* Beilschmiedia turbinata: A Newly Recognized but Dying Species of Lauraceae from Tropical Asia Based on Morphological and Molecular Data

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Abstract

China took great efforts to reforestation, even turned the long-term forest loss into a net gain, but this cannot hide the loss of species diversity due to destruction of primary forests, habitat loss, invasion of alien species, and over exploitation. Here we provide such a case by recording a dying tree species of Lauraceae from the evergreen forests of SE Yunnan of China and adjoining Vietnam. We made field collections and observations for four consecutive years from 2009 to 2012. Phylogenetic analyses were conducted based on a combined dataset from nrITS and plastid intron, rpl16 intron, and psbA-trnH spacer. The results indicate that the Asiatic Beilschmiedia and Syndicus are reciprocally monophyletic with Endiandra as a sister group, and both morphology and molecular phylogeny clearly suggest that the new species belongs to Beilschmiedia. Thus Beilschmiedia turbinata Bing Liu et Y. Yang is illustrated and described as new to science, color plates, line drawings, distribution map and comparison with related species are provided. This new species is similar to B. yunnanensis in the small and ferruginous-brown tomentose terminal buds, elliptic to oblong-lanceolate and alternate or subopposite leaves bearing the fine veinlet reticulation, but differs from the latter by the smaller flowers, the eglandular stamens of the third whorl, and the large turbinate furfuraceous fruits.

Introduction

Global biodiversity loss is a serious problem due to habitat loss, overexploitation, biological invasion, pollution, and global climate change according to the Millennium Ecosystem Assessment [1], and it is the case in China as well. Though China took great efforts in reforestation, and even turned the long-term forest loss into a net gain, the increased forest cover mainly consists of non-native tree crops but not regenerating natural forest [2]. The primary forest was continuously cut down and transformed into monoculture plantations of non-native economic trees [2–4]. After privatization of former collective forests since 2008, smallholders often cut natural forests for immediate income, then plant monoculture tree crops for long-term investment [2]. Because loss of native species diversity is masked under the increasing reforestation, it is more serious than what people thought [2,5], especially in areas with high species richness, e.g. Yunnan of China.

Southeast Yunnan of China, adjoining to Vietnam and Laos, preserves the northernmost tropical rainforests in Asia, and harbors extremely high diversity of plant species [6], with more than 7000 native species of flowering plants [7], and falls within one of the biodiversity hotspots in the world [8], but experienced a fast loss of species due to farming, logging, habitat loss, and vegetation destruction. Many species were thus eliminated from the earth before they were recognized and described. Here we describe a critically threatened tree species of Lauraceae from the evergreen forest of SE Yunnan of China based on field investigations in SE Yunnan and N Vietnam in the last four years and a detailed comparative study including morphology, anatomy, and molecular systematics analyses.

The Lauraceae are typical elements of SE Asia, and are also well represented in SE Yunnan of China [9–10]. The pantropical genus Beilschmiedia Nees is one of the largest genera within the family, containing ca. 250 species worldwide [11–13], and includes common trees in tropical Asia [9]. In addition to old publications of Liou [14] and Kostermans [15], a few regional revisions on this genus have recently been completed, e.g. America [16], Borneo [17], Madagascar [13], and China [18].

It is difficult to distinguish Beilschmiedia from its close relatives due to overlapping characters [19–20], e.g. Potamnea (Madagascar), Endiandra (mostly in Australia, three species in China), Syndicus (mostly in China), Sinopora (Hong Kong), and Yasunia (S America), which together belong to the Beilschmiedia group [20]. The genus Beilschmiedia has been recognized based on a combination of characters: cymose-paniculate inflorescences with the lateral flowers of the cymes not strictly opposite but somewhat alternate [21], trimerous flowers bearing usually nine but sometimes six
Morphological evidence. The new species, belonging to the

Beilschmiedia

species, could be divided into two groups: fine type and coarse

type [16]. Cuticular characters were sometimes used to determine
generic ascription [19–20, 22], and indeed, leaf cuticular morphol-
ogy is a good marker to define

Syndiclis

[22]. We tried several times to extract cuticles of our new species, but failed, because the epidermis of this new species is strongly thickened, in which it differs from all other known Asiatic species of the

Beilschmiedia

group [22].

