FIELD DISCRIMINATION BETWEEN LESSER SHORT-NOSED FRUIT BAT (CYNOPTERUS BRACHYOTIS MULLER, 1838) AND GREATER SHORT-NOSED FRUIT BAT (C. SPHINX VAHL, 1797) (CHIROPTERA: PTEROPODIDAE) IN SOUTHEAST THAILAND

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ABSTRACT

The fruit bats Cynopterus brachyotis and C. sphinx overlap in size throughout their sympatric distribution. The present study aimed to determine the most reliable characteristics for separating these two species and to produce discriminant functions for distinguishing between them in the field. Discriminant function analysis revealed that a combination of forearm length and ear length predicts the correct species in 98-100% of cases. The derived functions showed a relatively high accuracy and the morphometric dimensions of individuals identified by these discriminant functions agree with those used in previous studies. From a recent molecular study, the species in south east Thailand are identified as C. sphinx and the Sunda lineage of the C. brachyotis species complex. Since morphological dimensions of fruit bats may vary along ecological and geographical gradients, morphological discriminant functions based on individuals of known genotype are recommended. The status and distribution of Cynopterus in southern Thailand also warrants further investigation.

Key words: Cynopterus brachyotis, Cynopterus sphinx, field discrimination, fruit bat, Thailand.

INTRODUCTION

Positive identification of a study species is a critical first step in every ecological investigation. Although a few misidentifications may not alter the conclusions of some ecological studies at the community level, such as those on diet and reproductive biology (BRUSEO ET AL., 1999), it may result in misleading interpretations at the level of the individual (e.g. radiotracking studies of habitat use and home range). Although molecular techniques are now widely used to identify sibling species and morphologically similar sympatric organisms (BARRATT ET AL., 1997; KINGSTON ET AL., 2001; MAPATUNA ET AL., 2002; CAMPBELL ET AL., 2004), these techniques are still costly and time-consuming, and cannot be used in field conditions. Therefore, species identification based on morphological characters is still vital for field studies.

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Received 1 December 2004; accepted 13 June 2005.
Discriminant function analysis is widely applied for separating individuals of similar size, both between species and between sexes on the basis of morphological characters (Arlettaz et al., 1990, 1997; Kitchener & Maharadatunkami, 1991; Bruseo et al., 1999; Reutter et al., 1999; Bronner, 2000; Purkait & Chandra, 2004). This statistical technique is also capable of detecting the most reliable discriminating characters based on the known species.

*Cynopterus brachyotis* and *C. sphinx* are small fruit bats (Fig. 1) which occur sympatrically over a wide range from the Indian subcontinent to the Indo-Malayan region (Corbet & Hill, 1992; Bates & Harrison, 1997). These species overlap in body size throughout the area of sympathy, and a genetic study revealed that they have recently undergone speciation (Schmitt et al., 1995). They inhabit a variety of habitats and predominantly occupy secondary forests (review by Storz & Kunz, 1999). How these closely related phytotrophic bats partition their resources has recently been investigated in southern Thailand, together with aspects of their diet, reproductive biology and foraging behaviour (Bumrungsri, 2002; Bumrungsri & Racey, 2005). Such studies rely on positive field identification. The present study aims to determine the most reliable characteristics for separating these species and to produce discriminant functions to separate individuals of both species in the field in southern Thailand. First, however, we will review the literature on this problem further.

Bats currently in the genus *Cynopterus* were firstly recognised as *Vespertilio* in 1797 by Martin Vahl. Later, F. Cuvier named it *Cynopterus* in 1824 by the principal characteristics of the short rostrum and the absence of the second upper molar and the third lower molar. Although it has been studied extensively, this genus is also one of the most taxonomically uncertain. In his revision of *Cynopterus*, Andersen (1912) listed 30 proposed names and consolidated them into 6 species of 16 forms. In the Malaysian peninsula, he recognised...
2 subspecies of *C. brachyotis*: *C. b. brachyotis* with shorter ears and rostrum and *C. b. angulatus* with longer ears and rostrum. Both subspecies occur sympatrically in Sumatra and throughout much of peninsula Malaysia, but *C. angulatus* also extends to northern Thailand. According to ANDERSEN (1912), *C. sphinx* occurs in India, Java and the Asian mainland as far as northern Thailand, and is found sympatrically with *C. b. angulatus*.

