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Taxonomic study of the genus *Anzia* (*Lecanorales*, lichenized Ascomycota) from Hengduan Mountains, China

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Abstract: Analyses of morphological, anatomical, chemical and DNA sequences led to the recognition of ten species of *Anzia* in the Hengduan Mountains, which harbour all species known from China, including *A. pseudocolpota* sp. nov. and *A. hypomelaena* comb. & stat. nov. Furthermore, populations similar to *A. hypoleucoides* but with narrow lobes and a yellow-orange pigmented medulla may be a phylogenetically distinct species tentatively recognized as *A.* aff. *hypoleucoides*. The species are primarily distinguished by the presence or absence of a central axis, the colour and shape of the spongy cushion and the nature of the secondary compounds. A key to all known species of *Anzia* from China is presented.

Key words: lichens, molecular phylogeny, *Parmeliaceae*, taxonomy, Yunnan Province

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Introduction

Anzia Stizenb. (*Parmeliaceae*; Lumbsch & Huhndorf 2010; Thell *et al.* 2012) comprises foliose lichens characterized by a brown-black or pale brown spongiostratum (spongy cushion) covering the lower surface (Calvelo 1996), pale greyish white to greyish green narrow lobes, and asci with numerous crescent-shaped ascospores (Stizenberger 1861). The genus has a rather cosmopolitan distribution, and includes *c.* 38 species, occurring primarily between 1000 and 4000 m in subtropical or temperate latitudes of both hemispheres. *Anzia* may be closely related to *Pannoparmelia* (Müll. Arg.) Darb., which also has a spongy cushion on the lower surface, but differs by the asci containing eight spores and the yellow-green upper cortex (Darbishire 1912). Asahina (1935) divided *Anzia* into three sections (*Simplices*, *Duplices*, and *Nervosae*) based on the anatomy of the medulla. Section *Nervosae* was identified by the presence of a central axis. Sections *Simplices* and *Duplices* were defined by the presence of a single-layered or double-layered medulla, respectively. Yoshimura (1987) observed both of these medulla types within a single species (i.e. *A. japonica*) and therefore combined these two sections as section *Anziae*.

The circumscription of the genus and geographical ranges of the species have been revised for Macaronesia (Haugan 1992), Asia (Yoshimura & Elix 1993; Yoshimura 1995; Yoshimura *et al.* 1997; Jayalal *et al.* 2012; Liang *et al.* 2012) and South America (Calvelo 1996). Eight species are currently recognized from mainland China (Wei 1991; Liang *et al.* 2012), mostly from Yunnan Province (Wu & Wang 1992; Wang 1995), but the diversity, variation and hence boundaries of species have not been comprehensively investigated. *Anzia leucobatoides* (Nyl.) Zahlbr., for example, was described based on a single collection from Yunnan and has not been collected since.

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Here we critically revise the genus *Anzia* in China based on a morphological and chemical study of all collections held in KUN, and on phylogenetic inferences from ITS sequences obtained for a set of exemplars. We propose to recognize ten species, including *A. pseudocolpota* sp. nov. and *A. hypomelaena* comb. & st. nov., based on *A. leucobatoides* f. *hypomelaena*, and highlight under *A.* aff. *hypoleucoides* a set of populations that are closely related to *A. hypoleucoides* but differ by their narrower lobes and their pigmented medulla, and which may constitute an additional species. A key to all *Anzia* taxa in China is provided.

Materials and Methods

Morphological and chemical studies

Approximately 500 specimens were examined in the Lichen Herbarium of the Kunming Institute of Botany (KUN-L), including recent collections from 2013 and 2014, made mostly in the Hengduan Mountains. Specimens were examined using standard microscopy techniques and hand-cut sections under a NIKON SMZ 745 T dissecting microscope. Anatomical descriptions are based on observations of these preparations under a NIKON Eclipse 50i microscope and photographs were taken using a NIKON digital camera head DS-Fi2. Size of the thallus, apothecia and lobes are based on measurements for each specimen, and ascospore dimensions reflect ten measurements typically from a single apothecium per specimen. Size is in each case represented by the range between the smallest and largest single values.

Secondary metabolites of all the specimens were identified using spot test and thin-layer chromatography as described by Elix *et al.* (1987), White & James (1985) and Orange *et al.* (2001). Solvent C (toluene: acetic acid = 85:15) was used for TLC analysis.

DNA extraction

Total DNA was extracted from small fragments of fresh thallus tips following the protocol by Ekman (1999) using Axyprep Multisource Genomic DNA Miniprep Kit. The nrDNA ITS region (ITS1-5.8S-ITS2) was amplified by PCR using the ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) primers. Conditions previously described by Arup (2002) were used for PCR: 2 µl of genomic DNA was added to the following mix: 2 µl of primer (1 µl for each primer of a 10 mM solution), 12·5 µl of 2×Taq PCR Mastermix (Aidlab) (containing Taq DNA Polymerase: 0·1 unit/µl; $MgCl_2$: 4 mM; dNTPs: 0·4 mM) and 8·5 µl dH_2O for a total volume of 25 µl. Amplifications were carried out in a thermocycler (C 1000TM), with the following profile: initial denaturation at 94°C for 5 min, followed by 30 cycles, each of three steps (94°C for 1 min, 56°C for 1 min, 72°C for 1·5 min), and a final extension at 72°C for 7 min. The PCR products were sanger-sequenced by Sangon Biotech.

Phylogenetic analyses

All raw sequences were assembled and edited using SeqMan (DNAstar packages). The newly generated ITS sequences were complemented by sequences available in GenBank. Sequences were aligned using MUSCLE v3.6 (Edgar 2004), and ambiguous regions were excluded using Gblocks (Talavera & Castresana 2007) with the default settings. Phylogenetic relationships were inferred using Bayesian Inference (BI) and under the Maximum Likelihood criterion (ML), with *Pannoparmelia angustata* (Pers.) Zahlbr. selected as the outgroup.

BI analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The optimal substitution model implemented in MrBayes was determined based on the Akaike Information Criterion (AIC) using jModelTest 3.7 (Posada 2008). BI analyses were run for 1 000 000 Markov chain Monte Carlo (MCMC) generations and 4 incrementally heated chains; MCMC started from a random tree and trees were sampled every 1000 generations, with the first 10% of trees discarded as burn-in. Posterior probabilities (PP) were obtained from the 95% majority-rule consensus tree of all saved trees.

ML analyses were performed with an online version of RAxML v7.2.6 (Stamatakis 2006). All parameters in the ML analysis were set to their default setting, and statistical support values were estimated by 1000 non-parametric bootstrapping pseudoreplicates. Bootstrap support values (MLBS) were obtained from the 70% majority-rule tree of all saved trees, using RAxML.

Results

Phylogenetic analyses

Sixteen new ITS sequences were generated and the final matrix included 25 samples of *Anzia* and the outgroup exemplar (Table 1). The most likely tree is composed of one well-supported (MLBS = 100; PP = 1·0) and one unsupported clade (Fig. 1). The presence or absence of a central axis does not define a single clade wherein the character is conserved. All species producing lobaric acid, divaricatic or divaricatic and sekikaic acids form respective monophyletic lineages. By contrast, species producing anziaic and divaricatic acids compose a polyphyletic group (Fig. 1).