Molecular phylogenetics based on DNA sequencing nowadays
becomes a regular approach in plant systematics. In addition to
the big phylogeny of the Lauraceae [23–24], a few molecular
systematic studies were conducted on Lauraceae to discuss the
generic relationships, e.g.,

Actinodaphne

[25–27],

Neoillex

[26–28],

Neocinnamomum

[29–30],

Actinodaphne

[26–27, 31], and

Persia

group [32–33], but the

Beilschmiedia

group was poorly sampled. For the

Beilschmiedia

group in the ‘Tropical East Asia (Beilschmiedia, Sinopora,

Syndiclis,

and

Endiandra)’, it is difficult to separate

Endiandra

and

Syndiclis

from

Beilschmiedia.

This group is poorly represented in previous molecular phylogenetic studies of Lauraceae [23–24, 29].

To determine the generic ascription of our new species, we
sampled twelve species of Asiatic

Beilschmiedia, two

Endiandra,

and four

Syndiclis,

which is the most extensive sampling of the

Beilschmiedia

group thus far, no DNA analysis has been applied in Lauraceae to determine the taxonomic ascription of newly described species thus far. Both nuclear ribosomal ITS and chloroplast DNA fragments

(trnL-trnF, trnL-trnF, and

psbA-trnH)

were sequenced. This phylogenetic study is helpful to determine the taxonomic position of the newly recorded species in addition to
morphological evidence.

Materials and Methods

Ethics Statement

The field investigations were carried out in collective forests in
Sumawan of China and Thanh Thuy of Vietnam, which are
owned by the local village, but not protected area of state-owned
land. The village head of Sumawan, Mr. Maorong Tian, gave us
permissions to conduct the investigations and recommended two
villagers as guides for us. Dáo Thiệu assisted us collecting in
Thanh Thuy of Vietnam.

Morphology and Anatomy

We surveyed three sites which cover the whole known
distribution area of the new species. The voucher specimens,
FAA-pickled materials, measurements, and photographs of vegeta-
tive and reproductive characters were obtained in the field.
Anatomical observations of leaflet venation pattern and floral
structure were taken under light microscope (Zeiss Axio Imager
A1) in LSEB (State Key Laboratory of Systematic and Evolution-
ary Botany), IBCAS (Institute of Botany, the Chinese Academy of
Sciences).

Beilschmiedia yunnanensis

Hu is close to the new species in morphological; and the two species were compared in detail.

Assessment of Conservation Status

We estimated the population size in the field, investigated the
threat factors, and assessed the endangered category according to
IUCN red list criterion [34]. The distribution map and calculation
of EOO (extent of occurrence) were made by usingArgGIS ver.
9.3 [35].

Molecular Systematics

To determine the systematic position of our new species, thirty-
two samples belonging to nineteen species of four genera

(Beilschmiedia, Endiandra, Syndiclis, and

Cryptocarya)

were included in this study. All but the new one were recorded in
Flora of China
[18]. Leaf materials for DNA extraction were dried with silica gel.
Vouchers of samples (Tab. 1) were deposited in the National
Herbarium (PE), IBCAS. Cryptocarya calcicoxa

H. W. Li

is selected as the outgroup of

Beilschmiedia, Endiandra,

and

Syndiclis.

Due to lacking DNA samples and sequences in the Gene Bank,

Beilschmiedia

species outside China were not integrated into our
analyses.

Total DNA was extracted from silica-gel-dried leaves using
Tiangen® extraction kis. Polymerase chain reaction amplification
was accomplished using Prime Taq DNA Polymerase with
annealing temperatures of 50 °C. The ITS region was amplified
with primer pair ITSS4, ITS5 [36] and C26A, ITS [37]. The
chloroplast

trnL-trnF

region was amplified using primers designed by
Taberlet et al. [38]. For some special species, the sequences of

Beilschmiedia

and related genera were obtained by our own
designed primers, and the suitable annealing temperature is
50 °C. The

trnl-trnF

intron and

psbA-trnH

spacers were amplified using primers of Sang et al. [39] and Kelchner et al. [40], respectively.
All the primers used in this study are listed in Tab. 2.

