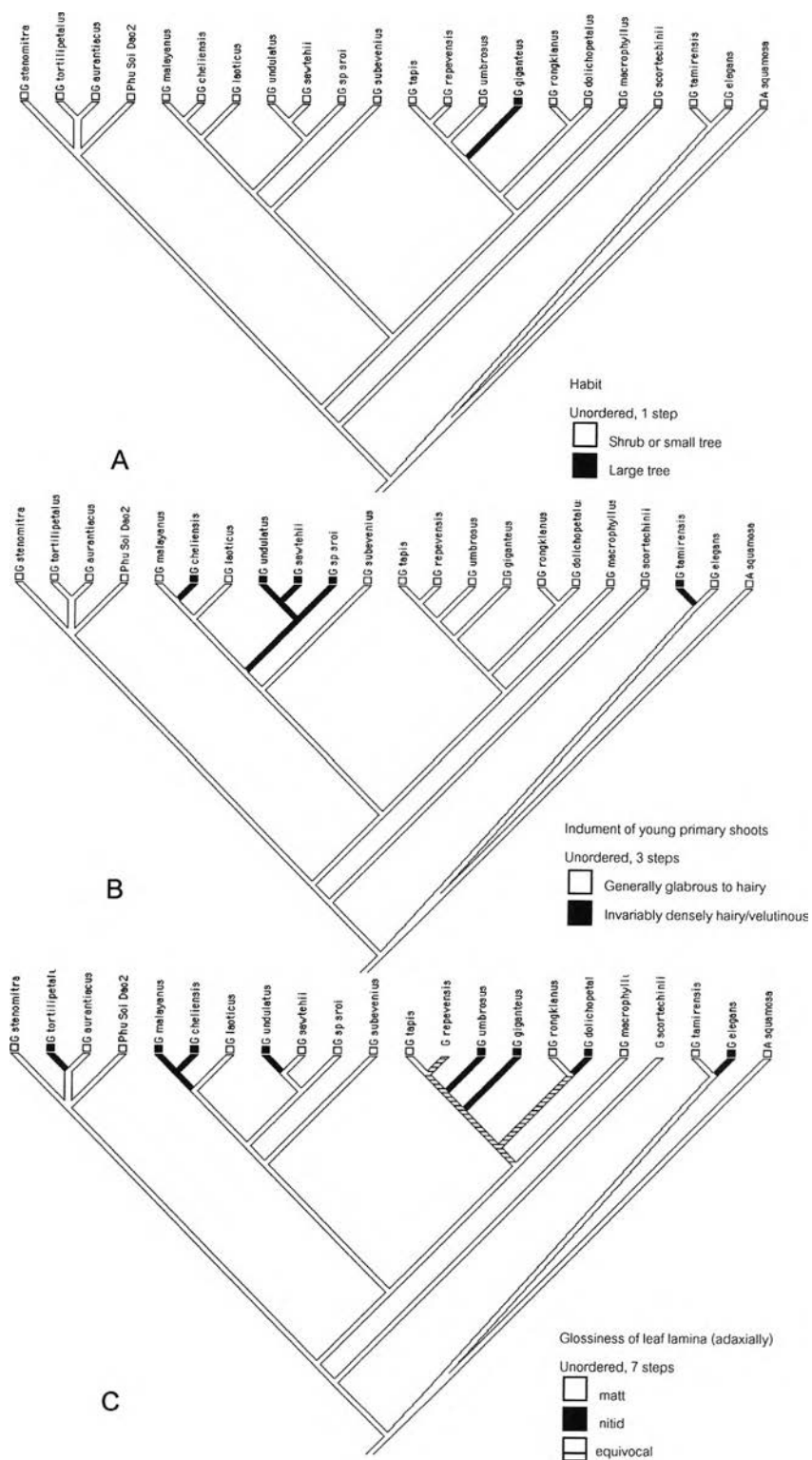
Figure 4.1 Trees showing inferred evolution of habit (A), indument of young primary shoots (B) and glossiness of leaf lamina (adaxially) (C) in *Goniothalamus*.
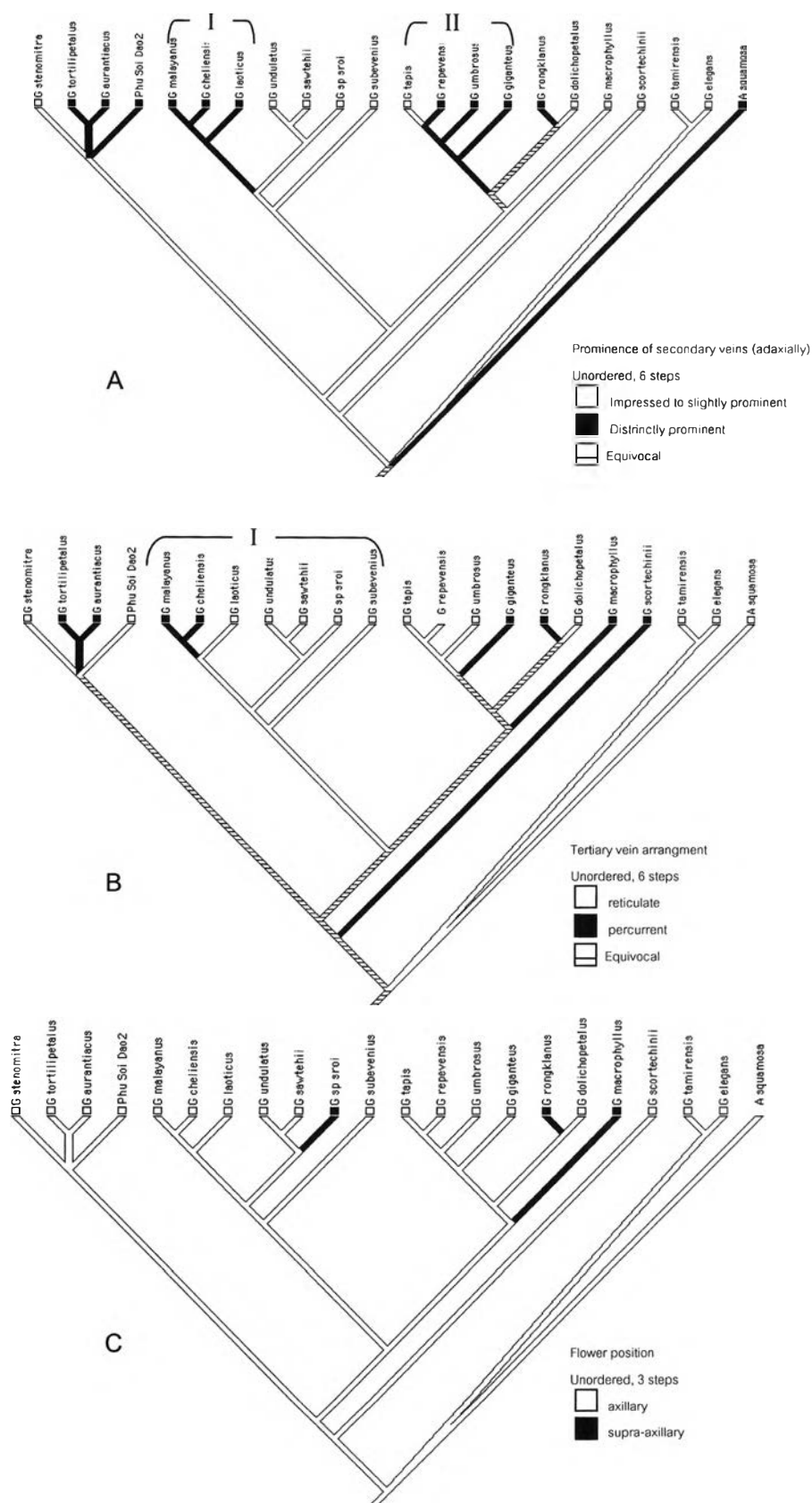
Figure 4.2 Trees showing inferred evolution of prominence of secondary veins (adaxially) (A), tertiary vein arrangement (B) and flower position (C) in *Goniothalamus*.