The three populations of a putative new species (i.e., *A. pseudocolpota*), similar to

TABLE 1. *Specimens used in this study, newly obtained sequences are in bold.*

Species name	Locality	Collector(s)	Voucher Specimens	GenBank accession number
Anzia colpota	S. Korea	*SO Oh, JS Hur & KM Lim*	*Hur* 041492	DQ068379
A. colpota	S. Korea	*SO Oh, JS Hur & KM Lim*	*Hur* 040952	DQ068380
A. colpodes	USA: Tennessee	*Lumbsch* 4.VI.04.	UPS	DQ980000
A. aff. *hypoleucoides*	China: Yunnan	*LS Wang, XY Wang & JW Li*	13–37933	**KJ486586**
A. aff. *hypoleucoides*	China: Yunnan	*LS Wang, XY Wang & JW Li*	13–37994	**KJ486587**
A. aff. *hypoleucoides*	China: Yunnan	*LS Wang & XY Wang*	13–38259	**KJ486588**
A. aff. *hypoleucoides*	China: Yunnan	*LS Wang & XY Wang*	13–37971	**KJ486589**
A. flavotenuis	Sri Lanka	*U. Jayalal, P. Wolseley et al.*	BM:1088238	JN873338
A. formosana	China: Taiwan	*A. Thell et al.*	TWN 267–666	AY251411
A. hypoleucoides	China: Yunnan	*LS Wang & XY Wang*	13–38280	**KJ486575**
A. hypoleucoides	S. Korea	*KM Lim, JS Hur & LS Wang*	*Hur* 040112	DQ001273
A. leucobatoides f. *hypomelaena*	China: Yunnan	*LS Wang & XY Wang*	13–38008	**KJ486574**
A. leucobatoides f. *hypomelaena*	China: Yunnan	*LS Wang & XY Wang*	13–38273	**KJ486576**
A. leucobatoides	China: Yunnan	*LS Wang & MM Liang*	11–32320	**KJ486584**
A. leucobatoides	China: Yunnan	*LS Wang & MM Liang*	11–32373	**KJ486585**
A. mahaeliyensis	Sri Lanka	*U. Jayalal, P. Wolseley et al.*	BM:1088237	JN873339
A. opuntiella	S. Korea	*SO Oh, JS Hur & KM Lim*	*Hur* 040489	DQ068381
A. opuntiella	China: Yunnan	*D Liu, DL Niu, JW Li et al.*	12–35597	**KJ486577**
A. ornata	China: Yunnan	*LS Wang & XY Wang*	13–38282	**KJ486578**
A. pseudocolpota	China: Yunnan	*LS Wang*	09–31151	**KJ486581**
A. pseudocolpota	China: Yunnan	*LS Wang & XY Wang*	13–38274	**KJ486579**
A. pseudocolpota	China: Yunnan	*LS Wang & XY Wang*	13–38940	**KJ486580**
A. rhabdorhiza	China: Yunnan	*LS Wang, XY Wang & D Liu*	12–34689	**KJ486582**
A. rhabdorhiza	China: Yunnan	*LS Wang & XY Wang*	13–38264	**KJ486583**
Pannoparmelia angustata	Australia	*O Blanco, A Crespo, RH Re & HT Lumbsch*	*Elix* 42640	AY785272

A. colpota but differing by the presence of a central axis, form a highly supported clade (MLBS = 94%; PP = 1·0; Fig. 1) sister to *A. colpota*, *A. formosana* and *A. ornata*. Specimens resembling *A. hypoleucoides* but distinguished by their yellow medulla and their mostly subtropical distribution, clustered (MLBS = 75%; PP = 0·99) within the *A. hypoleucoides* clade, and are subtended by a clade of two typical exemplars (Fig. 1). The two samples of *A. leucobatoides* f. *hypomelaena* form a robust clade (MLBS = 100%; PP = 0·99) separated from typical *A. leucobatoides* by *A. mahaeliyensis*.

Taxonomic treatment

Key to species of *Anzia* from China

1 Central axis present 2
Central axis absent 7

2(1) Spongiostratum white or pale brown, medulla C– . . . . . **A. leucobatoides**
Spongiostratum black to dark brown. 3

3(2) Central axis flat, buried in medulla, medulla C+ red . . . .**A. hypomelaena**
Central axis cylindrical, growing between medulla and spongiostratum, medulla C–. 4

4(3) Lobes wide and roundish, pruinose on the tips . . . . **A. pseudocolpota**
Lobe tips acute, without pruina 5

5(4) Rhizines covered with spongiostratum . . . . **A. rhabdorhiza**
Rhizines simple and bare, without spongiostratum 6

6(5) Medulla not yellow, lobes wider than 1 mm . . . . **A. hypoleucoides**
Medulla (orange-) yellow, lobes less than 1 mm wide. . . **A.** aff. **hypoleucoides**

7(1) Medulla C+ red, containing anziaic acid. 8
Medulla C–, without anziaic acid 9

8(7) Spongiostratum not continuous, forming roundish patches near the tips . . . . **A. japonica**
Spongiostratum continuous, medulla double-layered. . . . . . .**A. formosana**

9(7) Lobes pruinose near the apex, medulla single-layered . . . . **A. colpota**
Lobes without pruina, medulla double-layered 10

10(9) Soredia-like lobules present along the lobe margin . . . .**A. ornata**
Without lobules, lobes opuntia-shaped . . . . **A. opuntiella**

Anzia colpota Vain.

Botan. Magaz. **35:** 19 (1921); type: Japan, Rikuzen Province, Gamo, *A. Yasuda* 248 (TUR—holotype).

Morphology and chemistry. See Yoshimura (1974).

Ecology and distribution. Usually growing on *Pinus*, *Quercus* or *Rhododendron* bark, at altitudes between 2000 and 3500 m. Known from Japan, Korea and China (Yoshimura 1974). The Chinese specimens are mostly distributed in the Hengduan Mountains area.

Comments. This species is characterized by the pruinose upper surface, palmate (apically densely branched) lobes and a single-layered medulla (Fig. 2A), containing divaricatic and sekikaic acids.

Anzia colpota may be confused with *A. japonica*, but that species has a central axis, and the spongy cushion is clearly not continuous over the lower surface but forms roundish patches (Fig. 3G). Furthermore, *A. japonica* contains anziaic acid (medulla C+ red), whereas divaricatic and sekikaic acids are present in *A. colpota*.

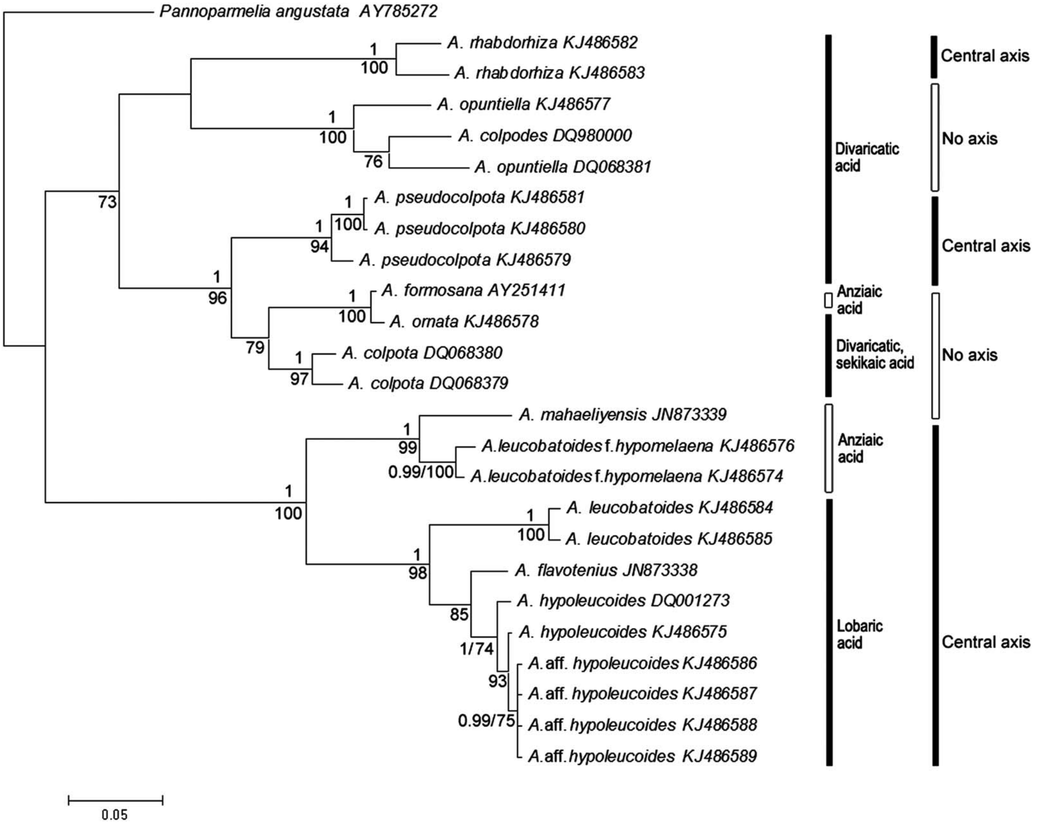

FIG. 1. Most likely phylogenetic relationships of *Anzia* samples from China inferred from ITS sequences using *Pannoparmelia angustata* as the outgroup. Posterior probabilities are shown above and ML bootstrap frequencies below branches.