Results

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a
published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names
containin this work have been submitted to IPNI, from where they will be made available to the
Global Names Index. The IPNI LSIDs can be resolved and the
associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and
available from the following digital repositories: PubMed Central, LOCKSS.

Description of the New Species

Beilschmiedia turbine
ata

Bing Liu & Y. Yang sp. nov. Figs. 1, 2 77128395-1.

Type.

China. Yunnan Province: Malipo County, Tianbao,
Sumawan, Long. 104.84 E, Lat. 22.98 N, 24 Sept 2011, flowering,
Bing Liu 1442 (holotype, PE).

Diagnosis. Hace species nova ramulis juvenilibus, gemmis
terminalibus foliisque juvenilibus dense fulvo-tomentosis, glandulis
staminum ordinis III absentibus, fructibus turbinatis et ferrugineo-
furfuraceis, valde magnis, ad 7.2 cm longis, 4.6 cm in diametro
inter alia distinguenda.

Description. Trees, up to 25 m tall, to 1 m in DBH (diameter at breast height). Bark brownish gray, Branchlets 1.6–
Table 1. Vouchers and accession numbers of sequences.

| Species                        | Voucher | Locality                  | Accession no. |
|-------------------------------|---------|---------------------------|---------------|
|                               |         |                           | mtrTS | trnL-trnF | rp16 | psbA-trnH |
| Beilschmiedia appendiculata (C.K. Allen) S.K. Lee et Y.T. Wei | B. Liu 1504 | Guangzhou, Guangdong, China. Cult. | KC958643 | KC958611 | KC958675 | KC958707 |
| B. fordii Dunn               | B. Liu 1080 | Shangsi, Guangxi, China | KC958625 | KC958593 | KC958657 | KC958689 |
| B. delicata S.K. Lee et Y.T. Wei | B. Liu 1451 | Gulin, Sichuan, China | KC958642 | KC958610 | KC958674 | KC958706 |
| B. delicata S.K. Lee et Y.T. Wei | F. Q. Liu s. n. | Enshi, Hubei, China | KC958641 | KC958609 | KC958673 | KC958705 |
| B. glauca var. glaucoides H.W. Li | B. Liu 1323 | Malipo, Yunnan, China | KC958638 | KC958606 | KC958670 | KC958702 |
| B. kweichowensis Cheng        | B. Liu 1419 | Libo, Guizhou, China | KC958631 | KC958599 | KC958663 | KC958695 |
| B. laevis C.K. Allen          | B. Liu 1270 | Guangzhou, Guangdong, China. Cult. | KC958626 | KC958594 | KC958658 | KC958690 |
| B. linocleroides H.W. Li      | B. Liu 1479 | Jinping, Yunnan, China | KC958627 | KC958595 | KC958659 | KC958691 |
| B. percoriaea C.K. Allen      | B. Liu 1254 | Xishuangbanna, Yunnan, China. Cult. | KC958628 | KC958596 | KC958660 | KC958692 |
| B. purpurascens H.W. Li       | B. Liu 1321 | Malipo, Yunnan, China | KC958629 | KC958597 | KC958661 | KC958693 |