4.3.2 Flowers

Three lineages evolved to flower at supra-axillary position which is the derived condition (character 6, Figure 4.2C). Our study is supported by Saunders (2002) that the position of flower is initially axillary during development. After that the supra-axillary position was derived due to the primordium of the axillary shoot is situated towards the main axis and becomes separated from the leaf axil resulting from the growth of the internode of the main axis.

In contrast, it is not possible to infer which condition is plesiomorphic in another flower position, flowering on young growth vs. on older growth, and this character is therefore inconclusive (character 8, Figure 4.3A). Likewise, the ancestral state of the pedicel length character is not known, although it appears to be homoplastic, with parallel evolution in three unrelated lineages, namely *G. tortilipetalus*, *G. malayanus* and *G. giganteus* (character 12, Figure 4.3B).

4.3.3 Sepals

The two sepal characters are inconclusive (character 13-14, Figure 4.3C and 4.4A), with the ancestral state unknown in both cases. Basally connate sepals appear to synapomorphic in the *G. stenomitra-G. tortilipetalus-G. aurantiacus*-Phu Soi Dao2 clade, the *G. malayanus-G. cheliensis* clade and clade I. However, it seems that *G. tapis* and *G. giganteus* reversed to have free sepals. On the other hand, sepal venation can not infer the evolutionary polarity because of equivocal nodes. But distinct venation on sepals is likely to be apomorphic.