This species, which is common in Eastern Asia, is morphologically identical to *A. colpodes* (Yoshimura 1974; Wu & Wang 1992; Hur *et al.* 2005), a species considered to be endemic to the East Coast of North America (Hale 1955). *Anzia colpodes* and *A. colpota* differ only by the presence or absence of sekikaic acid which, given the morphological similarity, led Culberson (1961) to suggest that they should perhaps be considered conspecific. We have been unable to obtain ITS sequences for *A. colpota* but inferences from publically available sequences for this species and *A. colpodes* suggest that they belong to two well-supported distinct clades (Fig. 1). We propose to retain the Asian and American populations in separate taxa until further sequence data can be analyzed.

Selected specimens examined. **China:** *Yunnan Prov.*: Deqin Co., Meili Snow Mt., 3200 m, 28°38'13·48"N, 98°39'47·01"E, on *Rhododendron* bark, 2012, *L-s. Wang* KUN-L 23961, 23962; Lijiang Co., Yulong Snow Mt., 3000 m, 28°24'N, 98°45'E, on *Pinus* bark, 1987, *L-s. Wang* KUN-L 17626, 17710; Gongshan Co., Dulongjiang, 3600 m, on *Quercus* bark, 2005, *L-s. Wang* KUN-L 11697.

Anzia formosana Asahina

J. Jap. Bot. **13:** 221 (1937); type: Taiwan, Mt. Alishan, leg. *M. Ogata* (hb. Asahina—holotype).

Morphology and chemistry. See Asahina (1937).

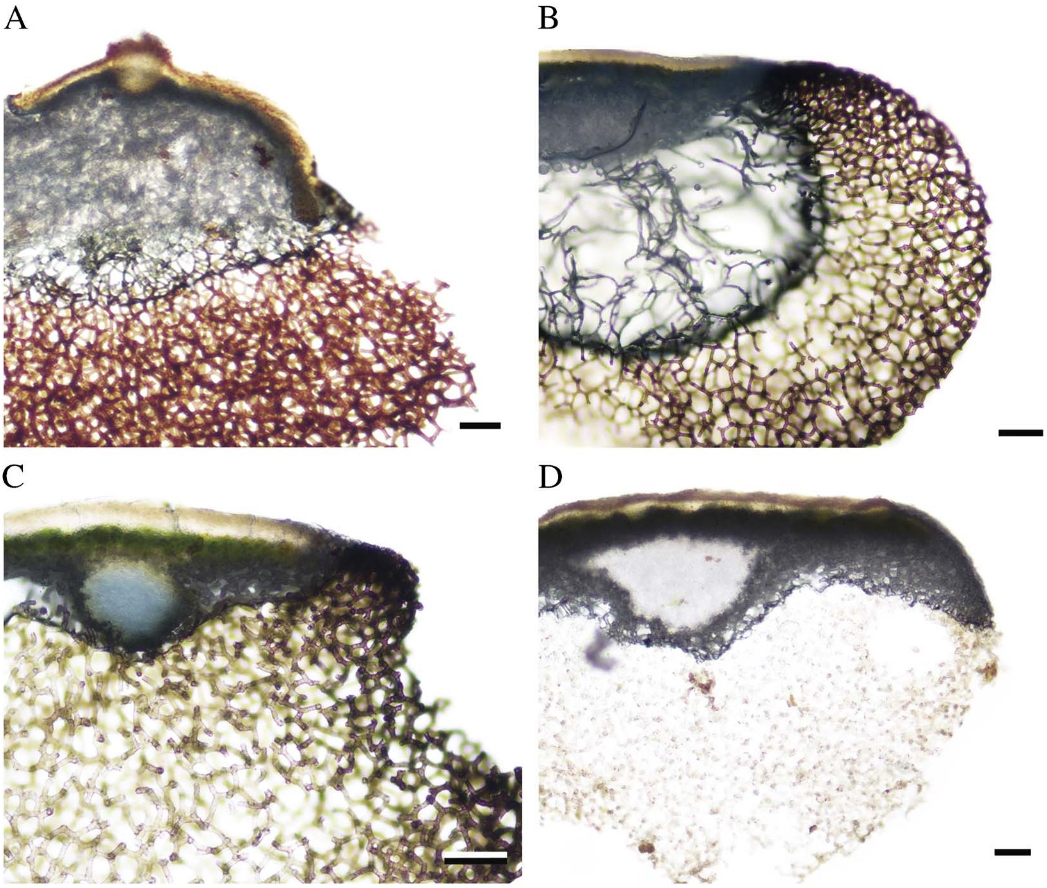

FIG. 2. Medulla types of *Anzia*. A, single-layered medulla of *A. colpota*; B, double-layered medulla of *A. formosana*; C, black and cylindrical central axis growing between medulla and spongiostratum (*A. hypoleucoides*); D, white and flattened central axis buried in medulla (*A. leucobatoides*). Scales: A–D = 100 µm. In colour online.

Ecology and distribution. Growing on *Picea*, *Pinus*, and *Rhododendron* between 1800 and 3000 m. Originally reported from Taiwan, and mainly distributed in the southern part of China (Sichuan and Yunnan; Yoshimura 1987).

Comments. This species endemic to China is characterized by the long linear lobes with pointed tips, two-layered medulla (Fig. 2B), and the presence of anziaic acid. *Anzia hypoleucoides* differs by the presence of a central axis in the medulla and by containing lobaric versus anziaic acid. *Anzia opuntiella* also has a two-layered medulla, but it contains divaricatic and sekikaic acids and has opuntia-like lobes rather than the linear branched lobes of *A. formosana*.

This species is related to *A. colpota* and *A. ornata*, with which it shares the lack of a central axis in the medulla and a similar ecological distribution.