| B. purpurascens H.W. Li       | B. Liu 1443 | Malipo, Yunnan, China | KC958630 | KC958598 | KC958662 | KC958694 |
| B. robusta C.K. Allen         | B. Liu 1466 | Maguan, Yunnan, China | KC958632 | KC958600 | KC958664 | KC958696 |
| B. robusta C.K. Allen         | B. Liu 1481 | Jinping, Yunnan, China | KC958633 | KC958601 | KC958665 | KC958697 |
| B. robusta C.K. Allen         | B. Liu 1428 | Malipo, Yunnan, China | KC958634 | KC958602 | KC958666 | KC958698 |
| B. rufohirtella H.W. Li       | B. Liu 1302 | Malipo, Yunnan, China | KC958635 | KC958603 | KC958667 | KC958699 |
| B. rufohirtella H.W. Li       | B. Liu 1493 | Xichou, Yunnan, China | KC958636 | KC958604 | KC958668 | KC958700 |
| B. rufohirtella H.W. Li       | B. Liu 1430 | Malipo, Yunnan, China | KC958637 | KC958605 | KC958669 | KC958701 |
| B. turbinata Bing Liu et Y. Yang | B. Liu 1442 | Malipo, Yunnan, China | KC958639 | KC958607 | KC958671 | KC958703 |
| B. turbinata Bing Liu et Y. Yang | B. Liu 1185 | Malipo, Yunnan, China | KC958640 | KC958608 | KC958672 | KC958704 |
| B. yunnanensis Hu             | B. Liu 1473 | Maguan, Yunnan, China | KC958644 | KC958612 | KC958676 | KC958708 |
| B. yunnanensis Hu             | B. Liu 1484 | Jinping, Yunnan, China | KC958645 | KC958613 | KC958677 | KC958709 |
| B. yunnanensis Hu             | B. Liu 1439 | Malipo, Yunnan, China | KC958646 | KC958614 | KC958678 | KC958710 |
| B. yunnanensis Hu             | B. Liu 1474 | Maguan, Yunnan, China | KC958647 | KC958615 | KC958679 | KC958711 |
| Cryptocarya calcicola H.W. Li | B. Liu 1457 | Malipo, Yunnan, China | KC958656 | KC958624 | KC958688 | KC958720 |
| Endiandra coriacea Merr.      | J. F. Ye s. n. | Taizhong, Taiwan, China. Cult. | KC958655 | KC958623 | KC958687 | KC958719 |
| E. dolichocarpa S.K. Lee et Y.T. Wei | B. Liu 1381 | Jinping, Yunnan, China | KC958653 | KC958621 | KC958685 | KC958717 |
| E. dolichocarpa S.K. Lee et Y.T. Wei | B. Liu 1480 | Jinping, Yunnan, China | KC958654 | KC958622 | KC958686 | KC958718 |
| Syndiclis anlungensis H.W. Li | B. Liu 1452 | Anlong, Guizhou, China | KC958648 | KC958616 | KC958680 | KC958712 |
| S. marilopesmis H.W. Li       | B. Liu 1282 | Malipo, Yunnan, China | KC958649 | KC958617 | KC958681 | KC958713 |
| S. marilopesmis H.W. Li       | B. Liu 1420 | Malipo, Yunnan, China | KC958650 | KC958618 | KC958682 | KC958714 |
| Syndiclis sp.                 | B. Liu 1472 | Maguan, Yunnan, China | KC958651 | KC958619 | KC958683 | KC958715 |
| Syndiclis sp.                 | B. Liu 1486 | Pingbian, Yunnan, China | KC958652 | KC958620 | KC958684 | KC958716 |