Many species possess not-reflexed sepals, but a few lineages evolved the derived state of reflexed sepal (character 15, Figure 4.4B). This state might serve as the synapomorphic for the clade I in which the subclade of one taxon has reversal condition, i.e. *G. repevensis*.
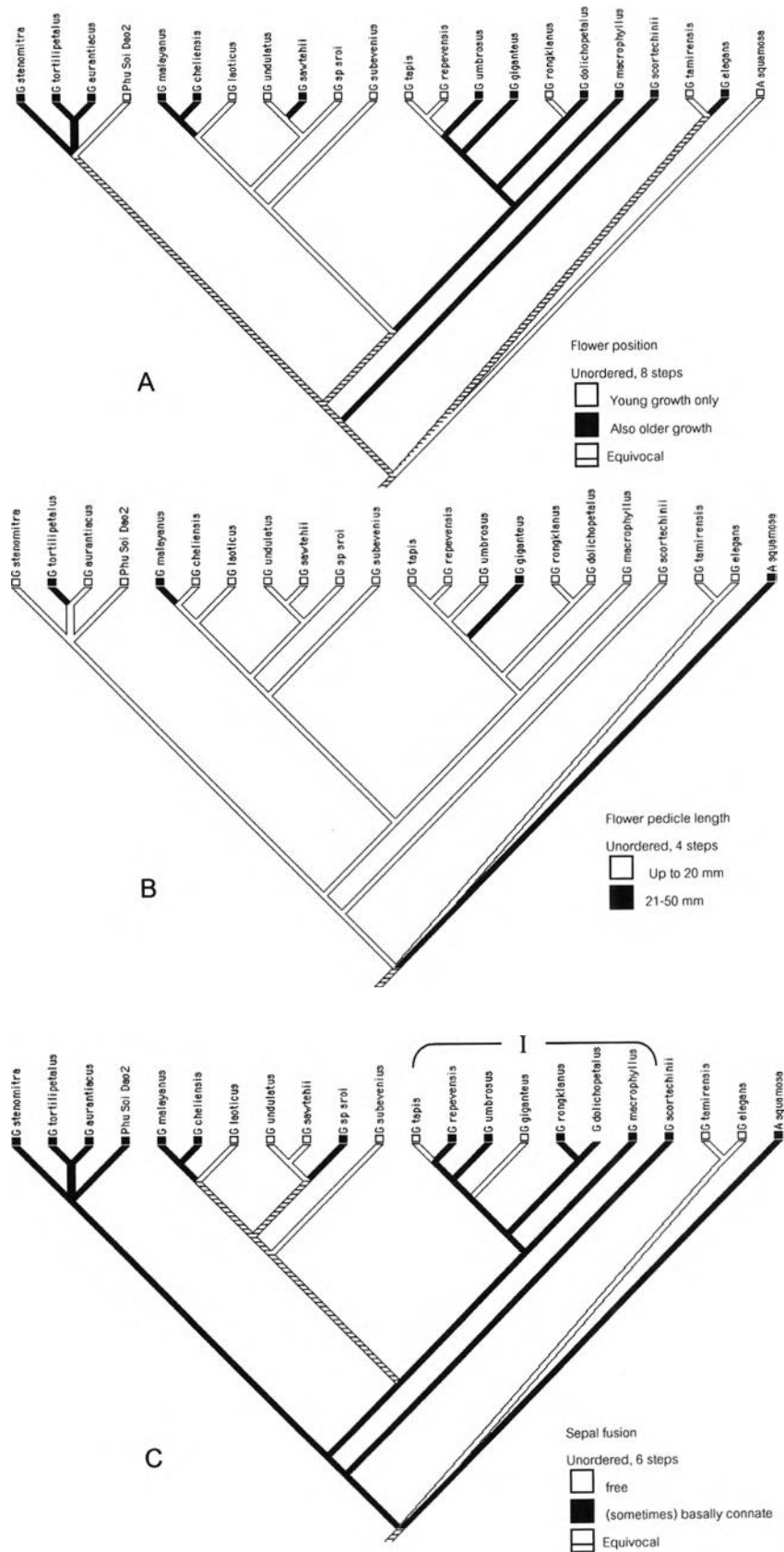
Figure 4.3 Trees showing inferred evolution of flower position (A), flower pedicle length (B) and sepal fusion (C) in *Goniothalamus*.
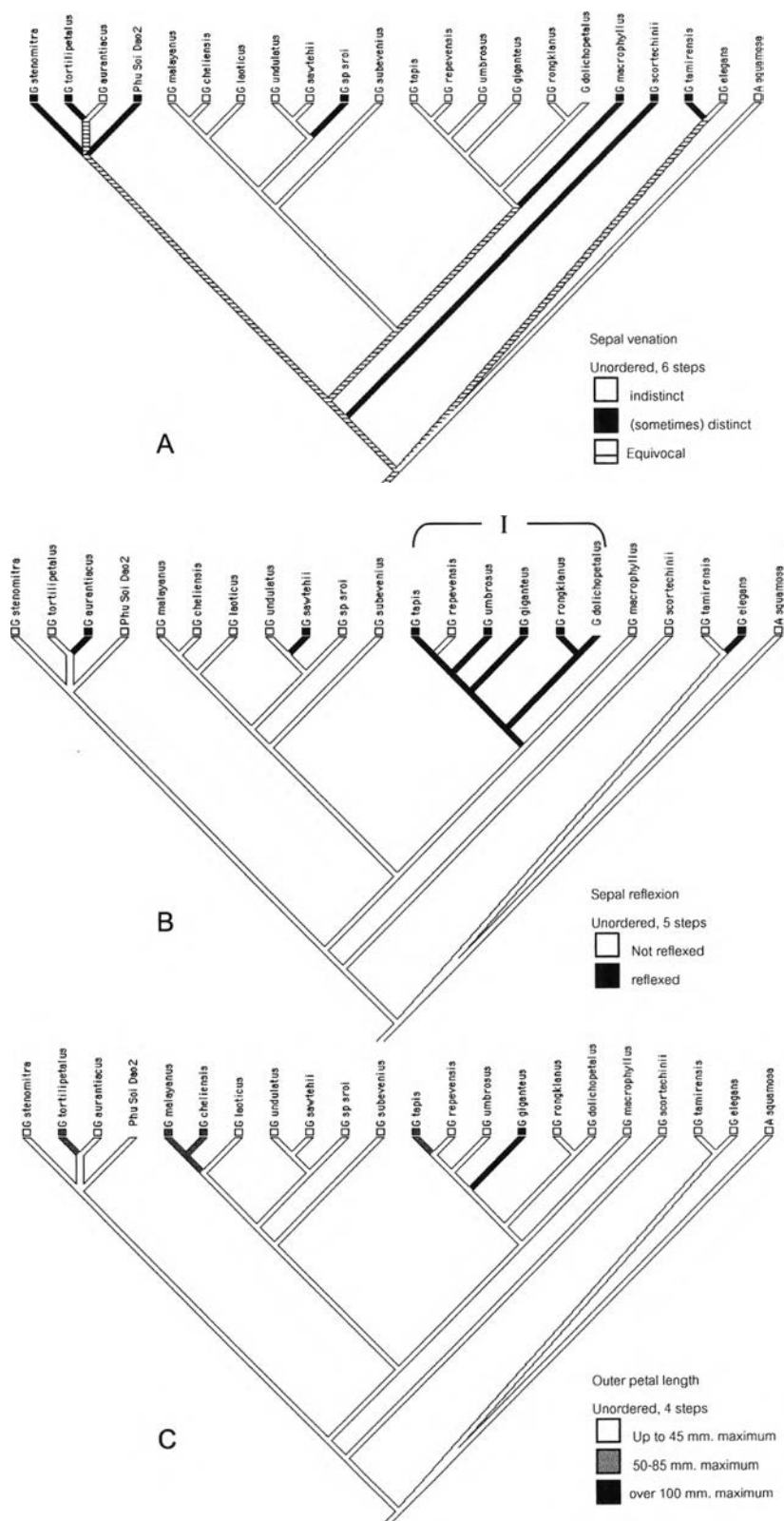
Figure 4.4 Trees showing inferred evolution of sepal venation (A), sepal reflexion (B) and outer petal length (C) in *Goniothalamus*.

4.3.4 Outer and inner petals

Outer petals that have length over 45 mm are a derived state found in four unrelated lineages (character 16, Figure 4.4C), of which *G. tortilipetalus*, *G. tapis* and the *G. malayanus-G. cheliensis* clade have medium size flowers and *G. giganteus* has the largest flowers. Saunders (2002) noted that *G. giganteus* is one of the most attractive species in this genus, with numerous very large yellow and fragrant flowers. Although the outer petals of *G. giganteus* are very large, the inner petals are still relatively small. These petals dictate the size of the mitriform dome and the size of apertures between adjacent inner petals, and hence dictate the size of the pollinator. Corlett (2004) inferred that Asian Annonaceae, having the tiny floral chamber, is pollinated by beetles.

Likewise, the presence of a claw on the outer petals is also homoplastic (character 17, Figure 4.5A) and found in five unrelated lineages. This character is synapomorphic for the *G. malayanus-G. cheliensis-G. laoticus* clade and clade I in which one taxon, *G. tapis*, reversed to have no claw (although it should be noted that the character state for *G. repevensis* is unknown). The flowers of *Goniothalamus* species as sweetly and often intensely fragrant and attract crowds of small beetles, so having clawed petal is suitable for beetles, attracted by a strong scent (Corner, 1988). The outer petal claws make beetles pass outer whorl easily and can enter the floral chamber.

In contrast, many basal or relatively basal clades did maintain plesiomorphic state, i.e. adaxial surface of basal region of outer petal with velutinous hair, but in clade I and II evolved to be less hairy or glabrous (character 18, Figure 4.5B) except few taxa, namely *G. cheliensis* and *Goniothalamus* sp. (sroi) that have the reverse condition.