Selected specimens examined. **China:** *Yunnan Prov.*: Jingdong Co., Ailao Mt., near Xujiaba reservoir, 2500 m, 24°32'28·81"N, 101°01'01·47"E, on *Quercus* bark, 2013, *L-s. Wang* KUN-L 23963, 23964; Lijiang

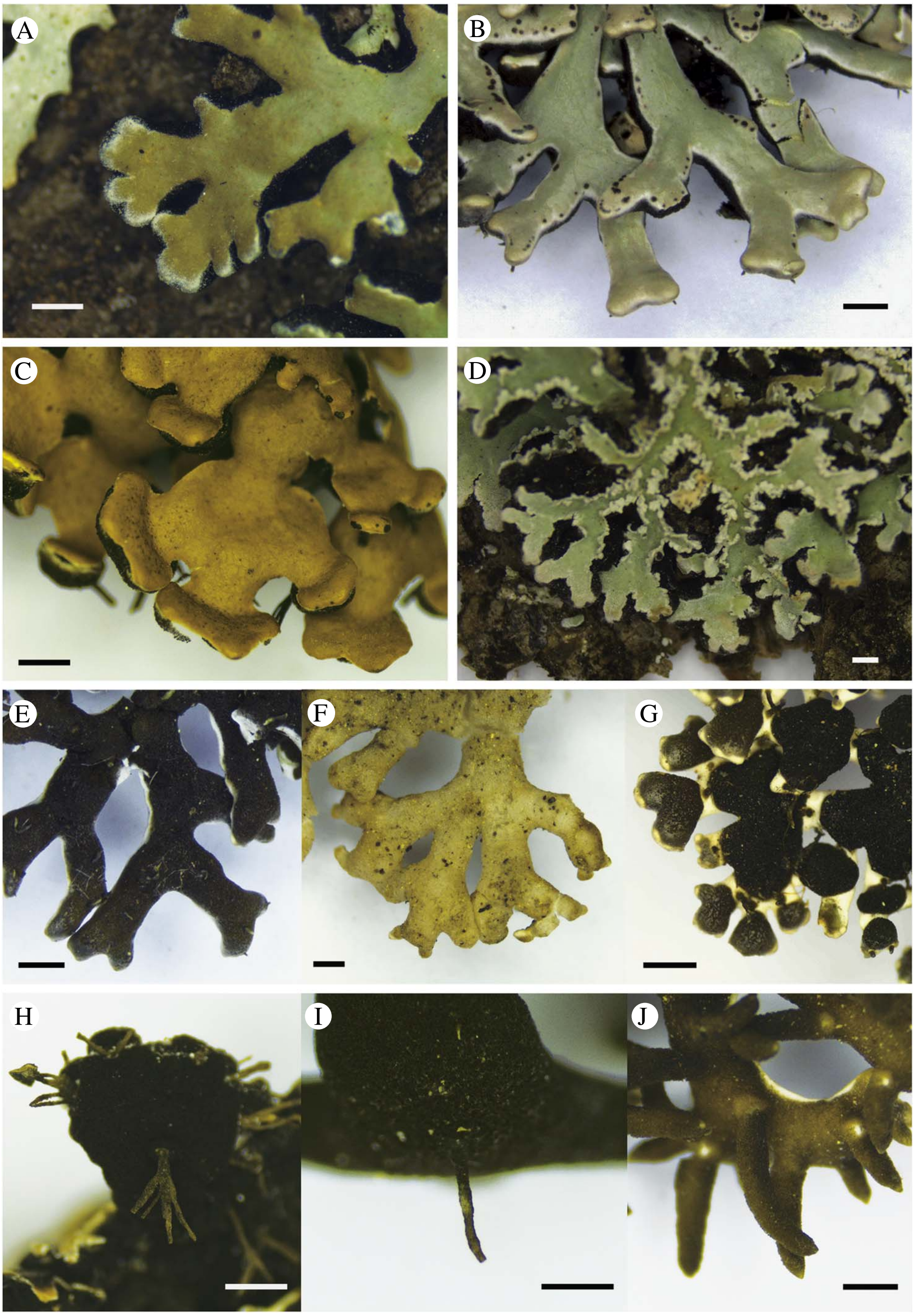

FIG. 3. Lobe (A–D), spongiostratum (E–G) and rhizine (H–J) types in *Anzia*. A, pruinose lobe (*A. colpota*); B, dichotomously branched lobe (*A. hypoleucoides*); C, opuntia-like lobe (*A. opuntiella*); D, lobulate lobe (*A. ornata*); E, black (*A. hypoleucoides*); F, white (*A. leucobatoides*); G, patchy spongy cushion (*A. japonica*); H, 3–5 bundled (*A. pseudocolpota*); I, single (*A. hypoleucoides*); J, covered with spongiostratum (*A. rhabdorhiza*). Scales: A–J = 1 mm. In colour online.

Co., Jiuhe Village, Laojun Mt., 3800 m, 26°37·940'N, 99°43·494'E, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23965. *Xizang Prov.*: Chayu Co., Ridong village, on bark, 2000 m, 1982, *J-j Su* KUN-L 11677. *Sichuan Prov.*: Muli Co., No. 915 woodland, 3700 m, 28°24·574'N, 99°46·685'E, on *Picea* bark, 2001, *L-s. Wang* KUN-L 18976.

Anzia hypoleucoides Müll. Arg.

Flora **74:** 111 (1891); type: Japan, Tosa, *Miyoshi* (G—holotype).

Morphology and chemistry. See Yoshimura (1974).

Ecology and distribution. Growing on the bark of *Rhododendron*, *Quercus* or *Abies* in subalpine regions in Japan, Korea and China (Sichuan and Yunnan; Yoshimura 1974).

Comments. This species is characterized by the black, cylindrical central axis lying between the medulla and the spongy layer, a dark brown spongy cushion, and the presence of lobaric acid.

Anzia hypoleucoides may be confused with *A. leucobatoides*, but it forms much smaller thalli (up to 8 cm in diam.) with rounded lobes *c.* 1 mm wide (Fig. 3B), whereas the latter species forms rather large thalli (to 12 cm in diam.) with pointed lobes to 2 mm wide. Furthermore, the central axis is black and cylindrical, developing under the medulla (Fig. 2C), versus white and flat, buried in the medulla in *A. leucobatoides* (Fig. 2D). *Anzia rhabdorhiza* also has a central axis and forms rather small thalli but differs by the spongy cushion covering the rhizines (Fig. 3J), the rather long rhizines (to 7 mm long vs to 2 mm in *A. hypoleucoides*), and the presence of divaricatic (instead of lobaric) acid.

Selected specimens examined. **China:** *Yunnan Prov.*: Lijiang Co., Alpine Botanical Garden, 3450 m, 27°00.447'N, 100°10.502'E, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23966; Jingdong Co., Ailao Mt., Xujiaba, 2500 m, 25°44·290'N, 99°03·565'E, on stump, 1994, *L-s. Wang* KUN-L 14527. *Sichuan Prov.*: Miyi Co., north slope of Malong Mt., 2800 m, on bark, 1983, *L-s. Wang* KUN-L 4972, 4818.

Anzia aff. hypoleucoides

(Fig. 4)

Thallus foliose, upper surface convex, greyish green to brownish green (herbarium stored), loosely adnate to the substratum, to 8 cm diam., without soredia or isidia; lobes linear, anisotomic dichotomously branched, lobes rather slim (0·5–1·0 mm wide), internodes 1·0–1·5 mm long, lobe tips roundish, cracks present on the marginal part of mature lobes; *medulla* yellow to orange-yellow (mature), single-layered, with black central axis growing between medulla and spongiostratum, cylindrical to oblate; lower surface covered with black to brownish black spongy cushions, continuous, spongy cushions narrower than the upper cortex, invisible from upper surface, hyphae brown in section, 7·5–9·0 µm thick; rhizines rather rare, simple, to 1 mm long (Fig. 3I).

Apothecia disc-shaped, rare, usually on the central part of the upper surface; *disc* reddish brown, 1–10 mm diam., roundish when young, becoming cracked and irregular when mature; *epihymenium* brownish yellow, 8–10 µm thick, hymenium hyaline, 25–30 µm thick, subhymenium 40–50 µm, colourless; *ascus* rod-shaped, *c.* 30 µm long, with numerous ascospores spirally arranged, spores simple and curved, 10·0–12·5 × 2·5 µm.

Pycnidia black, prominent above the surface, always on the margin of the lobe tips.

Chemistry. Cortex K+ yellow, medulla C+ yellow, KC–, containing atranorin, lobaric acid and an unknown pigment.

Ecology and distribution. Growing usually on *Quercus* or *Rhododendron*, in mesic montane forests (south end of the Hengduan Mountains), with only limited occurrence on *Rhododendron* in subalpine forests in NE Yunnan (Lijiang Co.), endemic to south-western China (Yunnan).