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2 mm in diam., grayish brown, lenticellate, green, ferruginous-brown pubescent when young and blackish brown when dry. Terminal buds small type, 4–5 mm long, densely ferruginous-brown tomentose. Leaves alternate or subopposite; leaf blade broadly lanceolate, elliptic to elliptic-lanceolate, 7–12(–16)×2.2–4.2(–6) cm, base broadly cuneate to obtuse, often oblique, apex acute to acuminate, midrib elevated on both surfaces, lateral veins 4–6(–7) pairs, immersed on upper surface but elevated on lower surface, minor veins slender, connected into fine type of minor venation pattern, covered with dense ferruginous pubescence on both surfaces when young, but glabrescent soon; petiole 1.4–2 cm long, densely ferruginous tomentose when young. Inflorescences paniculate; panicles terminal or axillary, 4.5–8×3–5.5 cm, few flowered; rachises slightly robust, sparsely ferruginous tomentose; bracts ovate, 2–3 mm long, caducous; flowers yellowish green, ca. 1.5 mm in length, 3 mm in diam.; pedicels 2–2.5 mm, sparsely tomentose; perianth lobes 6, ovate or broadly ovate, 1.2×1 mm, pubescent on both surfaces. Fertile stamens nine in three whorls, glandular, subequal, ca. 1 mm long, filaments short but present and densely pubescent; anthers 2-celled, cells of the first and second whorls of stamens introrse, and those of the third whorl extrorse, the tip of anthers glabrous; staminodes of fourth series long-triangular and pubescent, ca. 0.6 mm long; pistils pyriform, pubescent, style short, ca. 0.3 mm. Each infructescence usually bearing one single mature fruit; fruits maturing in two years. Young fruits of the first year ovoid, 2.5–3×1.5–1.8 cm. Mature fruits large and turbinate, 5.4–7.2×3.8–4.6 cm, apex obtuse, base attenuate, densely ferruginous-furfuraceous, having 16–20 irregular longitudinal ridges; fruiting pedicles conspicuously thickened and robust, 1.3–1.8 cm in diam. Young seedlings up to 40 cm in
from October to November; it takes two years for fruits to get 
Syzygium austroyunnanense 
H.T. Chang et R.H. Miao, 
C.Y. Wu, 
PLOS ONE | www.plosone.org 4 June 2013 | Volume 8 | Issue 6 | e67636 
Yang et P.H. Huang, 
Caryodaphnopsis 
of ca. 20 km² (EOO). The trees were cut down for construction 
individual trees are dispersed in the evergreen broad-leaved forest 
Lat. 22.98 N, 15 Nov 2010, seedling, Bing Liu 1184 (PE).

Distribution. This species was only found in the border of 
China (Malipo County of Yunnan Province) and Vietnam (Vi 
Xuyen County of Ha Giang Province) (Fig. 3).

Habit & Ecology. The species inhabits limestone soils of 
primary forests on hills with altitudinal ranges from 1000 to 
1200 m. Associated tree species include Beilschmiedia purparacens 
H.W. Li, B. delicata S.K. Lee and Y. T. Wei, Cinnamomum sabatul 
H.W. Li, Actinodaphne obtusa [Nees] Blume, Lithocarpus yunnanensis Y.C. 
Yang et P.H. Huang, Laderia gracilipes H.W. Li, Cryptocarya sp., Caryodaphnopsis sp., Cyclobalanopsis spp., Lithocarpus sp., Garcinia sp., 
Syzgium austroyunnanense H.T. Chang et R.H. Miao, Aglaia textilasci C.Y. Wu, Amoora sp., Pittosporum sp., and Osteos katharinei Pax. 
It blooms from September to October, and fruiting season is 
from October to November; it takes two years for fruits to get 
group.

Conservation. There is only one population with ca. 30 adult 
trees across the boundary of China and Vietnam. These mature 
individual trees are dispersed in the evergreen broad-leaved forest 
of ca. 20 km² (EOO). The trees were cut down for construction 
leading to the population decline. Moreover, increasing human 
economic activities, e.g. the surrounding reclamation of farmlands, 
construction of border roads, and rubber plantations, are resulting 
in the fragmentation of the population, and threatening the 
existence of the new species and its living habitat. Our field 
observation found dozens of seedlings nearby the parent trees, but 
no young sapling exists. Alien species (e.g. Amoora obtusa (L.) 
R. M. King et H. Rob, and Cassocephalum rubens (Jacq.) S. Moore) 
are also found nearby. Consequently, we here tentatively consider 
the new species to be “Critically Endangered” (CR Blab (v)+D), 
according to IUCN red list category and criterion [34].

Etymology. The specific epithet “turbinate” of this new species is 
from its turbinate shape of fruits.

Additional specimens examined. China. Yunnan Province: 
Malipo County, Tianbao, Sumawan, Long. 104.84 E, Lat. 
22.96 N, 15 Nov 2010, fruits, Bing Liu 1185 (PE); Vietnam. Ha 
Giang Province: Vi Xuyen County, Thanh Thuy, Long. 104.86 E, 
Lat. 22.98 N, 15 Nov 2010, seedling, Bing Liu 1184 (PE).

