

***ERIOBOTRYA FUSCA* SP. NOV. (ROSACEAE) FROM YUNNAN PROVINCE, CHINA**

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Abstract

Eriobotrya fusca Kuan ex Gao & Idrees, belonging to the family Rosaceae, a new species from Yunnan Province, China is described and illustrated based on both morphological and molecular data. The new species resembles closely morphologically to *E. salwinensis* Hand.-Mazz., but differs from it by having elliptic-oblong or oblong-ovate leaves, fewer pairs of lateral veins (12 - 15), lanceolate bracteoles, absent of pedicels, peduncles ca. 10 mm long, both densely brown tomentose, suborbicular petals with emarginate apices, triangular sepals with acute apices, and styles that are connate at their base. The phylogenetic analysis based on nrDNA ITS sequences confirmed the distinctiveness of this new species.

Introduction

The genus *Eriobotrya* Lindl., belongs to the family Rosaceae, subfamily Spiracoidea, tribe Pyreae, subtribe Pyrinae (Campbell *et al.* 2007, Potter *et al.* 2007). This genus is mainly distributed in China and Southeastern Asia (Lin *et al.* 1999). It includes more than 30 taxa, including species, varieties and forms (Gu and Spongberg 2003). Among these, 21 species and varieties are native to China, while other species are native to South-eastern Asia (Lin *et al.* 2004, Yang *et al.* 2005). However, there are still many Chinese species of *Eriobotrya* Lindl., believed to be unnamed (Lin *et al.* 1999). This new species, *Eriobotrya fusca*, was first collected by Liu Wei-Xin in 1999 from Lushui Xian, Yanzijiao, Yunnan Province and was so-named on the specimen sheet by Kejan Kuan (Fig. 2), but this name has apparently not published by him and could not be found in the International Plant Names Index (IPNI) at <http://www.ipni.org> and the plant list at <http://www.theplantlist.org>. The Institute of Botany (PE) and Kunming Institute of Biology (KUN) herbarium listed two collections in the name of *E. fusca*. In this paper, *Eriobotrya fusca* Kuan ex Gao & Idrees is being validated. Information about distribution and habitat, morphological and phylogenetic assessments against other congeners are provided.

Materials and Methods

The genus *Eriobotrya* Lindl., was reviewed on the basis of specialized literature and examination of all type specimens deposited in different herbaria (A, B, P, K, IBK, BM, IBSC, HUH, WU). During visits to different herbaria (CDBI, CANT, IBSC, KUN, PE and SYS) in China, an undescribed species was found collected from southwest Yunnan. The macromorphological and micromorphological features were analysed using an optical microscope (WILD, Heerbrugg; Nikon SMZ1000) and photographed. The morphological characters of studied specimens were compared with previously described species.

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The genomic DNA was extracted from dry specimen, using the Tiangen Plant Genomic DNA Extraction Kit (Beijing) according to instructions from the manufactures. Nuclear ITS primer pairs using ITSF and ITSr (Li *et al.* 2009) were used for amplification of the nuclear ITS regions. PCR amplification reactions were performed using SimpLiAmp thermo cycler (Applied Biosystem, Life technology). Conditions for amplification of the region consist of initial denaturation at 94°C for 3 min, then 35 cycles at 94°C for 30 sec (denaturation), annealing temperature at 48°C for 1 min, 72°C for 1 min (extension), with the final step of extension at 72°C for 7 min.

The sequence of new species was BLAST-searched (<http://www.ncbi.nlm.nih.gov/>). Closet sequences and others published sequences of *Eriobotrya* species were retrieved from Genbank and included in the final data set (Table 1). *Malus sieboldii* (Regel) Rehders (1915: 293), *Mespilus germanica* Linnaeus (1753: 478), *Photina beauverdiana* C. K. Schneid (1906: 309), and *Raphiolepis indica* (Linnaeus 1753: 477) Lindley (1820: t. 468) were used an outgroup for rooting purposes following Campbell *et al.* (1995, 2007). Sequences alignment were performed using ClustalW (Larkin *et al.* 2007) with default parameter as implemented in MEGA 6 (Tamura *et al.* 2013) and manually edited using Bioedit sequence alignment editor v 7.0.5.3 (Hall 1999). Phylogenetic analysis was undertaken using the Maximum Likelihood (ML) method. The K2+G model was selected as the best model for phylogenetic analysis. ML analysis was performed using MEGA 6 (Tamura *et al.* 2013), with gaps treated as missing data. Support values were assessed using the bootstrap option with 1000 replicates. All the sequences were deposited in Genbank (Table1).

Results and Discussion

Phylogenetic analyses identified a total of 14 taxa and together with the closest hit of the ITS sequence which were retrieved from NCBI and included in the final data set to construct the phylogenetic tree (Table 1).

The final ITS data set comprised of 309 aligned DNA characters, of which 64 were variable and 27 were informative. The phylogenetic analysis of the ITS sequences did not resolve the relationships of the new species, but it formed a trichotomy with *E. fragrans* and *E. serrata*, and with the rest of the genus. The new species had a long branch confirming its novelty in the genus (Fig. 1).