Although they appear identical in size, ANDERSEN (1912) indicated that only *C. b. angulatus* has an orbit-to-nares length less than 1/4 the total length of skull. This view was adopted by HILL (1961) but not by several other taxonomists (reviewed by HILL & THONGLONGYA, 1972). ANDERSEN (1912: 612-634) reported that *C. b. brachyotis* has a forearm length of 57–66 mm whereas in *C. b. angulatus* it is 65–72 mm. MILLER (1898) noted that the ear length of *C. b. angulatus* is 18–21 mm.

HILL & THONGLONGYA (1972) failed to separate *C. b. angulatus* and *C. sphinx* using the rostral length, a criterion used by ANDERSEN (1912), and concluded that angulatus was a subspecies of *C. sphinx*. They reported that the forearm length of *C. sphinx* from the Asian mainland and Sumatra generally exceeds 65 mm and the ear length is greater than 19 mm while *C. brachyotis* rarely exceed these values. HILL (1983) noted that the forearm length of *C. s. angulatus* from Thailand is 64.9–73.7 mm.

KITCHENER & MAHARADATUNKAMSI (1991), using multivariate analysis of external and skull morphology, proposed several new species of *Cynopterus* from the Indonesian archipelago and this was subsequently supported by molecular studies (SCHMITT ET AL., 1995). Consequently, *C. brachyotis* and *C. sphinx* were redefined. Using specimens from Sri Lanka, peninsula Malaysia, Sumatra, Borneo, Java and surrounding islands, KITCHENER & MAHARADATUNKAMSI (1991) pointed out that *C. brachyotis* has a forearm length of 55.9–66.7 in males (n = 71) and 54.7–66.2 mm in females (n = 70). They reported a forearm length of male *C. sphinx* of 59.2–75.0 mm (n = 38) and females 58.1–75.8 mm (n = 29). This view of speciation is not consistently accepted (CORBET & HILL, 1992). In reviewing *Cynopterus* in the Indo-Malayan subregion, CORBET & HILL (1992) recognised only four species of *Cynopterus* and indicated that *C. brachyotis* has a forearm length less than 67 mm and a condylobasal length less than 29.5 mm. The same measurements for *C. sphinx* are 66–78 mm and 29–35 mm respectively.

Recently, CAMPBELL ET AL. (2004), on the basis of mitochondrial DNA sequence data, suggested that *C. brachyotis* is a complex of lineages, 6 of which were well recognized: India/Sri Lanka, Myanmar, Sulawesi, Philippines, and 2 (Sunda and Forest) which broadly overlap in peninsular Malaysia and on Borneo.

**STUDY AREAS**

This study was carried out between March 1998 and March 2000 in 6 national forest reserves in Southeast Thailand (101°35'–102°20'E; 12°30'–13°30'N). The study area encompasses a variety of habitats, including lowland moist evergreen, lowland dry evergreen forest, beach forest and fruit orchards at the forest edge, ranging from sea level to 400 m asl. About 82% of specimens examined are from selectively logged dry evergreen forest interspersed with early successional vegetation (for site description see Bumrungsri, 2002). The average annual rainfall is 1,588 mm, 90% of which occurs during mid-April to October. The cool dry season is from October to January, with an average minimum temperature of
16°C, followed by a hot dry summer, when the average maximum temperature is 38°C. The average annual temperature is 26.5°C.