The shape of inner petal did not change in many species, although the apomorphic condition is observed in six species, *G. stenomitra*, *G. aurantiacus*, *G. cheliensis*, *G. repevensis*, *G. umbrosus* and *G. rongklanus*, whose extensive contiguous area of inner petal are developed (character 19, Figure 4.5C). The *G. undulatus-G. sawtehii-G.* sp. (sroi) clade maintains the plesiomorphic condition.
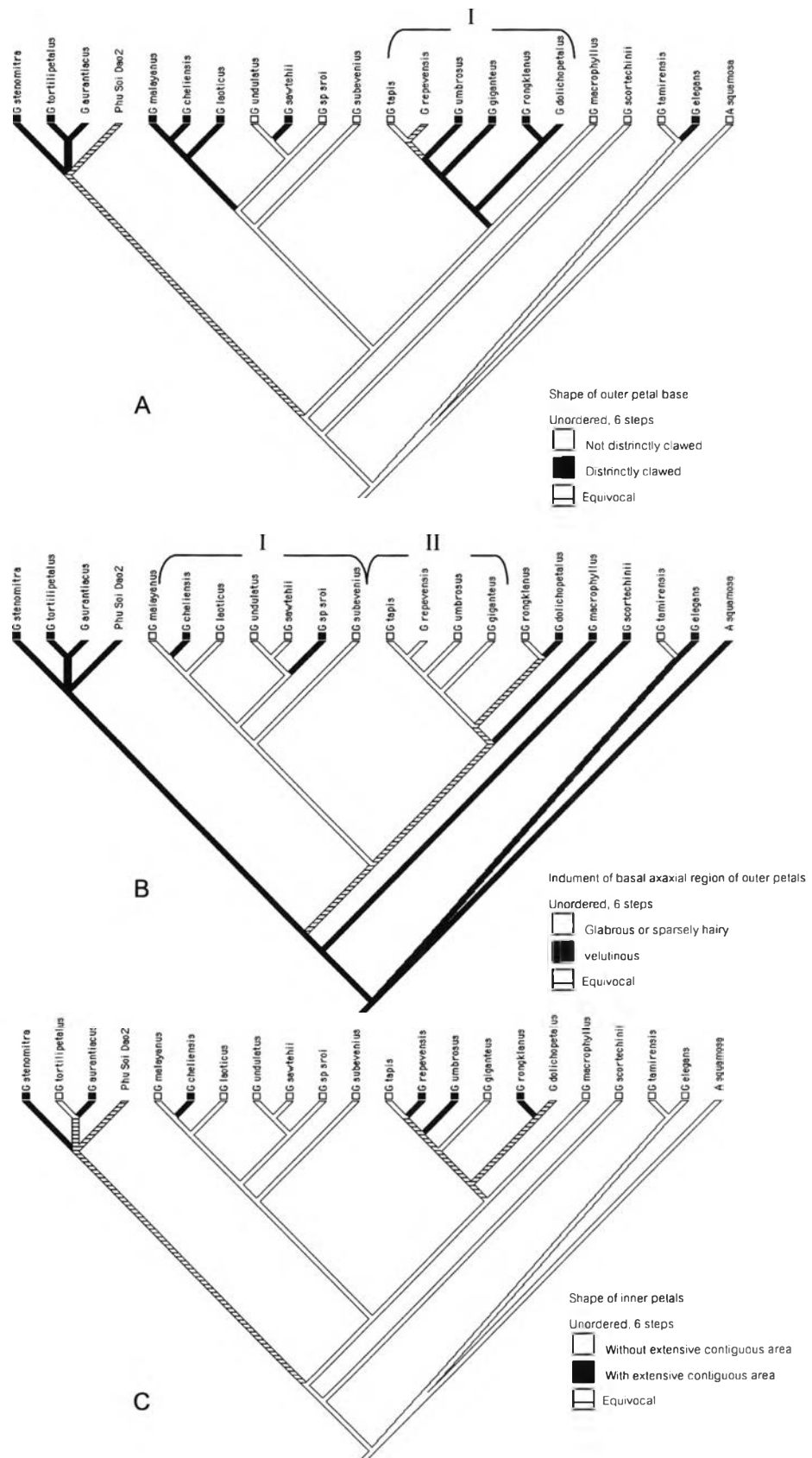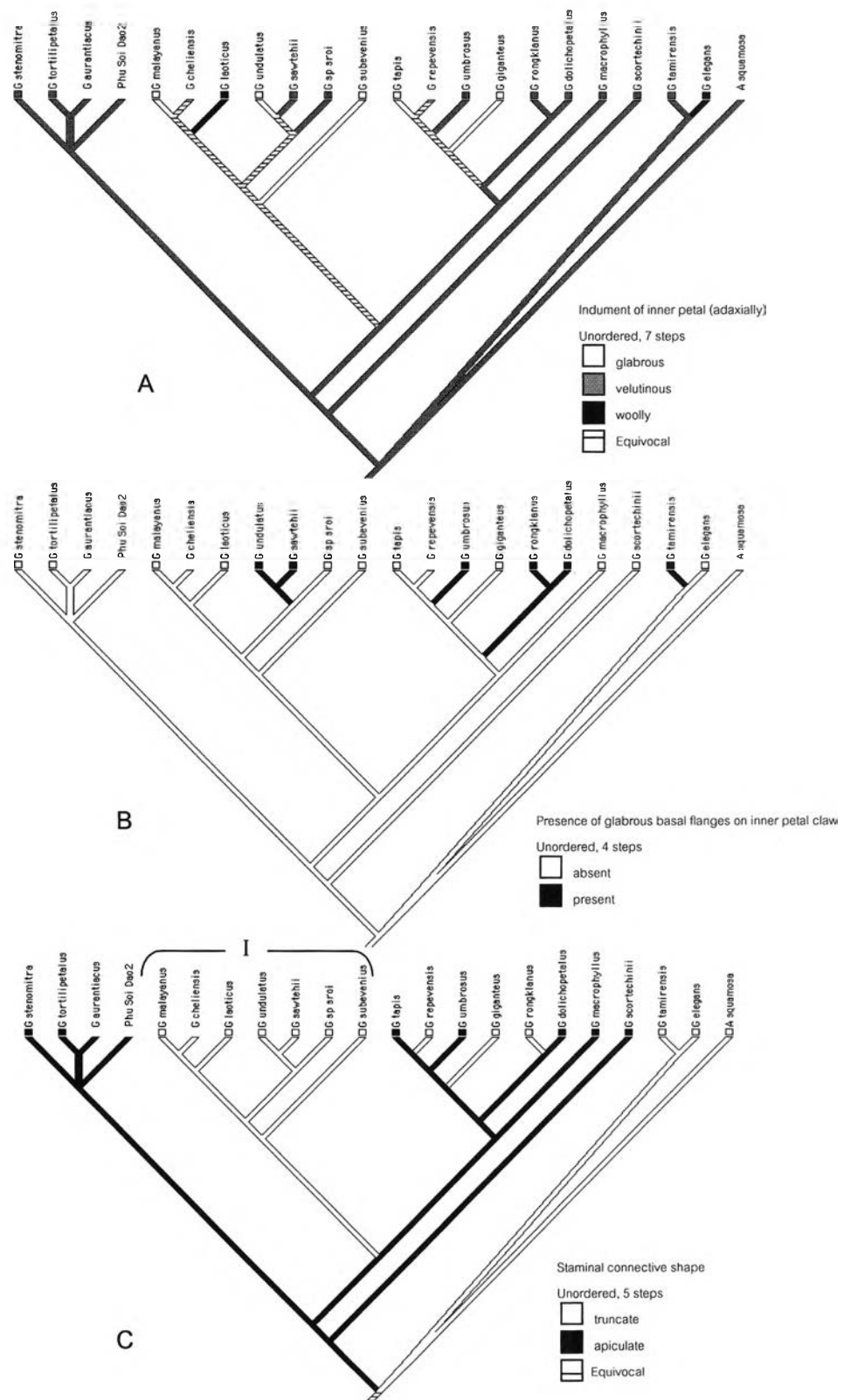
Figure 4.5 Trees showing inferred evolution of shape of outer petal base (A), indument of basal adaxial region of outer petals (B) and shape of inner petals (C) in *Goniothalamus*.