Comments. This potential taxon differs from all other Chinese *Anzia* by the yellow pigment in the medulla, and the rather small lobe size. *Anzia* aff. *hypoleucoides* may be

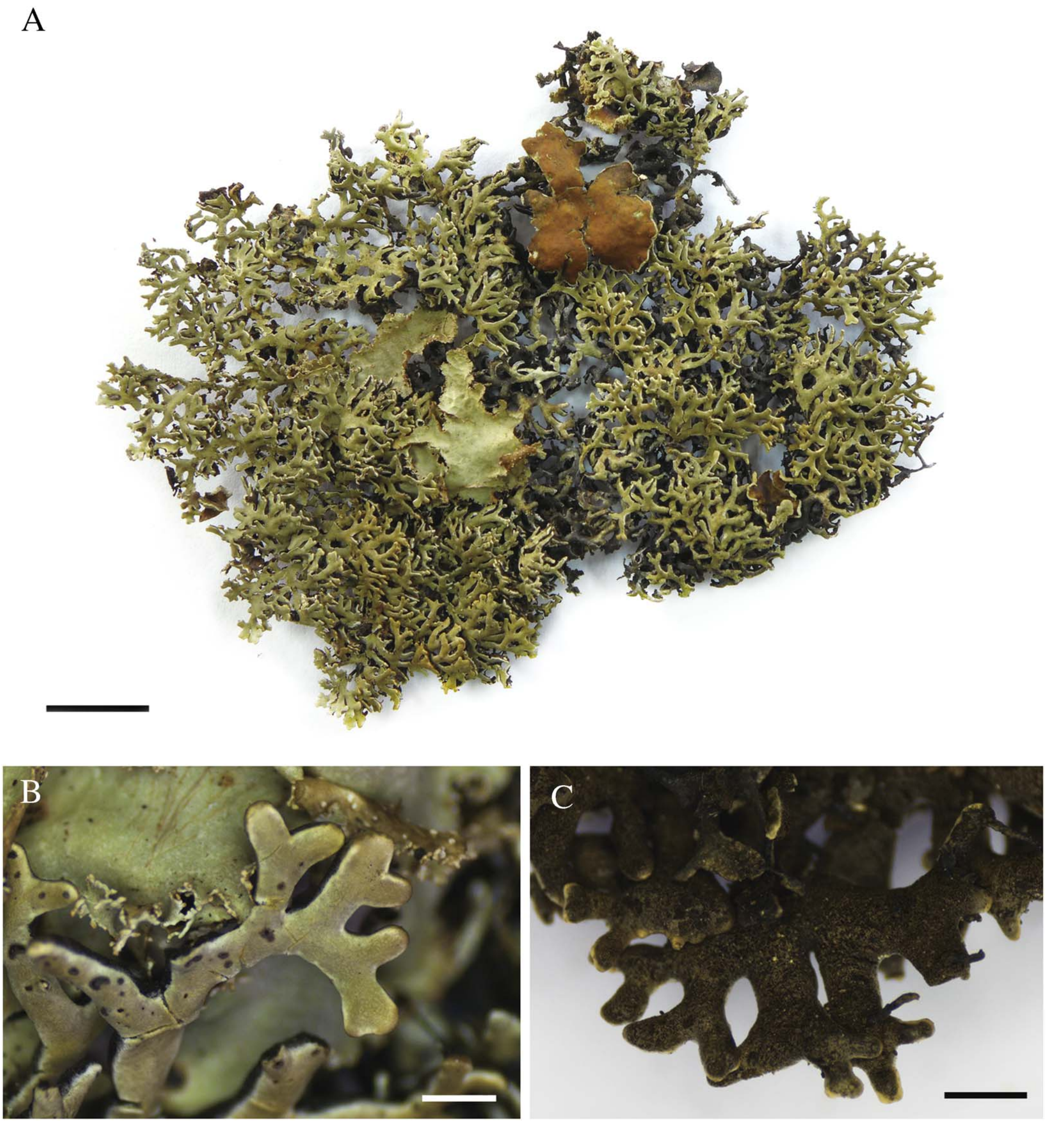

FIG. 4. *Anzia* aff. *hypoleucoides*, habit. A, thallus; B, rounded lobe apex; C, lower surface. Scales: A = 1 cm; B & C = 1 mm. In colour online.

confused with *A. hypoleucoides*, as both have a central axis and contain lobaric acid, but is distinguished by the yellow pigmented medulla, narrow lobes (less than 1 mm wide) and shallow hymenium (less than 30 µm thick). The two entities are thus morphologically and chemically distinct (Fig. 4). Substitutions in the ITS sequences distinguish *A.* aff. *hypoleucoides* from *A. hypoleucoides*, but the latter is not recovered as monophyletic. Paraphyly is not inconsistent with the recognition of a taxon and is known from cases of budding evolution or recent cladogenic events (Zander 2013). Our sampling is limited and the molecular differentiation of *A.* aff. *hypoleucoides* is weak in comparison to other species pairs, and we therefore only tentatively highlight its uniqueness until inferences from a more exhaustive sampling can be completed.

Anzia endoflavida Yoshim., *A. gregoriana* Müll. Arg., and *A. tianjarana* Yoshim. & Elix also comprise individuals with a yellow medulla. The variation in medullar pigmentation is not paralleled by changes in other traits, as seen here in *A. hypoleucoides*, and hence the yellow medulla was not given any taxonomic weight. Yoshimura (1995) noticed that the yellow pigmentation occurred only in species producing anziaic acid, and proposed that perhaps the yellow pigment may be metabolically related to anziaic acid. *Anzia* aff. *hypoleucoides*, however, lacks anziaic acid, suggesting that either these compounds are not chemically similar or that the yellow pigment in var. *flavohypoleuca* differs from that in other *Anzia* species.

Selected specimens examined. **China:** *Yunnan Prov.*: Jingdong Co., Ailao Mt., Xujiaba water reservoir, 2400 m, 24°32'22·60"N, 101°01'62·91"E, on *Quercus* bark, 2013, *L-s. Wang* KUN-L 23995; Ailao Mt., Xujiaba protection station, 3200 m, 26°38·324'N, 99°49·978'E, on *Rhododendron* bark, 2008, *L-s. Wang* KUN-L 23996; Wuliang Mt., Gaofeng, 2460 m, 24°32·808'N, 101°01·367'E, on stump, 2005, *L-s. Wang* KUN-L 23997; Gongshan Co., Yeniugu, 2950 m, 27°48·045'N, 98°49·518'E, on *Rhododendron* bark, 2000, *L-s. Wang* KUN-L 19012, 19015.

Anzia hypomelaena (Nyl.) Xin Y. Wang & Li S. Wang comb. & stat. nov.

MycoBank No.: MB 807710

Anzia leucobatoides (Nyl.) Zahlbr. f. *hypomelaena* Zahlbr. in *Symb. Sin.* **3:** 196 (1930); type: China, Yunnan, Lijiang Co., *Rock* 11575, 11778 (Y—syntypes).

Thallus foliose, greyish green to brownish green (herbarium stored), closely adnate to the substratum, thallus size medium, 3–7 cm diam., without soredia or isidia, upper surface slightly convex, sometimes with cracks along the margin in old parts, lobes 1·0–1·5 mm wide, dichotomously branched, internodes 1–3 mm long, tips blunt, rounded; *medulla* white, single layered, with flat and white central axis in the middle; lower surface covered with a black to brownish black spongy cushion, hyphae brown in section, 7–9 µm thick; rhizines rare, simple and black, 1–2 mm long, growing from the middle of the spongy cushion.

Apothecia disc-shaped, rare, usually in the centre of the surface, 1–8 mm wide; *disc* chestnut brown, epihymenium brownish, 8–10 µm thick, hymenium hyaline, 35–40 µm thick, subhymenium 50–60 µm thick, colourless; *ascus* rod-shaped, *c.* 30 µm long, with numerous ascospores spirally arranged, spores simple and curved, 10–12(± 1·0) × 2·5(± 0·5) µm.