METHODS

After bats were caught, measurements of forearm length (from the extremity of the elbow to the extremity of the carpus with the wing folded), ear length (from the lower border of the external auditory meatus to the tip of the pinna in a natural shape), tail length and anterior palatal width (across the outer borders of the upper canines) were taken using dial calipers to the nearest 0.01 mm. The taxonomic terms follow BATES & HARRISON (1997). Bats were weighed with a 100-gram Pesola spring balance. Sex and reproductive status were assessed following RACEY (1988) and KOFRON (1997). Mature bats were identified with numbered rings on ball-chain necklaces (HANDLEY ET AL., 1991) Only mature individuals, including nulliparous females with a light yellow collar pelage, were subjected to analysis. For those recaptured bats from which measurements were taken more than once, the means of all variables except body mass were applied. The first record of body mass from each individual except pregnant females was employed.

Bats were classified into 3 groups: C. b. brachyotis, C. s. angulatus and ‘unclassified Cynopterus’ based on ANDERSEN (1912), MILLER (1898), HILL & THONGLONGYA (1972), and HILL (1983). Since the overlap of forearm length between C. b. brachyotis and C. s. angulatus occurs between 64.9–66.0 mm, those individuals with forearms less than 64.4 mm were assigned a priori as C. b. brachyotis, and those with forearms more than 66.5 mm were assigned as C. s. angulatus. Bats were allocated to an ‘unclassified’ group when their forearm lengths were between 64.4 and 66.5 mm. As the ear length of C. s. angulatus is longer than or equal to 18.00 mm, then those individuals with a forearm length of more than 66.5 mm but an ear length less than 17.00 mm, and those with a forearm length less than 64.4 mm and an ear length more than 19.00 mm, were also placed in an ‘unclassified’ group.

All variables were investigated for outliers or any irregular data, and these were eliminated. Subsequently, they were subjected to stepwise discriminant function analysis to select the significant variables for group separation. The correlation between these variables was also investigated. Where there was a high correlation between a pair of variables, one of them was eliminated. These selected variables were checked for sexual dimorphism in each species. As bats showed sexual dimorphism in some variables, males and females were separated for subsequent analysis.

Levene’s test for homogeneity of variance and Box’s M test for equality of covariance metrics (MCGARIGAL ET AL., 2000) were used for all variables in each sex of both a priori C. brachyotis and C. sphinx. Subsequently, a linear discriminant function analysis was carried out, and the percentage of samples classified correctly was indicated. This percentage is a direct measure of predictive accuracy (MCGARIGAL ET AL., 2000). The leave-one-out classification technique was used as it is relatively unbiased (LANCE ET AL., 2000). These functions also separated the ‘unclassified group’ into each species. These individuals were remixed to their species and the discriminant functions recalculated. The discriminant score was plotted to determine multivariate normality. All statistical analysis was performed with SPSS 9.0 (SPSS Inc. USA).
RESULTS

Preliminary stepwise discriminant function analysis selected 4 variables: forearm length, ear length, anterior palatal width and body mass which separated individuals significantly into 2 groups (F-test, \( p < 0.001 \)). Body mass was highly correlated with forearm length (\( r = 0.48 \)) but since it varies considerably with the quantity of food ingested, season and reproductive status, it was eliminated. Both species showed sexual dimorphism. Females of both species had longer forearms than males (t-test, \( C. \) brachyotis, \( t = -1.63, p = 0.052; C. \) sphinx, \( t = -2.54, p = 0.006 \)), and female \( C. \) brachyotis had a narrower anterior palatal width (t-test, \( t = 3.43, p < 0.001 \)) (Table 1). This led to separate analysis of the sexes. The percentages of correct classification from a discriminant function analysis with three variables (forearm length, ear length, and anterior palatal width) was no better than that using only the first two variables, so discriminant functions based on two variables were presented. In total, 482 individuals with measurements of forearm length and ear length were classified into three a priori groups (\( C. \) brachyotis, 206 cases; \( C. \) sphinx, 184 cases; unclassified, 92 cases). Among these, 250 were male and 232 were female.

