

CHAPTER 6

GENERAL CONCLUSION

Hoya parasitica (Roxb.) Wall. ex. Wight (Asclepiadaceae) is a long climbing epiphyte with opposite leaves, predominantly occurs in Southeast Asia. In Thailand, it is common and extremely variable species. This plant was recalled as the “*H. parasitica* complex” due to its great variations (Thaithong, 1995). This species has never been studied for a long time. Thus, the taxonomic status of “*H. parasitica* complex” in Thailand is still doubtful.

The results of this study showed that “*H. parasitica* complex” is a highly variable species. These variations supported the suggestions of Kerr (1956) and Thaithong (1995). Nine morphological and anatomical forms can be observed based on shape, leaf base, venation, and indumentum of leaves; sepal and coronal scale shapes. They are named here as form I, II, III, IV, V, VI, VII, VIII, and IX.

Naturally, the members of the complex occur in a wide range of habitats which are more or less overlap in Thailand. Form I, form II and IV grow only in evergreen forests and never been found in other habitats. Form VIII is common in beach and mangrove forests, along coastal areas of islands or mainland, while the other forms are mostly found on trees along stream banks, common in mixed deciduous and dry dipterocarp forests, and rarely occur in evergreen forests. The habitats and geographical distributions are significant characters and support separation of forms I, II, IV and VIII into distinct taxa in the complex.

From the extreme variability of morphological characters, both vegetative and reproductive parts, two techniques of numerical taxonomy were used to investigate variations of the nine forms in the “*H. parasitica* complex” in Thailand. It is evident that there are three groups within the “*H. parasitica* complex”, i.e. a group of form I, a group of form II, and a group of form III-IX. The significant characters used for segregation of the three groups are: the length of petiole, sepal, and corolla; the width of leaf and corpusculum; the diameter of corona, coronal-receptacle, and corolla. Furthermore, the

results of morphological and anatomical studies show close relationships among Forms III-IX.

Genetic variations are studied using the PCR-RFLP technique to detect the possible diversity of chloroplast DNA of "*H. parasitica* complex" in Thailand. The results show the low variation of cpDNA in this complex. Only nine amplified cpDNA/enzyme combinations show polymorphic patterns of chloroplast DNA. Twelve haplotypes (types of chloroplast DNA) are found in this complex, i.e. H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, and H12. Four of the twelve haplotypes (H1, H7, H10 and H11) are dominant and present in high frequency. Their wider geographical distributions along with the high frequency reflect the ancient origin. On the other hand, low frequency of the unique haplotypes (H2, H3, H4, H5, H6, H8, H9, and H12) indicated that they might be the result of recent mutations.

The overall results showed that "*Hoya parasitica* complex" in Thailand had high morphological and genetic variations. These detected variations did not correspond to the taxonomic status of plants, but directed to the conclusion that these variations should not be the results of an infraspecific variations of a single species, *Hoya parasitica*. So, the complex should be segregated into species, subspecies or varieties and forma.

The criteria being used to delimit species should be chosen carefully. The species represents the basic unit of systematic, evolution, genetics and most other areas of biology. At present, there is no universal definition of species concept. A group of individual plants that is fundamentally alike is generally treated as a species in taxonomic or morphological concepts. Ideally, a species should be separated by distinct morphological differences from the other closely related species (Jones and Luchsinger, 1987) was used in this study.

Furthermore, the International Code of Botanical Nomenclature (ICBN) recognizes five infraspecific taxa, i.e. *subspecies*, *variety*, *subvariety*, *form*, and *subform* (Stace, 1984). Three of them (*subspecies*, *variety* and *form*) are commonly used by plant systematics to provide formal taxonomic recognition of variation within species (Jones and Luchsinger, 1987). The categories *subspecies* and *variety* are applied to populations of species in various stages of differentiation. In taxonomic

practice, *subspecies* and *variety* are recognizable by morphological variations within species. Their divergence is usually related to adaptation to different geographical areas or climates or to local but sharply distinct ecological habitats. Whether the category *subspecies* or *variety* is employed often depends on the custom or philosophy of taxonomists. While the category *form* is generally used to recognize and describe sporadic variations in a single morphological feature. It is commonly used in early taxonomic treatments or in treatments of horticultural interest (Jones and Luchsinger, 1987).

The previous taxonomic treatments of the genus *Hoya* and the "*H. parasitica* complex" based primarily on discrete characters of floral characters (Rintz, 1978; Hooker, 1883; Forster and Liddle, 1991 and Kiew, 1995). In this study, floral characters were used to define a species, while vegetative characters and their geographical distribution are used to specify a variety. Hence, it is recognized that the nine forms of the "*H. parasitica* complex" are best treated as *H. parasitica* and its varieties together with a cryptic species. Form I and form II of the "*H. parasitica* complex" are ranked in species level, due to their discontinuity in floral, vegetative and ecological characters.