Figure 4.6 Trees showing inferred evolution of indumenta of inner petal (adaxially) (A), presence of glabrous basal flanges on inner petal claw (B) and staminal connective shape (C) in *Goniothalamus*.

It appears that for the indument of the adaxial surface of the inner petal, the velutinous state is plesiomorphic, whereas glabrous and woolly are derived states, which are only observed in a few taxa (character 20, Figure 4.6A). Moreover, this study found that the indument of outer petals and inner petals is closely related. That is to say the basal region of the adaxial surface of the outer whorl of petals generally has the same indument as the adaxial surface of the inner petals (Figure 4.5B and Figure 4.7A).

The absence of basal flanges on inner petal claw is plesiomorphic but many terminal lineages as well as one taxon in the early branching clade are present, possess such flanges (character 21, Figure 4.6B). It seems that presence of basal flanges is synapomorphic for the *G. undulatus-G. sawtehii* clade and the *G. rongklanus-G. dolichopetalus* clade (although it should be noted that the latter clade has only 57% bootstrap support).

4.3.5 Stamens and stamen connectives

Although the ancestral state is equivocal, the majority of the genus had evolved to have the apiculate stamen (character 23, Figure 4.6C) after the departure of early branching clade, the *G. tamirensis-G. elegans* clade. Then this character state in the clade I and other taxa reversed to truncate stamens. My study was supported by Doyle and Le Thomas (1996) inferred that the truncate-peltate state is characteristic of most Annonaceae, which arose in the core group of the family. Moreover, Scharaschkin and Doyle (2006) presented that pointed stamens are ancestral state and truncate stamens are derived state in *Anaxagorea*, the basal grade of Annonaceae. Therefore, truncate stamens are typically stamens in this family.

4.3.6 Carpels and pistils

The ancestral state of ovary indument (character 26, Figure 4.7A) in the genus *Goniothalamus* is equivocal. Many lineages have hairy ovaries, whilst only four lineages have glabrous ovaries, i.e. the *G. tamirensis-G. elegans* clade in early branching clade, the *G. undulatus-G. sawtehii* clade, *G. laoticus*, *G. subevenius*. This character may be
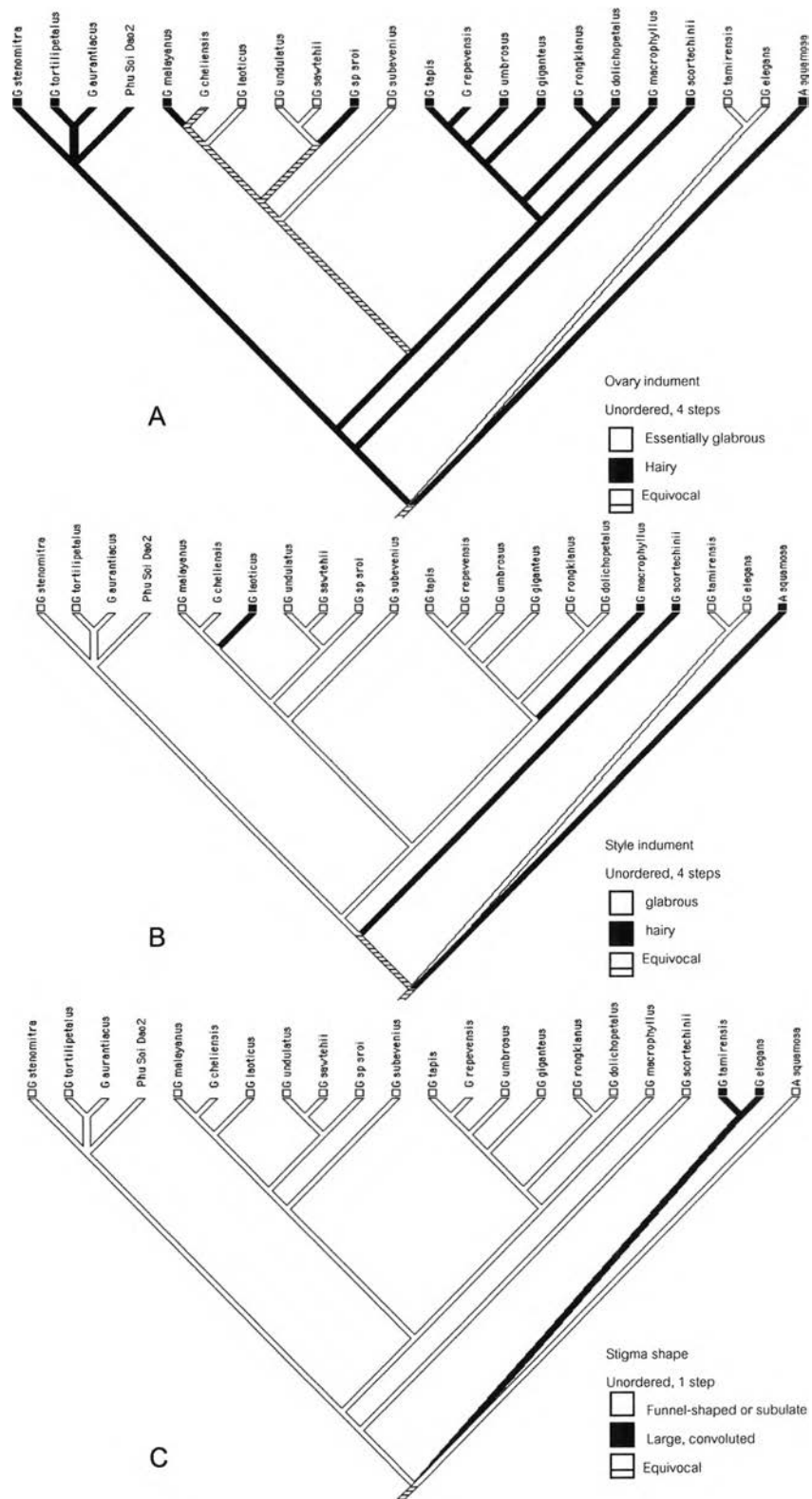
Figure 4.7 Trees showing inferred evolution of ovary indument (A), style indument (B) and stigma shape (C) in *Goniothalamus*.
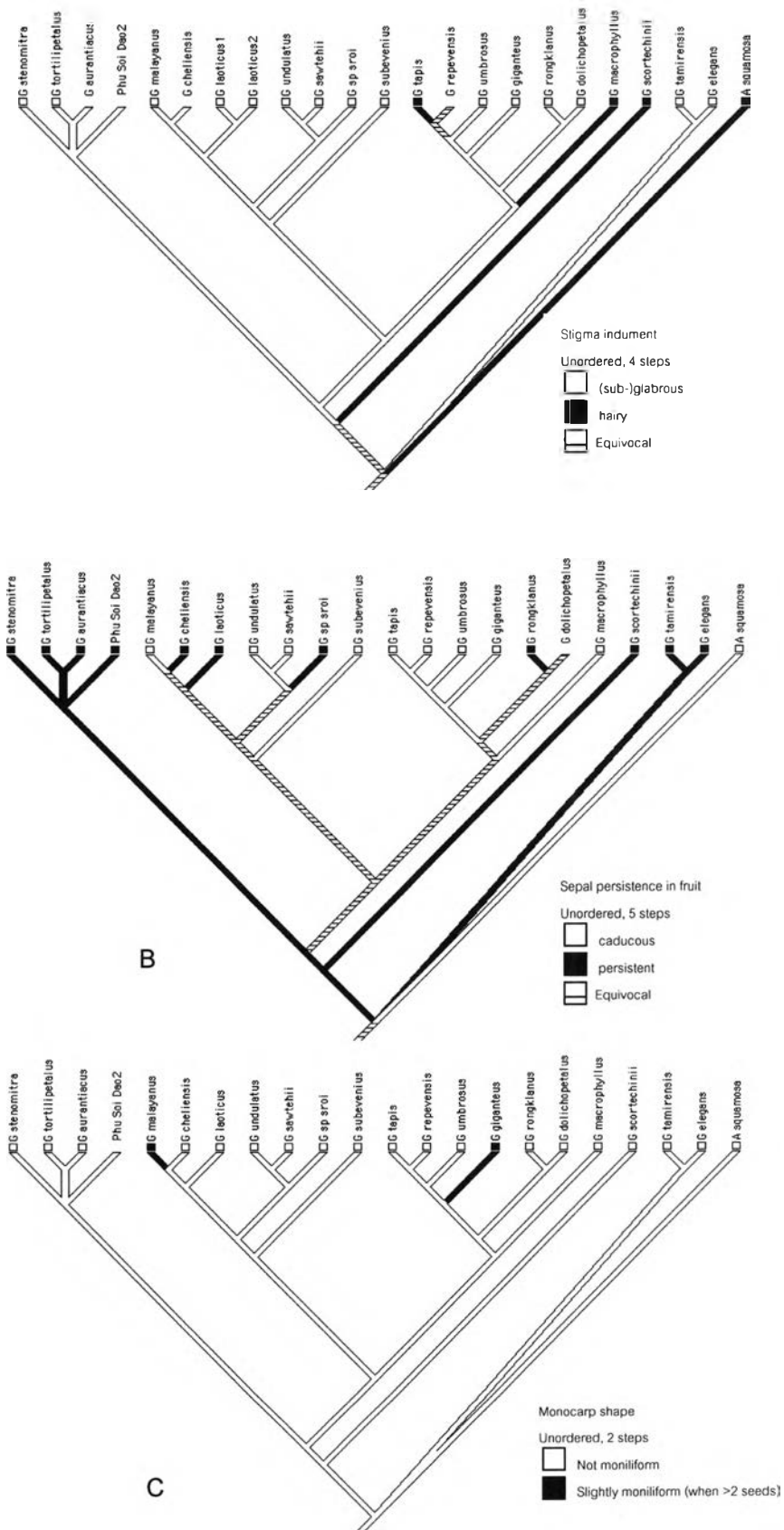
Figure 4.8 Trees showing inferred evolution of stigma indument (A), sepal persistence in fruit (B) and monocarp shape (C) in *Goniothalamus*.