Homogeneity of variance for each variable of each sex in both a priori species was rejected (Levene’s test, \( p < 0.01 \)). Equality of covariance matrices was met in females (Box’s M test, \( p = 0.24 \)) but not in males (\( p = 0.003 \)). This Box’s M test is sensitive to sample size and also to violation of the multivariate normality (McGarigal et al., 2000). In both a priori species, the normality of forearm length is rejected (Kolmogorov-Smirnov test with Lilliefors significance correction, \( p < 0.05 \)) which indicates that the equality of covariance is probably not grossly violated. Linear discriminant function analysis was carried out, resulting in the first significant discriminant function with high canonical correlation (male: Wilks’ lambda = 0.15, \( p < 0.001 \), eigenvalue = 5.91, canonical correlation = 0.93; female: Wilks’ lambda = 0.14, \( p < 0.001 \), eigenvalue = 6.04, canonical correlation = 0.93). With equal prior probability for each species, the leave-one-out classification using these discriminant functions revealed that 100% of cases from both a priori groups were correctly classified in both sexes.

In addition, 54 cases in an unclassified group were assigned by these discriminant functions to \( C. \) brachyotis and 38 cases to \( C. \) sphinx. These samples were then mixed with their species and the discriminant function analysis repeated. Homogeneity of variance was met in both males and females (Levene’s test, \( p > 0.05 \)). These final samples had equality of covariance matrices (Box’s M test, male, \( p = 0.52 \); female, \( p = 0.80 \)) which resulted in linearity of canonical function and efficiency of parameter estimation. Discriminant analysis derived the first significant discriminant function with high canonical correlation (male, eigenvalue = 3.04, Wilks’ lambda= 0.25, \( p < 0.001 \), canonical correlation = 0.87; female, eigenvalue = 3.49, Wilks’ lambda = 0.22, \( p < 0.001 \), canonical correlation = 0.88, Table 2). Forearm length accounted for the higher correlation with functions for both sexes (male, 0.86; female, 0.76).

With equal prior probability for each species, the classification function with cross validation (leave-one-out method) resulted in 100% correct classification of males and 98.7% of females. Only 3 females were misclassified. Discriminant scores in each species suggests multivariate normality was met in both sexes (Komogorov-Smirnov test, \( p > 0.01 \)), however, a minor overlap between species still existed (i.e. \( z = 0 \), Fig. 2).

Functions derived from discriminant function analysis are:
Table 1. The dimensions of individuals classified as *C. brachyotis* (a) and *C. sphinx* (b) before carrying out discriminant function analysis. Sexual variation in each morphometric dimension is examined with a t-test and a one tailed p-value is presented. Abbreviations: FA = forearm length (mm), Ear = ear length (mm), C1–C1 = anterior palatal width (mm), BM = body mass (g).

a) *C. brachyotis*

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SD</td>
<td>min-max</td>
<td>n</td>
</tr>
<tr>
<td>FA</td>
<td>104</td>
<td>61.73</td>
<td>1.62</td>
<td>58.40–64.35</td>
<td>102</td>
</tr>
<tr>
<td>Ear</td>
<td>104</td>
<td>17.06</td>
<td>1.02</td>
<td>14.59–19.00</td>
<td>102</td>
</tr>
<tr>
<td>C1–C1</td>
<td>64</td>
<td>5.71</td>
<td>0.34</td>
<td>4.39–6.60</td>
<td>70</td>
</tr>
<tr>
<td>BM</td>
<td>102</td>
<td>33.49</td>
<td>2.93</td>
<td>27.0–41.0</td>
<td>64</td>
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</table>

b) *C. sphinx*

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
<th>p-value</th>
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<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SD</td>
<td>min-max</td>
<td>n</td>
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<tr>
<td>FA</td>
<td>95</td>
<td>68.57</td>
<td>1.30</td>
<td>66.62–73.15</td>
<td>89</td>
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<tr>
<td>Ear</td>
<td>95</td>
<td>20.08</td>
<td>1.34</td>
<td>17.10–24.19</td>
<td>89</td>
</tr>
<tr>
<td>C1–C1</td>
<td>53</td>
<td>6.43</td>
<td>0.33</td>
<td>5.35–7.10</td>
<td>60</td>
</tr>
<tr>
<td>BM</td>
<td>90</td>
<td>46.97</td>
<td>3.56</td>
<td>36.0–56.0</td>
<td>61</td>
</tr>
</tbody>
</table>