Form I corresponds to the previous described species, *Hoya rigida* Kerr (Kerr, 1939). The result from morphological and anatomical studies show good diagnostic characters for recognizing this species based on leaf shape, leaf venation, leaf indumentum, and sepal shape. This corresponds to the results obtained from numerical study. The length of sepal, and petiole, diameter of corona and corolla are larger than those of the others. This species will be separated from *H. parasitica* s. l. from this study. Corresponding result was presented by Kerr (1939), and eliminated the suspicion by Veldkamp et al. (1995) that this species should be included in *H. parasitica* s. l. However, the results of PCR-RFLP indicated a closely relationship between *H. rigida* Kerr and *H. parasitica* s.l.. Therefore, we agreed with Kerr (1939) to separate *H. rigida* Kerr from *H. parasitica* s.l. and treated them as closely related taxa.

Form II was rather similar to *H. parasitica* (Roxb.) Wall. ex Wight var. *critina* (Ridl.) Rintz from its appearances. However, after intensive studies showed

that this form is differed from the *H. parasitica* (Roxb.) Wall. ex Wight var. *critina* (Ridl.) Rintz in some aspects. It has scattered minute hairs on the abaxial surface of leaves and oblanceolate-oblong corpusculums. While, Rintz (1978) described to *H. parasitica* (Roxb.) Wall. ex Wight var. *critina* (Ridl.) Rintz, and from his illustrations showed the lower surface of leaves with dense hairs and triangular-oblong corpusculum. The result from this morphological and anatomical study indicated that form II is clearly distinguished from the *H. parasitica* s. l. Its diagnostic characters are broad ovate leaf with cordate base; 3-5 prominent nerves, extending from base to apex, minute hairs, scattering on the abaxial surface; oblanceolate-oblong corpusculum. These characters do not match any previously described taxa. Therefore, form II may be treated as a new species of the genus *Hoya*. The results of numerical taxonomy supported the separation of this form from the others (Figure 4.2 and Figure 4.5). The two most important characters are broad leaves and narrow corpusculums. However, the molecular-study result shows a close relationship between form II and some other forms in the northern and western populations. Furthermore, the cpDNA variations support the difference between form II and *H. parasitica* (Roxb.) Wall. ex Wight var. *citrina* Rintz. They represented different haplotypes, H1 and H7, respectively. At present, form II was treated as a cryptic and undescribed species (*H. sp.*) within the "*H. parasitica* complex" and was considered as a closely related taxon of *H. parasitica* (Roxb.) Wall. ex Wight and *H. rigida* Kerr.

Form III and form IV have conspicuously discrete vegetative characters and share common characters of the flower. Thus, they should be treated in varietal level of this complex. Nevertheless, difference in leaf venation of a few number of specimens are still not sufficient to distinguish these two forms as the distinct varieties of the species. The difference of form III and form IV from the others might be more or less an effect of gene mutation or an environmental variation. Due to their common characters of leaves, flowers as well as geographical distribution of the closely related forms, V and IX, respectively. The results of numerical taxonomy suggested that the two forms were not distinct. This is in agreement with the variations found in cpDNA. Therefore, they were only some variable forms of *H. parasitica* (Roxb.) Wall. ex Wight. Whether or not these two forms are new varieties of *H. parasitica* s.l. more specimens will be needed for further study.

Due to a small discontinuity in flower and leaf characters in form V-IX, they do not clearly distinguish to different taxa. From this study, it was found that forms V-VII did not properly fit to *H. parasitica* var. *parasitica*, due to their slight differences in leaf and flower characters. In contrast, members of form VIII and IX are best fitted to the distinct taxa, i.e. *H. parasitica* (Roxb.) Wall. ex Wight var. *parasitica* and *H. ridleyi* King & Gamble, respectively. However, they are different only in size of leaves. Therefore, this variation might happen due to the environmental difference of littoral plant (full or partial expose to sun-light and dry habitat) and inland plant (partial shade and moist habitat). It was accepted that light condition has a pronounce effect on leaf development (Forster and Liddle, 1991). In shady habitat leaf is usually larger than that occurs in open habitat. The results of numerical taxonomy also show no significance in this character to recognize form VIII and IX as a separate taxa. This study agreed with Rintz (1978) who reduced *H. ridleyi* King & Gamble to a variety of *H. parasitica* (Roxb.) Wall. ex Wight var. *parasitica*. The results of numerical taxonomy also showed that forms V-IX are not distinct. Furthermore, the result of cpDNA variations revealed some relationships among forms V-IX. Forms V-VII are unique in cpDNA pattern (haplotype H1), which are shared with some individuals of the form IX. While cpDNA haplotypes (H7, H10, H11) of form VIII are shared with some individuals of the form IX. In all, results from this study indicated clearly that members of forms V-IX should be treated as the variable forms of a single species and variety *H. parasitica* (Roxb.) Wall. ex Wight var. *parasitica*.