related to the presence of a velutinous indument on the outer petals and inner petals, as discussed above.

The state of style indument in the ancestor is inconclusive (character 27, Figure 4.7B). Yet, this character seems uninformative and homoplastic as three taxa, namely *G. laoticus*, *G. macrophyllus* and *G. scortechinii*, independently evolved more hairy styles.

Stigma shape (character 28, Figure 4.7C) is the only character that appear to be synapomorphic for the *G. tamirensis-G. elegans* clade. In full morphological data set, however, *G. tomentosa* also have this derived state and because of its position, renders this character homoplasious.

Stigma indument (character 29, Figure 4.8A) is homoplastic and ancestral state is equivocal, but it appears that this character is uninformative and homoplastic as three lineages, namely *G. tapis*, *G. macrophyllus* and *G. scortechinii*, independently evolved more hairy styles. Glabrous stigmas are synapomorphic for *G. tamirensis-G. elegans* clade.

4.3.7 Monocarps and seeds

The ancestral state of sepal persistence in fruit (character 30, Figure 4.8B) is equivocal. This character is homoplastic. The persistent sepal in fruit appears in the early branching clades, namely the *G. tamirensis-G. elegans* clade, the *G. stenomitra-G. tortilipetalus-G. aurantiacus*-Phu Soi Dao2 clade and *G. scortechinii*. Then, few lineages had evolved to have fruit with caducous sepal, few taxa reversed to persistent sepal.

Moniliform monocarp shape (character 31, Figure 4.8C) is the derived state and found independently in two clades, the *G. malayanus* and the *G. giganteus* clades.