Partition homogeneity test between nrITS and chloroplast 
sequences was carried out using PAUP and the P value is 0.07. 
Therefore, we combined them into one dataset, representing 2618 
characters, including 163 characters that were parsimony-unin- 
formative and 135 characters that were parsimony-informative. 
Analyses of nrITS, trnL-trnF, rpl16, psbA-trnH and combine 
regions were carried out using MP (heuristic search) and the results 
are shown in Tab. 3, and the Bayesian consensus tree based on 
combined sequence data is shown in Fig. 4.

The result indicates that Syndicus and Beilschmiedia are recipro- 
ally monophyletic with the monophyletic Endiandra as outgroup, 
and Beilschmiedia turbinata clearly belongs to Beilschmiedia, though 
the position of the species is not well resolved within the genus (Fig. 4). 
In addition, this new species has typical Beilschmiedia morphology 
such as the trimerous flowers, nine fertile stamens in three whorls, 
fruits lacking persistent perianth lobes. This suggests that the new 
species is a Beilschmiedia but markedly differs from Syndicus and 
Endiandra. Beilschmiedia turbinata shows great similarities to B. 
yunnanensis in morphology, but it is not conclusive whether B. 
turbinata and B. yunnanensis are basal to other Beilschmiedia species or 
not due to the low bootstrap value (<50%).

Discussion

Morphological Comparison

Beilschmiedia turbinata possesses a unique set of morphological 
characteristics, e.g. the small type of terminal buds densely pubescent, 
eliptic to lanceolate elliptic leaves having fine veinlet reticulations, 
the trimerous flowers bearing nine eglandular fertile stamens in 
three whorls, and the large ferruginous-furfuraceous turbinate 
fruits. This new species is similar to B. yunnanensis in the small type 
and ferruginous-brown tomentose terminal buds, alternate or 
subopposite elliptic to oblong-lanceolate leaves, the elevated 
midrib, and the fine veinlet reticulation (Fig. 5), but differs from 
the latter by the smaller flowers (3 mm in diam. in the new species 
vs. up to 8 mm in B. yunnanensis), the eglandular stamens of the 
third whorl (vs. each of the three fertile stamens of the third whorl 
bearing two sagittate glands in B. yunnanensis), and the ovoid 
to turbinate, large, 5.4–7.2×3.8–4.6 cm, and densely ferruginous-
furfuraceous (vs. ellipsoid to globose or subglobose, smaller, 2– 
4×1.5–2.7 cm in B. yunnanensis) (Fig. 5).

Beilschmiedia turbinata also shares similar characters with B. 
dactyoneura Kosterm. from Borneo in having eglandular stamens 
[17], but they are easily distinguished: B. dactyoneura bears glabrous 
twigs and leaves, larger terminal buds, and smaller fruits, 
1.5 cm×1 cm [17].

A few species from Southeast Asia also bear large fruits, e.g. 
Beilschmiedia macrocarpa A. Chev. ex H. Liou from S Vietnam, with 
the ovoid fruits up to 6.2 cm [14]; it differs from B. turbinata by its 
large terminal buds (vs. small terminal buds) and coarse veinlet 
reticulation (vs. fine veinlet reticulation). Beilschmiedia gigantocarpa 
Kosterm. from Sulawesi, B. glaucophylla Kosterm., B. gyrotochoides 
Kosterm. and B. kiaul barsens Kosterm. from Borneo, all bear fruits 
larger than 5 cm in diam. [17,44]. Beilschmiedia turbinata can be 
easily distinguished from them by the turbinate shape of fruits vs. 
spindle-shaped shape of fruits in B. glaucophylla and B. gyrotochoides, 
globose to subglobose shape of fruits in B. gigantocarpa and B. 
kiaul barsens.