This investigation was a one of taxonomic study in the complex species of genus *Hoya* Br. The genus *Hoya* Br. has still some variable species that show highly variations in both vegetative and reproductive characters. In the past, only *Hoya australis* complex in Australia that there had intensive morphological and numerical studies; five infraspecific taxa were recognized within *Hoya australis* complex: i.e. subsp. *australis*, subsp. *tenuipes*, subsp. *oramicola*, subsp. *sanae*, and subsp. *rupicola* (Forster and Liddle, 1991).

In conclusion, based on the investigation of morphological, anatomical and molecular variations, the "*H. parasitica* complex" in Thailand is composed of 3 distinct species. i.e. *H. rigida* Kerr (form I), *H. sp.* (form II), and *H. parasitica*

(Roxb.) Wall. ex Wight (form III-IX). A key to species, descriptions are presented below.

Key to the species of "*H. parasitica* complex"

- 1. Sepals lanceolate, longer than the corolla tubes *H. rigida*
- 1. Sepals ovate, equal in length or shorter than corolla tubes
 - 2. Corpusculums narrowly oblanceolate-oblong *H. sp.*
 - 2. Corpusculums broadly triangular-oblong *H. parasitica*

1. *Hoya rigida* Kerr. Kew Bull. (1939) 463. Fl. Siam. Enum. 3 (1951) 42. Type: Thailand, Put 3034 (holotype, K; isotype, BK)

Leaves rigidly coriaceous, broad ovate to oblong with rounded to cordate base, 13.8-(17.8)-19.1 cm long, 6.8-(8.7)-10.1 wide; nerves 3-5, prominent, extending from base to apex, the other veins conspicuous; glabrous on both surface; petiole slender, 2.7-(3.4)-3.9 cm long. **Inflorescence** 7-(30)-65 flowered, peduncle 1.5-(2.9)-4.7 cm long; pedicel glabrous, 2-(2.3)-3 cm long. **Sepal** lanceolate, longer than corolla tube, 4.9-(5.4)-5.6 mm long, 2-(2.1)-2.2 mm wide. **Corolla** creamy white, 1.6-(1.9)-2.1 cm diam. **Coronal scale** broad ovate, 4.2-(4.5)-4.8 mm long, 2.2-(2.4)-2.6 mm wide, the outer angle slightly erected and white, apiculus of the inner angle raised and pink. **Pollinia** oblong, 0.62-(0.63)-0.64 mm long, 0.21-(0.23)-0.24 mm wide; translator short; corpusculum triangular-oblong, 0.16-0.18 mm long, 0.12-0.13 mm wide.

Ecology and distribution: a long climbing epiphyte, usually on trees along stream banks, in moist evergreen forest, in the eastern, western and southern parts of the country at 700-900 m elevations.

Vernacular name: Tao roi pla (Thai)

Specimens examined: see form I in CHAPTER 3; Khao Kuap, Krat (Trad), Put 3034 (isotype, BK); Nakorn Ratchasima, Khao Yai, Kasem 457 (BK).

2. *Hoya* sp. (Form II), sp. nov.

Leaves coriaceous, broadly ovate or rhombic with cordate base, 14.7-(15.9)-16.7 cm long, 11.3-(12.3)-13.2 cm wide; nerves 3-5, prominent, extending from base to apex, veins conspicuous; hairs minute, scattering on the abaxial surface; petiole

slender, 1.8-(2.1)-2.2 cm long. **Inflorescence** 10-(26)-49 flowered, peduncle 2.5-(3.3)-4.3 cm long; pedicel glabrous, 1.7-(1.9)-2.0 cm long. **Sepal** ovate, 1.8-(1.9)-2.0 mm long, 1.5-(1.7)-1.8 mm wide. **Corolla** creamy to yellowish white, 1.4-1.5 cm diam. **Coronal scale** ovate-lanceolate, 3.5-(3.8)-4.0 mm long, 1.5-(1.6)-1.7 mm wide, the outer angle flattened and pinkish white, apiculus of the inner angle raised and pink. **Pollinium** oblong, 0.45-0.46 mm long, 0.15-0.16 mm wide; translator short; corpusculum narrowly oblanceolate-oblong, 0.2-0.22 mm long, 0.07-0.08 mm wide.