Also, larger monocarps (character 32, Figure 4.9A) seem to be the derived condition with four (probably five) lineages, *G. aurantiacus*, *G. giganteus*, *G. dolichopetalus*, *G. malayanus* and *G. laoticus*, evolving this condition independently,
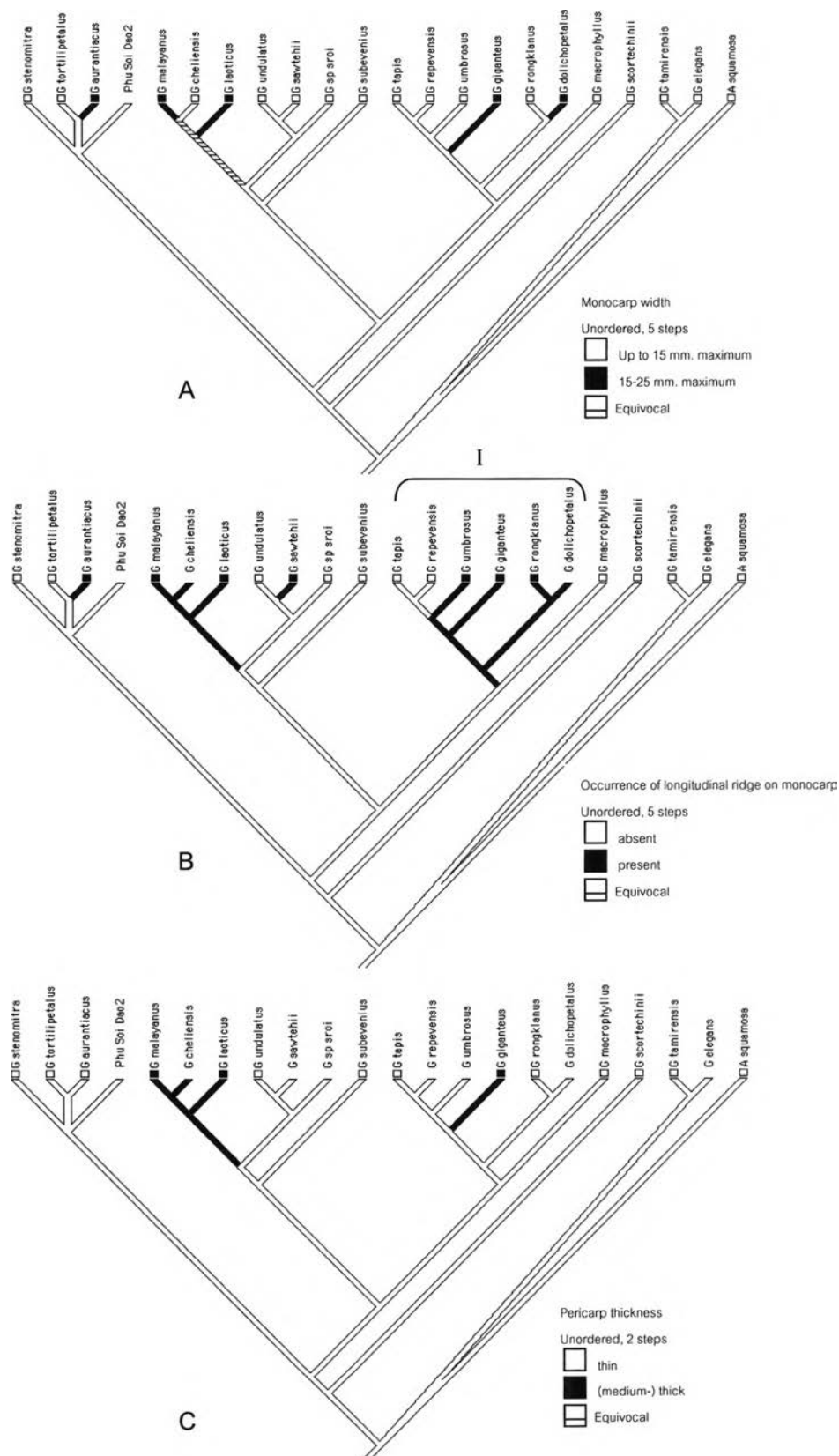
Figure 4.9 Trees showing inferred evolution of monocarp width (A), occurrence of longitudinal ridge on monocarp (B) and pericarp thickness (C) in *Goniothalamus*.

however, *G. malayanus* and *G. laoticus* may have evolved this character together. Due to the equivocal node, it is inconclusive. Monocarp size may be correlated with dispersal: small monocarps are easily carried by birds so they distribute far from the origin, whilst larger monocarps are carried by larger animals and so not distributed so far. The observational data that support this come from the report that fruits of *G. giganteus* are eaten by orangutans in Sumatra (herbarium specimen field notes: E. Sterk 185, L; cited by Saunders, 2003). In addition, Dr. Shumpei Kitamura (personal communication, April 27, 2006) said fruits of *G. laoticus* in Khao Yai National Park, Thailand are consumed by squirrels and monkeys.

The presence of a longitudinal ridge along the pericarp (character 33, Figure 4.9B) is a derived state but homoplastic. However, it is somewhat informative as it forms a partial synapomorphy for the *G. malayanus-G. cheliensis-G. laoticus* clade and for clade I, in which two taxa have reversal condition, i.e. *G. tapis* and G. *repevensis*.

Thicker pericarps is clearly the derived state (character 35, Figure 4.9C). Although this character is homoplasious, the thick pericarp state is partially informative as a synapomorphy for the *G. malayanus-G. cheliensis-G. laoticus* clade. Pericarp thickness may also be correlated with dispersal agents. The monocarps with thin pericarp are suitable to be eaten by birds, whereas the monocarps with slightly thicker pericarp are consumed by larger animals, including primates, as discussed above.

The number of seeds per monocarp (character 37, Figure 4.10A) is apomorphic for three unrelated lineages, *G. aurantiacus*, *G. laoticus* and *G. dolichopetalus*, although, this character is homoplastic.