Table 2. The characteristics of the first significant discriminant function and the percent of correct classification from final discriminant function analysis.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Eigenvalue</th>
<th>Wilks' lambda</th>
<th>Canonical correlation</th>
<th>Percent of correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>3.04</td>
<td>0.25</td>
<td>0.87</td>
<td>100</td>
</tr>
<tr>
<td>Females</td>
<td>3.49</td>
<td>0.22</td>
<td>0.88</td>
<td>98.7</td>
</tr>
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</table>
Figure 2. Distribution of discriminant scores of males (top) and females (below) *Cyopterus* (*C. brachyotis*, < 0; *C. sphinx*, > 0). There is a degree of taxonomic uncertainty for individuals with discriminant scores close to 0.
Table 3. The dimensions of individuals classified as *C. brachyotis* (a) and *C. sphinx* (b) after discriminant function analysis. Abbreviations are as Table 1.

a) *C. brachyotis*

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
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<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SD</td>
<td>min-max (mm-min-max)</td>
</tr>
<tr>
<td>FA</td>
<td>131</td>
<td>62.20</td>
<td>1.79</td>
<td>58.40-65.65</td>
</tr>
<tr>
<td>Ear</td>
<td>131</td>
<td>17.32</td>
<td>1.27</td>
<td>14.59-21.00</td>
</tr>
<tr>
<td>C¹-C¹</td>
<td>82</td>
<td>5.75</td>
<td>0.35</td>
<td>4.39-6.85</td>
</tr>
<tr>
<td>BM</td>
<td>128</td>
<td>34.19</td>
<td>3.83</td>
<td>27.00-49.00</td>
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b) *C. sphinx*

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<th>Males</th>
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<tr>
<td></td>
<td>n</td>
<td>mean</td>
<td>SD</td>
<td>min-max (mm-min-max)</td>
</tr>
<tr>
<td>FA</td>
<td>119</td>
<td>67.96</td>
<td>1.71</td>
<td>64.31-73.15</td>
</tr>
<tr>
<td>Ear</td>
<td>119</td>
<td>19.90</td>
<td>1.38</td>
<td>16.00-24.19</td>
</tr>
<tr>
<td>C¹-C¹</td>
<td>69</td>
<td>6.43</td>
<td>0.32</td>
<td>5.35-7.10</td>
</tr>
<tr>
<td>BM</td>
<td>115</td>
<td>46.10</td>
<td>4.37</td>
<td>31.50-56.00</td>
</tr>
</tbody>
</table>

For males, \( z = 0.489 \) forearm length + 0.256 ear length − 36.478.

For females, \( z = 0.440 \) forearm length + 0.415 ear length − 35.188.

When \( z \) is the standard canonical score calculated from the discriminant function If \( z > 0 \), then the individual is *C. sphinx* and if \( z < 0 \), then it is *C. brachyotis*. The morphometric dimensions of individuals identified by discriminant functions were presented in Table 3.

**DISCUSSION**

From the present study, the length of forearm and ear of both *C. brachyotis* and *C. sphinx* conform to those recorded for *C. b. brachyotis* and *C. s. angulatus* in earlier studies (Miller, 1898; Andersen, 1912; Hill & Thonglongya, 1972; Hill, 1983). Ear length of *C. brachyotis* in the present study generally reaches 20 mm, which is a little longer than that noted by Hill & Thonglongya (1972; 19 mm). This may be due to the larger sample size in the present study.