Pycnidia rare, at the tip of the lobes.

Chemistry. Cortex K+ yellow, medulla C+ red, containing atranorin and anziaic acid.

Ecology and distribution. Common on *Rhododendron*, in subalpine to alpine elevations, between 3000 and 3800 m; known only from Yunnan and primarily from the Hengduan Mountains.

Comments. Anzia leucobatoides f. *hypomelaena* was originally separated from the type form by the colour of the spongy cushion (black vs white; Fig. 3E & F), and the smaller thallus size. Examination of the type specimen and collections from the type locality confirmed these differences and also revealed that f. *hypomelaena* differs by containing anziaic acid (medulla C+ red) rather than lobaric acid, as in f. *leucobatoides*. Such differentiation is consistent with the phylogenetic inferences from ITS sequences whereby these two entities are not most closely related (Fig. 1), and f. *hypomelaena* shares a common ancestor with the Sri Lankan endemic *A. mahaeliyensis*, which also produces anziaic acid. We therefore propose to recognize f. *hypomelaena* at the species rank, as *A. hypomelaena*.

Anzia formosana, which also contains anziaic acid, differs from *A. hypomelaena* by the two-layered medulla. Furthermore, the lobes of *A. formosana* are rimmed by a white margin and the tips are pointed, whereas they lack a differentiated margin and are rounded in *A. hypomelaena*.

Anzia hypoleucoides is also similar to *A. formosana* but differs in the black, cylindrical central axis below the medullar layer, and in the presence of lobaric acid (C–).

Selected specimens examined. **China:** *Yunnan Prov.*: Luquan Co., Zhuanlong Vil., Jiaozi Snow Mt., 3814 m, 26°04'45·6"N, 102°50'18·6"E, on *Rhododendron* bark, 2007, *L-s. Wang* KUN-L 23967; Lijiang Co., Jiuhe Vil., Laojunshan Mt., 3860 m, 26°37·940'N, 99°43·494'E, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23968; Weixi Co., Lidiping Mt., 3350 m, 27°11'01·53"N, 99°24'50·44"E, on *Loranthus* bark, 2013, *L-s. Wang* KUN-L 23969.

Anzia japonica (Tuck.) Müll. Arg.

Flora **72:** 507 (1889); type: Japan, Musahi, Mt. Ryogami, *Kurokawa* 550620 (FH—holotype).

Morphology and chemistry. See Yoshimura (1974).

Ecology and distribution. Common on *Rhododendron* and *Abies* in the subalpine region, above 3000 m; known from China (Sichuan and Yunnan) and Japan (Yoshimura 1974).

Comments. This species is characterized by the palmate (apically densely branched) lobes, the patchy and discontinuous spongy cushion (the lower surface of terminal branches appears paw-shaped), and the presence of anziaic acid (C+ red).

Anzia formosana also has a two-layered medulla and contains anziaic acid, but the thallus is larger (to 10 cm vs 5 cm in diam. in *A. japonica*), the lobe is acute rather than broadly obtuse, and the spongy cushion is continuous.

Anzia japonica may be confused with the new species *A. pseudocolpota*, which also has palmate lobes (Fig. 5B), and a patchy, discontinuous spongy cushion, but it differs in the cylindrical central medullar axis, the consistently pruinose upper surface and the presence of divaricatic acid.

Selected specimens examined. **China:** *Yunnan Prov.*: Caojian Co., Ziben Mt., 3245 m, 27°05'N, 100°11'E, on *Rhododendron* bark, 2000, *L-s. Wang* KUN-L 17893; Chuxiong City, Zixishan Mt., 2500 m, 25°44·290'N, 99°03·565'E, on *Rhododendron* bark, 1994, *L-s. Wang* KUN-L 15540; Luquan Co., Jiaozi Snow Mt., 3700 m, 26°03'N, 102°05'E, on *Abies* bark, 2006, *L-s. Wang* KUN-L 23998. *Sichuan Prov.*: Huili Co., Louzhou Mt., 3500 m, 26°03'N, 102°05'E, on *Salix* bark, 1996, *L-s. Wang* KUN-L18982.

Anzia leucobatoides (Nyl.) Zahlbr.

Nat. Pflanzenfam. **1:** 214 (1907); type: China, Yunnan, 1887, *P. J. M Delavay* (H9505563—lectotype!).

Thallus foliose, loosely adnate to the substratum, 5–10(–12) cm diam., upper surface convex, greenish grey to brownish grey (herbarium stored), without soredia or isidia, with clear cracks along the margin when mature, especially on the central part; lobes usually rather large, 1–2 mm wide, isotonic dichotomously branched, internodes 3–5 mm long, tips acute, with an angle smaller than 30°; *medulla* white, single-layered; central axis present, white and flattened, always buried inside the medulla, 200–300 μm thick, white in section (Fig. 2D); lower surface covered with continuous spongy cushion, loosely composed, narrower than upper surface, white to pale brown (Fig. 3F), hyphae hyaline in section, 12–15 μm thick; rhizines rare, simple and black, 2–4 mm long, growing from the centre of the spongy cushion.

Apothecia disc-shaped, on the central part of the upper surface, 2–15 mm wide; *disc* pale yellowish brown, epihymenium yellow, 10–12 μm thick, hymenium hyaline, 50–60 μm thick, subhymenium 70–80 μm, colourless; *ascus* rod-shaped, *c.* 50 μm long, with numerous ascospores spirally arranged, spores simple and curved, 13–15(± 1·0) × 2·5(± 0·5) μm.

Pycnidia black, prominent above the surface, usually along the lobe margin near the tips.

Chemistry. Cortex K+ yellow, medulla C–, KC+ pale red, containing atranorin and lobaric acid.

Ecology and distribution. On *Larix*, *Picea*, *Quercus* or *Rhododendron* in the subalpine region between 2500 and 3700 m; endemic to south-western China (Sichuan, Yunnan),