Ecology and distribution: a long climbing epiphyte in moist evergreen forest, usually on trees along stream banks, restricted to Bala forest, peninsular Thailand at about 200 m elevations.

Vernacular name: -

Specimens examined: see form II in CHAPTER 3.

Notes: it is closely related to *H. parasitica* var. *citrina* (Ridl.) Rintz but differs in having a narrowly oblanceolate corpusculum and scattering hair on abaxial surface of leaves. It is a rare species in Thailand.

3. *Hoya parasitica* (Roxb.) Wall. ex Wight. Contrib. Bot. Ind. (1834) 37. F.B.I. iv. (1883) 57; F.M.P. 2 (1923) 396; F.I.C. 4 (1912) 136; Fl. Siam. Enum. 3 (1951) 40-41. Malay. Nat. J. 30 (1978) 514. Type: India, *Hb. Roxburgh* (BM)

Asclepias parasitica Roxb. Hort. Beng. (1814) 20; Fl. Ind. ii, (1832) 42. Type: India, *Hb. Roxburgh* (BM).

H. parasitica var. *geoffrayi* Constantin, F.I.C. 4 (1912) 136. Type: *Geoffray* 382 (P)

H. parasitica var. *spirei* Constantin, F.I.C. 4 (1912) 136. Type: *Spire* 1529 (P)

H. ridleyi King & Gamble. J.A.S. Beng. IV (1903) 575. Type: Malaysia, *Ridley* (K).

Leaves coriaceous to succulently coriaceous, ovate or elliptic to oblong, cuneate or obtuse to subcordate at base, 8.8-(11.5)-14.2 cm long, 3.6-(5.1)-6.6 cm wide, nerves 3, extending from base about half way to apex; veins obscure (occasionally the lowest pair extending from base to apex and veins conspicuous); covered with dense hairs on abaxial surface; petiole stout, 0.8-(1.3)-1.8 cm long. **Inflorescence** 25-(40)-55 flowered, peduncle 3.1-(4.5)-5.9 cm long; pedicel glabrous to pubescent, 1.74-(1.9)-2.24 cm long. **Sepal** ovate, 1.6-(1.9)-2.2 mm long, 1.4-(1.6)-

1.8 mm wide. Corolla creamy, yellowish, greenish and pinkish white, 1.2-(1.4)-1.6 cm diam. **Coronal scale** ovate to ovate or elliptic to lanceolate, 3.2-(3.5)-3.8 mm long, 1.7-(1.9)-2.1 mm wide, the outer angle slightly erected to erected and white, apiculus of inner angle short raised and white to pink. **Pollinium** oblong, 0.45-(0.50)-0.55 mm long, 0.16-(0.18)-0.20 mm wide; translator short; corpusculums triangular-oblong, 0.15-(0.17)-0.19 mm long, 0.11-(0.12)-0.13 mm wide.

Ecology and distribution: a long climbing epiphyte on tree branches in beach forests (coastal areas of island or mainland) and along stream banks in mixed deciduous to dry dipterocarp forests, sporadically occurs throughout northern, north-eastern, eastern, and western to peninsular Thailand.

Vernacular name: Lin hia, Nom mia, Nom pichit (Thai); Nua matawm (Laos, Chiang Mai)

Specimens examined: see form III-IX in CHAPTER 3.

Notes: the variable forms (III, IV, V, VI, VII, VIII, and IX) of *H. parasitica* (Roxb.) Wall. ex Wight var. *parasitica* could be separated into two groups by using their coronal scale. The first group consisted of forms III, V, and VII which have ovate-elliptic coronal scale. The second group is composed of forms IV, VI, VIII and IX which have ovate-lanceolate coronal scale. We treated these groups as “form” in taxonomic categories of *H. parasitica* (Roxb.) Wall. ex Wight var. *parasitica*.

Finally, this study shows great variations in “*H. parasitica* complex”, a wild plant that is commonly found in Thailand which is more than I expect. Unfortunately, due to the limited sample size of some variable forms, I can not clearly distinguish them in the present study. It is likely that additional studies of *Hoya parasitica* s.l. from neighboring countries, including enough sample sizes and/or a powerful method for the delimitation of taxonomic status will be available. However, this research is the first report of the intensive taxonomic study of the *Hoya parasitica* complex and its related species, the detailed results from this study can be served as a basis for further applied studies of this species and/or the other species of the genus *Hoya* can be based on.