A combination of forearm length and ear length allows field discrimination between *C. brachyotis* and *C. sphinx* in Southeast Thailand. Both characteristics are reliable and efficient for using in the field (Arletta et al., 1990). Discriminant functions from the present study are highly accurate, as suggested by a high percentage of individuals correctly classified (McGarigal et al., 2000). Furthermore, the size of identified bats in the present

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study is consistent with those of earlier workers (e.g. ANDERSEN, 1912; HILL & THONGLONGYA, 1972; HILL, 1983). HILL & THONGLONGYA (1972) pointed out that the majority of specimens can be separated readily by a combination of forearm length and ear length. ARLETTAZ ET AL. (1990, 1997) also used discriminant functions from these parameters to separate sibling mouse-eared bats, Myotis myotis and M. blythii. In the Indo-Malayan region, CORBET & HILL (1992) classified Cynopterus using forearm length and condylobasal length and other qualitative characteristics. In the Indian subcontinent, a combination of all the above characteristics and the length of the upper maxillary toothrow and the shape of the ear tip were used (BATES & HARRISON, 1997; MAPATUNA ET AL., 2002). Alternatively, YOON & UCHIDA (1989), using the morphology of the humerus, stated that C. sphinx has a concave pectoral ridge whereas the pectoral ridge is not concave in C. brachyotis.

As mentioned by several authors, taxonomic revision using molecular techniques is needed to clarify the species within Cynopterus. For nearly a century, the taxonomic debate about Cynopterus focused on the apparent overlap in size between C. brachyotis and C. sphinx. Recently, using mitochondrial DNA, CAMPBELL ET AL. (2004) suggested that C. sphinx is well defined, whereas taxa referred to C. brachyotis comprised a species complex. Six lineages were identified: Sulawesi, Philippines, India, Myanmar, Sunda and Forest. The latter 2 lineages are sympatric in peninsular Malaysia and on Borneo. Most of recognized lineages may warrant species recognition. The Thai specimens from the present study were included and were identified as C. sphinx and the Sunda lineage of the C. brachyotis species complex. This lineage is distributed from Southeast Thailand to North Vietnam and as far south as Singapore, Borneo and Java. For the distribution of C. sphinx, ANDERSEN (1912) AND HILL (1961) reported that this species occurs in peninsular Malaysia, as far south as South Perak, but CORBET & HILL (1992) noted that it is present on the entire peninsular. Although their study covered all of peninsular Malaysia, CAMPBELL ET AL. (2004) showed, using molecular genetics, the presence of C. sphinx only as far south as the Cameron Highlands, Pahang. As they indicated the presence of the Forest lineage of C. brachyotis in Perlis (North Malaysia), further study on the possible distribution of this bat in southern Thailand is required. Individuals in the Forest lineage are smaller (forearm length < 63.7 mm) than those in Sunda lineage (> 64 mm), the former is restricted to primary and mature secondary forest, while the latter is common in highly disturbed habitats (CAMPBELL ET AL., 2004).

Although discriminant function analysis is one of the most frequently used statistics for systematic classification (e.g. ARLETTAZ ET AL., 1990, 1997; KITCHENER & MAHARADATUNKAMSI, 1991; BRUSEO ET AL., 1999; REUTTER ET AL., 1999; BRONNER, 2000), in some cases, the results of such analyses do not always correspond to those of molecular taxonomy. For example, BRUSEO ET AL. (1999) found that one individual of Peromyscus was assigned by discriminant functions to P. leucopus but electrophoresis of salivary amylase indicated that it was P. maniculatus. Since morphological dimensions of fruit bats could vary along ecological and geographical gradients (CAMPBELL ET AL. 2004; KITCHENER & MAHARADATUNKAMSI, 1991), morphological discriminant functions based on individuals of known genotype are recommended for further study.
ACKNOWLEDGMENTS

We wish to thank the Thai Government for funding the PhD studentship of Sara Bumrungsri and Bat Conservation International for additional financial support. Sawai Wangkhongsa, David Raffaelli and Polly Campbell provide valuable discussion. Wacharee Leelapaibul, Mongkol Chaipakdee, Sunate Kampun assisted with the field study. Staff of the Royal Forest Department also provided generous help.

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