Phylogenetic and Morphological Implications

Beilschmiedia is mixed with allied genera (e.g. Potamnia and 
Endiandra) according to previous studies [24,29]. Sampling in these

| Table 2. Primers used for polymerase chain reaction 
| amplification and sequencing. |
| Primer | Sequence 5’-3’ | Reference |
| ITS 4 | TCTCCGGCTATATGATATGC | White et al., 1990 |
| ITS 5 | GGAAGTAACGTTCAACAAGG | White et al., 1990 |
| ITS C26A | GTTCTTTCTTCGCGT | Wen et al., 1996 |
| ITS 18S | AGGAGAAGTGCTAACAG | Wen et al., 1996 |
| trnL-trnF f | ATTGGAACCTGGAACAGAG | Taberlet et al., 1991 |
| trnL-trnF c | CGAATCGTGAGACGGCTACG | Taberlet et al., 1991 |
| trnL-trnF FC | CTAAATCCCGGAGGGTGACGTCC | this study |
| trnL-trnF RA | TAACAGGTTTCTGATGTTGAC | this study |
| rpl16 f71 | GTATGCTTATGTTGACCTCGTGT | Kelchner, 1997 |
| rpl16 r1516 | CTTCTACCTTCTCTTTTGTG | Kelchner, 1997 |
| psbA 3F | GTATTGATGAAACGGTATGCTC | Sang et al., 1997 |
| trnH 3R | CGCGCATGTTGGACTCAATTCC | Sang et al., 1997 |

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studies is poor, there are only six species of *Beilschmiedia*, two of *Potameia*, and one of *Endiandra* in Chanderbali et al. [24], seven species of *Beilschmiedia*, one of *Potameia*, and three of *Endiandra* in Rohwer & Rudolph [29], and these samplings are common in lacking Asiatic species. We sampled nineteen Asiatic species of the group (mostly from Yunnan) with the highest sampling density of

Figure 1. Illustrations of *Beilschmiedia turbinata* Bing Liu & Y. Yang showing morphological details. A, seedling; B, Flowering branch; C, flower; D, flower (front part removed); E, the first whorl perianth lobe; F, the second whorl perianth lobe; G, the first whorl stamen (adaxial view); H, the second whorl stamen (adaxial view); I, the third whorl stamen (abaxial view); J, the fourth whorl staminode (abaxial view); K, pistil showing the pubescent ovary; L, terminal bud; M, venation pattern (abaxial view); N, fruiting branch in the second year; O, a young fruit in the first year (Drawn by Y. B. Sun, A from Bing Liu 1184, B–M from Bing Liu 1442, N–O from Bing Liu 1185, PE). doi:10.1371/journal.pone.0067636.g001
the Beilschmiedia group so far. Our new phylogenetic analysis suggested that the new species belongs to the genus Beilschmiedia.

Morphology of the terminal buds is useful in classification of the genus Beilschmiedia [16–18,45–46]. Hooker [44] even divided Beilschmiedia into two sections mainly by the type of terminal buds: Sect. I, leaves opposite or alternate, terminal buds very small, pubescent or tomentose, not enclosed in coriaceous scales; Sect. II, leaves usually opposite, terminal buds enclosed in large glabrous coriaceous scales. According to the phylogenetic tree of this study, species bearing large type of terminal buds do constitute a well-supported clade (Fig. 4), and are considered to be derived from the species of small type. Beilschmiedia yunnanensis and B. turbinata, occupying the basal position of this genus, both have the small type terminal bud. Worldwide sampling of the Beilschmiedia group is
necessary to test and verify whether this character is useful for subgeneric classification or not.

Conservation Significance

Conservation status of *Beilschmiedia turbinata* warrants our attention. The new species is an endangered species and listed as CR, which is attributed to population decline, habitat loss, and alien species invasions because of increasing human activities in the area. Large trees of the species were cut down for construction. Expansion of farmlands and rubber plantations results in deforestation, and of course, habitat loss and fragmentation of population of the species. Road building causes fragmentation of population of the new species. Alien species invasions had potential impacts on both seed germination and growth of seedlings, and young seedlings might be difficult to grow up because of the lean soils and pressure of competition from both native and alien species. These factors are also major threats of regional biodiversity in Tropical Asia [5,9].