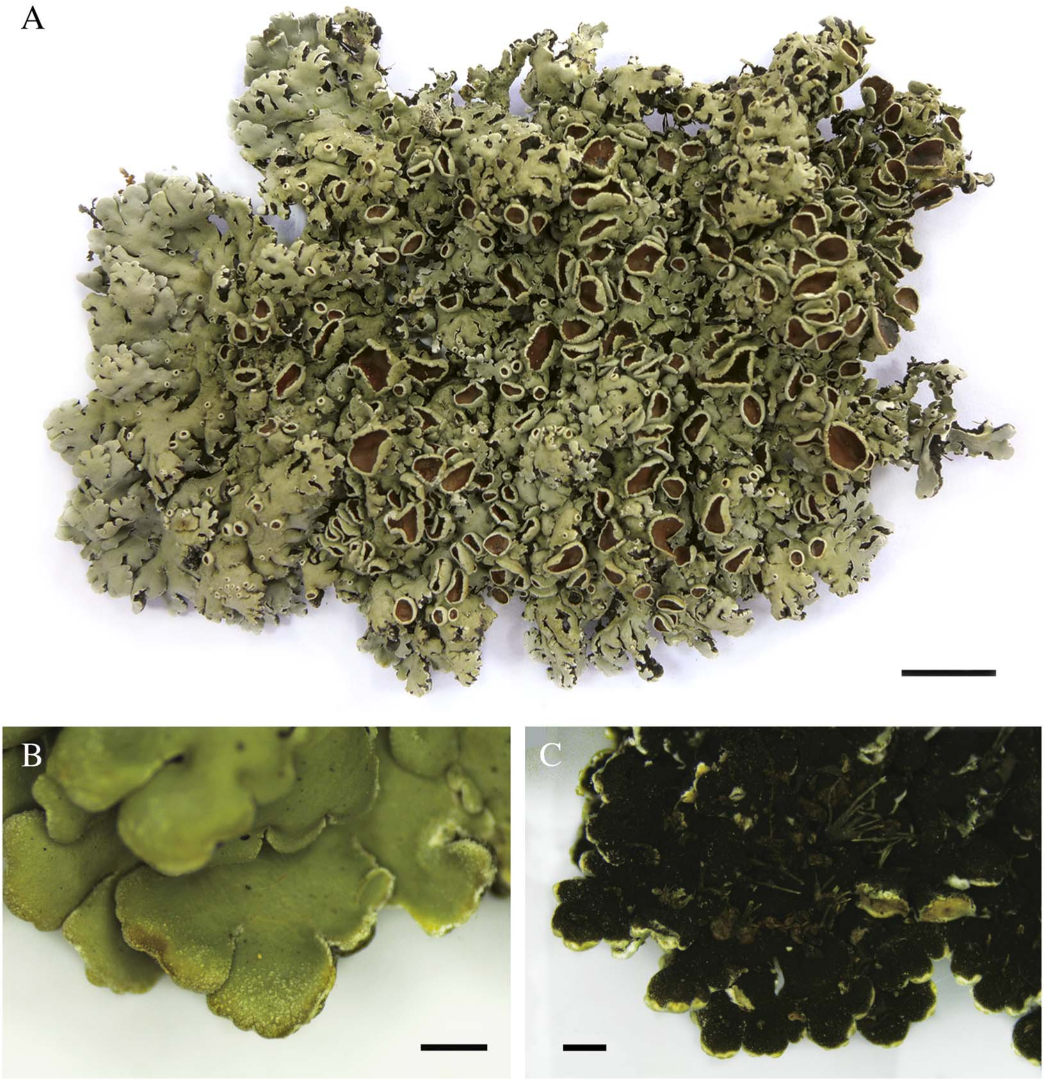

FIG. 5. *Anzia pseudocolpota*, habit. A, thallus; B, lobe apex with pruina; C, lower surface. Scales: A = 1 cm; B & C = 1 mm. In colour online.

and occurring primarily in the north-eastern part of the Hengduan Mountains.

Comments. This species is characterized by a large thallus with elongate lobes, with a thick central axis in the medulla (Fig. 2D), so that its thallus is rather flexible. The axis is always flat with a whitish colour. It is most easily distinguished from its sympatric congeners by the white to pale yellowish brown spongy cushion.

This species was first described by Nylander in Hue (1887) from Yunnan Province, Lijiang Co., without information regarding its medullary chemistry. Sato (1954) distinguished this species from *A. hypoleucoides* by the presence of anziaic acid in the medulla (C+ red), but none of our specimens collected from that region contains anziaic acid. In fact, the type specimen (H) and all other collections from the region of the type locality lack anziaic acid, but contain lobaric acid.

Anzia leucobatoides resembles *A. hypoleucoides*, which also has a central axis and contains lobaric acid, but the latter species has a black to brown spongy cushion, a black and cylindrical central axis below the medullar layer, forms smaller thalli (to 8 cm in diam.), and has red-brown (vs pale yellowish brown) apothecial discs.

Selected specimens examined. **China:** *Yunnan Prov.*: Lijiang Co., Alpine Botanical Garden, 3370 m, 27°00·204'N, 100°10·826'E, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23970; Binchuan Co., Jizu Mountain, 3220 m, 25°58·402'N, 100°21·254'E, on *Quercus* bark, 2012, *L-s. Wang* KUN-L 23971; Jingdong Co., Ailao Moutain, Xujiaba reservoir, 2500 m, 24°32'28·81"N, 101°01'01·47"E, on bark, 2013, *L-s. Wang* KUN-L 23972. *Sichuan Prov.*: Muli Co., Mahuanggou village, 2650 m, on decaying bark, 1983, *L-s. Wang* KUN-L 5179, 5280.

Anzia opuntiella Müll. Arg.

Flora **74:** 112 (1891); type: Japan, Tosha, *Miyoshi* (G—holotype).

Morphology and chemistry. See Yoshimura (1974).

Ecology and distribution. Usually on *Abies*, *Acer* or *Quercus* in the temperate mountains between 800 and 2000 m; known from Korea, Japan and China (Guizhou and Yunnan; Yoshimura 1974).

Comments. This species is characterized by and named for the opuntia-like lobes (Fig. 3C), a double-layered medulla, and the presence of divaricatic acid. It may be confused with *A. japonica*, which also has a double-layered medulla, but the latter species produces anziaic acid (C+ red), has palm-shaped lobes, and a consistently patchy and, especially near the tip, roundish spongy cushion. *Anzia ornata* also has a two-layered medulla, but is distinguished by soredia-like lobules along the margin, and regular linear rather than opuntia-like lobes. Based on the current taxon sampling, *A. opuntiella* shares a unique common ancestor with *A. colpodes* and *A. rhabdorhiza*. All three species produce divaricatic acid.

Selected specimens examined. **China:** *Yunnan Prov.*: Lijiang Co., Baishuihe village, 3000 m, on *Quercus* bark, 1985, *L-s. Wang* KUN-L 9171. *Guizhou Prov.*: Jiangkou Co., Fajingshan Mt., 2100 m, on bark, 1988, *L-s. Wang* KUN-L10703, 10840. *Zhejiang Prov.*: Hangzhou city, Linan village, Xitianmushan Mt., on bark, 1956, *Lu Ding-an* KUN-L 2580, 2538.

Anzia ornata (Zahlbr.) Asahina

J. Jap. Bot. **13:** 221 (1937); type: Taiwan, Mt. Niitaka, 1927, *Sasaki* (W—holotype; TNS—isotype).

Morphology and chemistry. See Yoshimura (1974).

Ecology and distribution. Usually on *Abies*, *Quercus* or decaying bark, and sometimes on rock in the temperate mountains between 1500 and 2900 m; known from Japan, China (Guizhou, Taiwan and Yunnan) and North America (Yoshimura 1974).

Comments. Anzia ornata differs from all other Chinese species by the marginal soredia-like, roundish lobules (Fig. 3D). It has a double-layered medulla, and contains both sekikaic and divaricatic acids. This taxon was initially known as *A. japonica* var. *ornata.* It resembles *A. japonica* s. str. due to its double-layered medulla, but the latter species produces anziaic acid (C+ red), lacks lobules on the margin, and develops a spongy cushion that is always patchy and roundish, especially near the tips. *Anzia ornata* resembles *A. ornatoides* in having lobules, but the latter species has much more linear and narrow lobes (*c.* 1 mm), contains lobaric acid and has a single-layered medulla with a central axis rather than a double-layered medulla.

Selected specimens examined. **China:** *Yunnan Prov.*: Weixi Co., Lidiping Mt., 3450 m, 26°39·207'N, 99°46·588'E, on bark, 2006, *L-s. Wang* KUN-L 23973; Lijiang Co., Alpine Botanical Garden, 3174 m, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23974. *Guizhou Prov.*: Jiangkou Co., Fanjingshan

Mt., 1400 m, 1995, *L-s. Wang* KUN-L 14346. *Taiwan Prov.*: Taizhong Co., Xueshan Mt., 3200 m, on *Rhododendron* bark, 1977, *Lai Ming-zhou* KUN-L 9329.

Anzia pseudocolpota Xin Y. Wang & Li S. Wang sp. nov.

MycoBank No.: MB 807711

Resembles *A. colpota*, from which it differs by the presence of a central axis, patchy spongiostratum and containing divaricatic acid.

Type: China, Yunnan Prov., Weixi Co., Lidiping Mt., on *Loranthus* bark, 3350 m, 15 June 2013, *Li S. Wang* 13–38274 (KUN-L 22479—holotype).