Hairy seed testa is clearly apomorphic and homoplastic but it is partially synapomorphic for the clade I and may be present in the clade II with reversal in *G. rongklanus*, although the character state at the node at the base of the clade is equivocal (character 39, Figure 4.10B). Saunders (2002) noted that the function of testal hair is unclear, but may possibly be involved in mucilage production. This study supported a positive correlation between testal hairs and mucilage production around
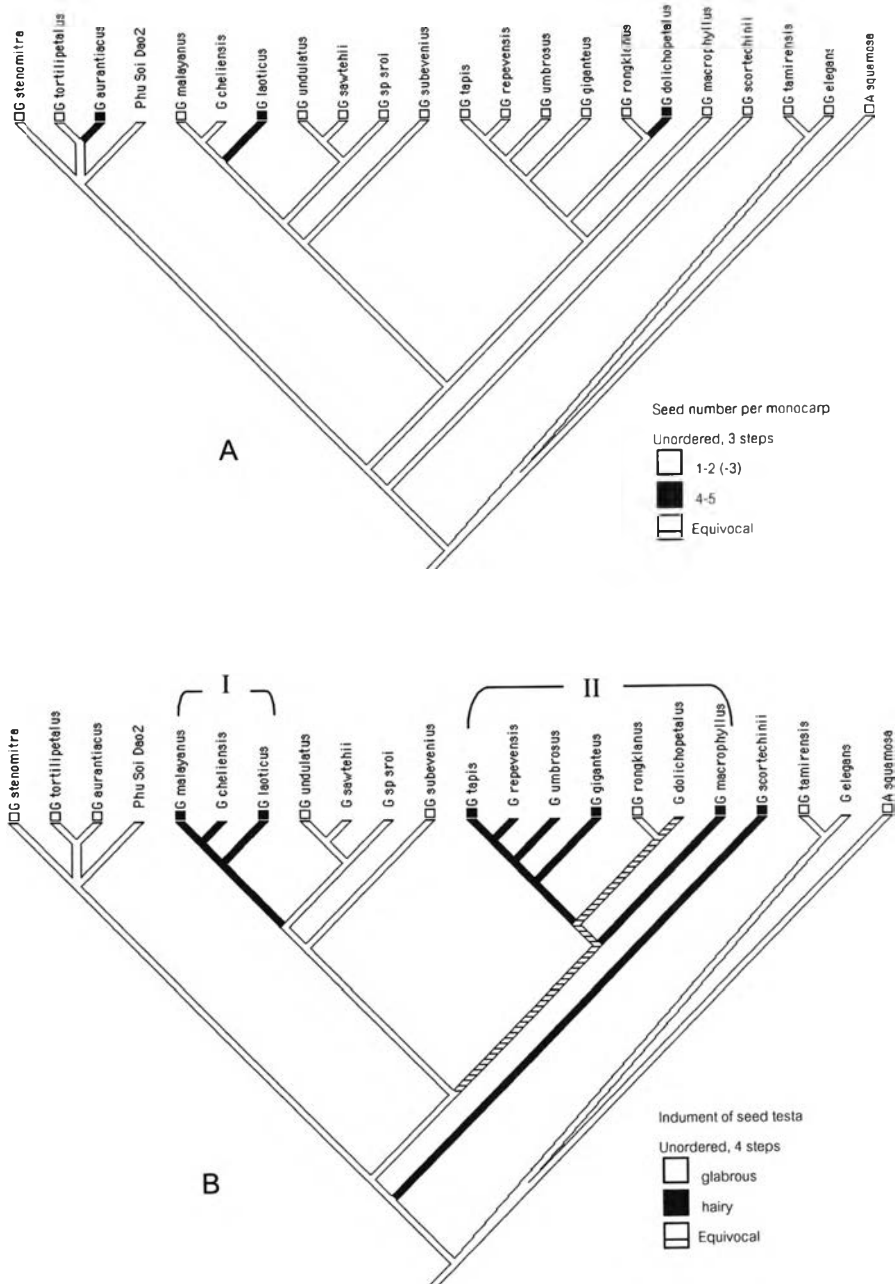
Figure 4.10 Trees showing inferred evolution of seed number per monocarp (A) and indument of seed testa (B) in *Goniothalamus*.
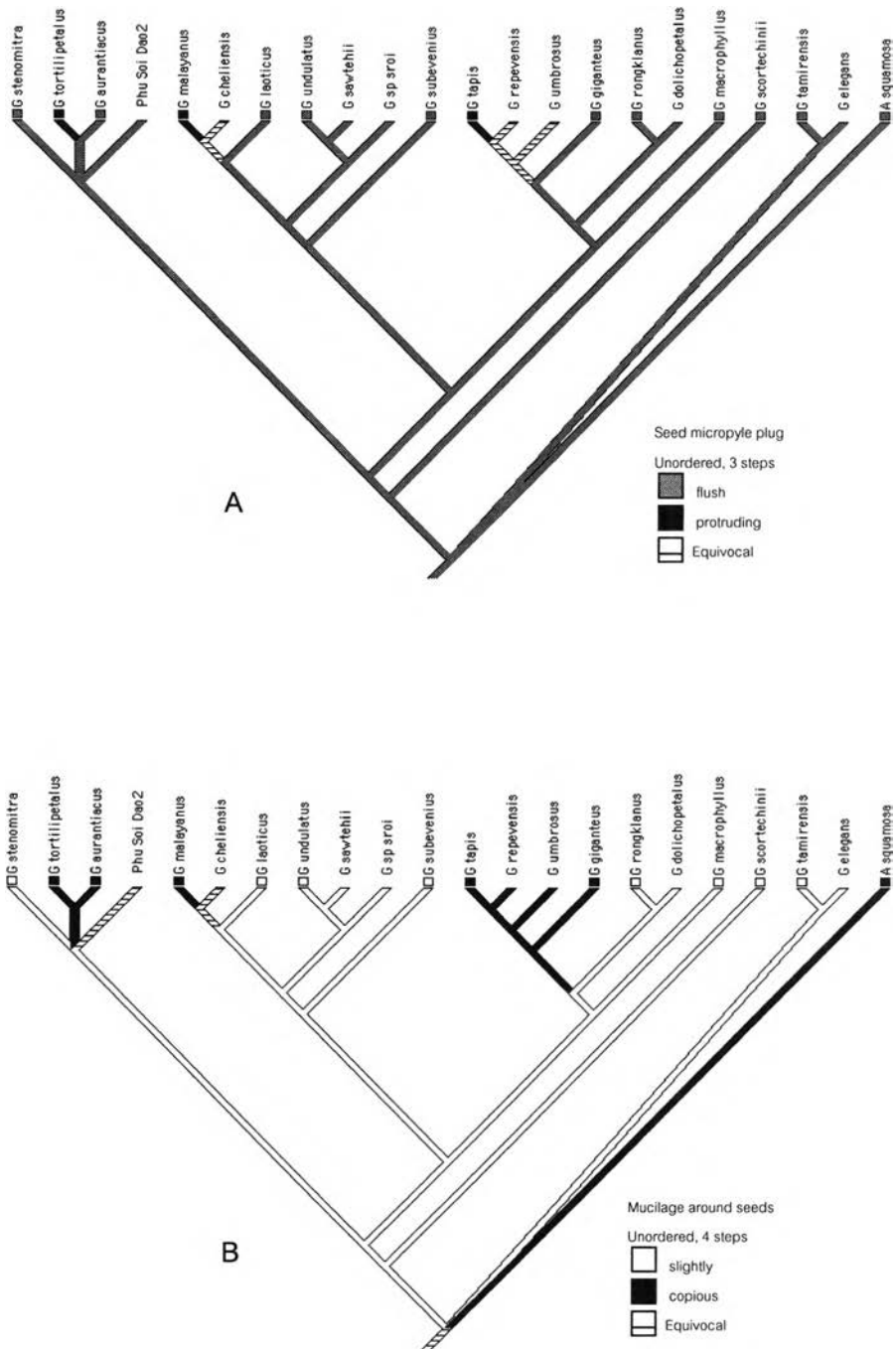
Figure 4.11 Trees showing inferred evolution of seed micropylar plug (A) and mucilage around seeds (B) in *Goniothalamus*.

seed, as in the *G. tapis-G. repevensis-G. umbrosus* clade, *G. giganteus* and *G. malayanus* produce copious mucilage (Figure 4.11B)

A protruding seed micropylar plug is apomorphic in three unrelated lineages, *G. tortilipetalus*, *G. malayanus* and *G. tapis*, although, this character is homoplastic (character 40, Figure 4.11A).

It is not clear which state of mucilage around seeds is plesiomorphic but it is partially informative (character 41, Figure 4.11B). This character may be related to hairs on seed testa as discussed above.