Yunnan Province is located in the SW China, harbors more than half the total species in China, and is the province with the highest floristic richness [47]. Within Yunnan Province, the

![Figure 3. Geographical occurrences of Beilschmiedia turbinata Bing Liu et Y. Yong and B. yunnanensis Hu. Triangles represent B. turbinata, and circles refer to B. yunnanensis.](https://doi.org/10.1371/journal.pone.0067636.g003)

Table 3. Summary of parsimony analyses and data properties for nuclear ribosomal internal transcribed spacer (nrITS), chloroplast trnL-trnF, rpl16, psbA-trnH, and combined regions.

| DNA region | ITS | trnL-trnF | rpl16 | psbA-trnH | combined |
|------------|-----|-----------|-------|-----------|----------|
| Length of sequences [bp] | 600–642 | 597–614 | 775–803 | 463–502 | 2496–2547 |
| Length of alignment [bp] | 668 | 626 | 774 | 508 | 2618 |
| Variable sites (%) | 70 (10.48) | 35 (5.59) | 36 (4.65) | 22 (4.34) | 163 (6.22) |
| Parsimony-informative sites (%) | 78 (11.68) | 36 (5.73) | 6 (0.78) | 15 (2.95) | 135 (5.16) |
| Consistency index (CI) | 0.7577 | 0.6847 | 1.0000 | 0.9268 | 0.7466 |
| Retention index (RI) | 0.8476 | 0.6196 | 1.0000 | 0.9483 | 0.7910 |
| Rescaled consistency index (RC) | 0.6423 | 0.4242 | 1.0000 | 0.8789 | 0.5906 |

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southeastern and southern regions are the most important and abundant center of plant diversity. More than 7000 species of flowering plants are native to the Southeast Yunnan alone [7], and over 4000 species are reported from southern Yunnan [48]. However, Yunnan remains an active area for plant taxonomic studies, there are 55 publications every year related to “new species” + “Yunnan” + “plant” during 2000 and 2012 according to ISI website searching. The floristic inventory of Yunnan is far from being completed, and description of new species may not catch up with the extinction of local plants due to over exploitation, habitat loss, and invasion of alien species.

The local governments have made great efforts to develop cash crops in the tropical areas, especially in southern Yunnan and lowlands of southeast Yunnan. Plantations occupy a large area previously occupied by old-growth primary forests, leading to deforestation and environmental destruction much more serious than several decades ago. Wang [49] took part in the China-Russia joint expedition to southern Yunnan in 1950’s, and revisited the same area in 1990’s. According to his observation in the air and on-the-spot investigation, both appearance and composition of the forests in southern Yunnan had greatly changed within the 40 years. The canopy color of the forest was dark green with very thick forests in 1950’s, but was changed into light green with many open places in 1990’s. Many of the primary forests with abundant species were cut down and transformed into economic monoculture forests (e.g. rubber), and the local biodiversity in southern Yunnan was precipitously reduced. Transformation of forest structure has negative effects not only on plant species, but also on insects [3]. The rubber plantations are less than 5 kilometers away from the forest where our new species was collected. Local villagers are unaware of protection, but still utilize the timbers by logging the large trees and substituting the forests.

Figure 4. Bayesian consensus of 18001 trees based on combined sequence data of nrITS and trnL-trnF, rpl16, psbA-trnH analysis. Bayesian posterior probability values greater than 0.9 (left) and bootstrap support value greater than 50 (right) are shown on the branches. The different types of terminal buds are illustrated on the right.
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with economic trees. The rapid economic development mode at the price of destruction of native vegetation was called into questions and even caused a lot of criticism [2,49–50]. Vegetation shifts caused by human disturbance have led to changes of floristic components and severe species loss [1]. *Beilschmiedia turbinata*, probably once a more widespread species, but became a dying species in SE. Yunnan, sounds the alarm again that conservation of the primary forests is urgent. *Nyssa yunnanensis* W.Q. Yin ex H.N. Qin & Phengklai of Nyssaceae also experienced a similar process [51]. As a result, conservation of primary vegetation and
endangered species of this biodiversity hot spot is quite worrying, conservation action is necessary. This finding furthers that botanical investigations are urgently needed before the primary forests are cut down and replaced by economic crops, e.g. rubber, eucalyptus, and banana.

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