(Fig. 5)

Thallus foliose, upper surface convex, light green to greyish green, turning yellowish green when herbarium stored, closely adnate to the substratum, to 6 cm diam., without soredia or isidia; lobes linear, irregularly branched, narrow at young stage (0·5–1·0 mm wide), then widening (1–2 mm wide), tips roundish, palm-shaped, usually broadened towards the ends of the tips; pruina always present on the margin of the lobes, especially near the lobe tips, sometimes extending to the lower surface; *medulla* white, single-layered, with black central axis growing between the medulla and spongy cushion, cylindrical to oblate, *c.* 200–300 μm diam.; lower surface covered with a black spongy cushion, roundish near the lobe tips, sometimes patchy, mature lobes lacking a spongy cushion in the central part, exposing the black central axis; a spongy cushion extending beyond the lower surface when young, narrower than the surface when mature; rhizines simple, forming 3–5 bundles, 1–2 mm long (Fig. 3H).

Apothecia disc-shaped, common, usually on the central part of the upper surface; *disc* chestnut brown, 1–6 mm diam.; *epihymenium* yellow, 10–12 μm thick; *hymenium* hyaline, 70–80 μm thick, subhymenium 30–40 μm, colourless; *ascus* rod-shaped, 50–70 μm long, with numerous ascospores spirally arranged, spores simple and curved, 13–15(± 1·0) × 2–3(± 0·5) μm.

Pycnidia black, prominent above the surface, rare.

Chemistry. Cortex K+ yellow, medulla C−, KC−, containing atranorin and divaricatic acid.

Ecology and distribution. On *Larix, Picea, Quercus* or *Rhododendron* between 2500 and 3700 m in the subalpine region; known only from south-western China (Sichuan and Yunnan).

Comments. *Anzia pseudocolpota* is diagnosed, and differs from the morphologically similar *A. colpota*, by the following combination: presence of a central axis (vs a single-layered medulla without central axis), a roundish or patchy spongy cushion (vs a continuous and thick spongy layer), and wide, roundish, white pruinose lobe tips (Fig. 5B; vs narrower, acute tips, Fig. 3A). The three populations sampled for their ITS sequence form a well-supported monophyletic group of ambiguous affinities to either *A. colpodes* or the clade comprising *A. formosana* and *A. ornata* (Fig. 1).

Anzia pseudocolpota resembles *A. centrifuga* Haugan, endemic to Madeira, by the chondroidal band, pruinose lobe tips and chemistry, but 1) *A. centrifuga* is saxicolous growing on bare rock in dry, sun-exposed habitats, whereas *A. pseudocolpota* grows on bark in the subalpine region; 2) *A. centrifuga* forms large thalli (30 cm in diam.) compared to *A. pseudocolpota*, which forms much smaller thalli (*c.* 6 cm in diam.) and 3) the hymenium is deep in *A. centrifuga* (i.e., to 125 μm high; Haugan 1992), and shallower in *A. pseudocolpota* (i.e., 80 μm high). *Anzia japonica*, which has a patchy spongy cushion and roundish lobes, may be confused with *A. pseudocolpota*, but it contains anziaic acid instead of divaricatic acid, and has no central axis but a double-layered medulla.

Selected specimens examined. **China:** *Yunnan Prov.*; Lijiang Co., Alpine Botanical Garden, 3370 m, 27°00·204'N, 100°10·826'E, on *Rhododendron* bark, 2011, *L-s. Wang* KUN-L 23974; Zhongdian Co., Haba Snow Mt., 2800 m, 27°20·356'N, 100°04·776'E, on *Picea* bark, 2002, *L-s. Wang* KUN-L 23975; Weixi Co., Lidiping village, 3350 m, 27°11'01·53"N, 99°24'50·44"E, on *Loranthus* bark, 2013, *L-s. Wang* KUN-L 22479. *Sichuan Prov.*: Yanyuan Co., er-da-dui, 3250 m, on *Quercus* bark, 1983, *L-s. Wang* KUN-L 7045.

Anzia rhabdorhiza Li S. Wang & M. M. Liang

Bryologist **115:** 383 (2012); type: China, Yunnan Prov., Lijiang Co., 2011, *Wang* 11–32047 (KUN–L 20000—holotype!; HMAS—isotype).

Morphology and chemistry. See Liang *et al.* (2012).

Ecology and distribution. Growing on branches and twigs of *Rhododendron*, also on bark of *Abies*, *Picea* and *Salix*, between 2400 and 3900 m; endemic to China (Yunnan).

Comments. *Anzia rhabdorhiza* is distinguished by the long and thick rhizines wrapped by spongiostratum (Fig. 3J), a flat central axis in the medulla, and the presence of divaricatic acid. The rhizines wrapped with spongiostratum resemble those of *A. physoidea*, which is found in the eastern Himalayas. However, *A. physoidea* clearly differs from *A. rhabdorhiza* by the lack of a central axis, and the presence of lobaric rather than divaricatic acid. *Anzia hypoleucoides* also has a central axis and is similar in morphology, but differs in containing lobaric acid, and its rhizines are simple without spongiostratum wrapped around.

Selected specimens examined. **China:** *Yunnan Prov.*: Lijiang Co., Jiuhe village, Laojunshan Mt., 3516 m, 26°38·540'N, 99°45·992'E, on *Rhododendron* bark, 2010, *L-s. Wang* KUN-L 23976; Alpine Botanical Garden, 3450 m, 27°00·447'N, 100°10·502'E, on *Sorbus* bark, 2011, *L-s. Wang* KUN-L 23977; Jingdong Co., Ailao Mt., Xujiaba water reservoir, on *Salix* bark, 1994, *L-s. Wang* KUN-L 23978. *Sichuan Prov.*: Miyi Co., Malong village, Beipo Mt., 2800 m, on bark, 1983, *L-s. Wang* KUN-L 4818.

Discussion

Species of *Anzia* in China are distinguished on morphological (e.g., size and distribution of spongy tissue), anatomical (medulla with or without a central axis) and chemical characters. Characters such as medullary colour and size of the lobes have previously been neglected as taxonomic traits.

Yoshimura (1987) had rejected the concept proposed by Asahina (1935) whereby species with single versus two-layered medulla should be separated into two sections. This view is supported here as the phylogenetic structure is not congruent with the distribution of these states: *A. colpota* (single layer) is closely related to *A. formosana* and *A. ornata*, which have two layers, and *A. colpodes* (one layer) shares a putatively unique common ancestry with *A. opuntiella* (two layers; Fig. 1).

Yoshimura (1987) thus accommodated the species of *Anzia* in two subgenera based on the presence (*Nervosae*, erected by Asahina 1935) or absence (*Anzia*, which included the sections *Simplices* and *Duplices* of Asahina 1935) of a central axis. Such classification is, however, not supported by the present inferences from ITS data, as both traits define polyphyletic assemblages. The poor phylogenetic value of the central axis is best illustrated by the highly supported shared ancestry between *A. pseudocolpata* (axis present) and a clade comprising *A. formosana*, *A. ornata* and *A. colpota*, which all lack the axis, or between *A. mahaeliyensis* (no axis) and *A. hypomelaena* (with axis; Fig. 1), as already suggested by Jayalal *et al.* (2012). As both character states define polyphyletic groups, polarity of character transformation is not obvious; the central axis could be plesiomorphic for the genus and subsequently be lost in at least two lineages, or be gained independently, a hypothesis less favoured if the axis is considered a complex trait.

Among the main secondary compounds only lobaric acid currently defines a monophyletic group of three species. The ability to synthesize anziaic acid clearly evolved twice, whereas that of sekikaic acid may have arisen once but was lost again in *A. formosana* (Fig. 1).

A reconstruction of the evolutionary history of *Anzia*, and hence a better understanding of the morphological, anatomical and chemical transformations that characterize cladogenic events, must await inferences from an exhaustive taxon sampling.

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