Eriobotrya fusca Kuan ex Gao & Idrees (Figs 2 - 3)

Diagnosis: The new species morphologically closely resembles to *E. salwinensis* Hand.-Mazz., but differs from it by having elliptic-oblong or oblong-ovate leaves, fewer pairs of lateral veins (12 - 15), lanceolate bracteoles, pedicels absent and peduncle ca. 10 mm long, both densely brown tomentose, suborbicular petals with emarginate apices, triangular sepals with acute apices, and style with connate base. In contrast, *E. salwinensis* has obovate-lanceolate leaves, more pairs of lateral veins (15 - 20), ovate bracteoles, pedicel 2 - 3 mm and peduncles absent, both densely rusty tomentose, obovate petals with rounded apices, ovate sepal with obtuse apices, and style with free base.

Type: SW China, Yunnan Province, Lushui Xian, Yanzijiao, alt. 1450 m, March 1957, Liu Wei-Xin 103 (holotype: PE!, Isotypes: PE!, KUN!).

Etymology: The specific epithet *fusca* derived from the Latin for brown referring to the brown tomentose inflorescence.

Table 1. List of species, and Genbank Accession number of DNA sequences used in present study. New sequences are marked bold.

Species Name	Locality	Vouchers	Herbarium	Genbank Acc. No. ITS
<i>E. bengalensis</i> (Roxb.) Hook. f.	China		IBSC	MH246941 ^a
<i>E. bengalensis</i> var. <i>angustifolia</i> Card.	China, Yunnan		IBSC	MH246942 ^a
<i>E. bengalensis</i> var. <i>intermedia</i> Vidal	"		KUN	MG938047 ^a
<i>E. fusca</i> Kuan ex Gao & Idrees	"		PE	MF595535 ^a
<i>E. cavaleriei</i> (H.Lévl.) Rehd.	China, Guangxi		PE	MH246944 ^a
<i>E. deflexa</i> (Hemsl.) Nakai	China, Taiwan		PE	MG938042 ^a
<i>E. deflexa</i> var. <i>buisanensis</i> (Hay.) Hayata	"		IBSC	MG938043 ^a
<i>E. fragrans</i> Champ. ex Benth.	China, Guandong		IBSC	MH246945 ^a
<i>E. henryi</i> Nakai	GenBank		-	KJ170777 ^b
<i>E. hookeriana</i> Decne.	Bhutan		PE	MG938046 ^a
<i>E. japonica</i> (Thunb.) Lindl.	China, Sichuan		PE	MG938044 ^a
<i>E. malipoensis</i> K.C. Kuan	China, Yunnan		IBSC	MH246947 ^a
<i>E. obovata</i> W.W. Sm.	"		PE	MH246948 ^a
<i>E. petiolata</i> Hook. f.	Bhutan		PE	MH246949 ^a
<i>E. prinooides</i> Rehder & E.W. Wilson	China, Yunnan		IBSC	MH246950 ^a
<i>E. salwinensis</i> Hand.-Mazz.	"		KUN	MG938048 ^a
<i>E. seguinii</i> (H.Lév.) Card. ex Guill.	"		-	FJ571507 ^b
<i>E. serrata</i> Vidal	"		KUN	MG938049 ^a
<i>E. tengyuehensis</i> W.W.Sm.	"		-	FJ796915 ^b
<i>Mespilus germanica</i> L.	USA, Chicago	M645-80	-	EF127040 ^{ab}
<i>Photinia beauverdiana</i> C.K. Schneid.	GenBank	Voucher 1733-80A	-	JQ392492 ^{ab}
<i>Rhaphiolepis indica</i> (L.) Lindl.	GenBank		-	KP093148 ^{ab}
<i>Malus sieboldii</i> (Regel) Rehder	Japan, C. & E. China	East Malling A1406	-	AF186505 ^{ab}

^a Stands for sequences used in the present study, ^b stands for sequences from Genbank, ^{ab} stands for sequences used as an outgroup, - stands for no information about the specimen.

Shrub or small tree, up to 5 m tall. Branchlets brownish grey, smooth, slender, tomentose when young, later glabrescent; Stipules caducous, not seen; Petioles 2.5 - 4.0 cm long, stout, initially tomentose, soon glabrous; Leaf blade elliptic-oblong or oblong-ovate, 11 - 20 × 6.5 - 8 cm, coriaceous, base cuneate, leaf margins remotely serrate apically, space 4 - 5 mm, entire at base, tomentose when young, glabrous when mature, apex acute or shortly acuminate; lateral veins 12 -15 pairs, craspedo-dromous, tertiary veins conspicuously reticulate, abaxially brown tomentose, glabrescent when mature, adaxially lustrous. Inflorescence terminal panicle, 12 - 16 cm long, spreading, brownish tomentose, many flowered. Peduncle ca. 10 mm long, densely brown tomentose; Bracts and bracteoles lanceolate, densely brown tomentose; Flowers sessile, densely brown tomentose, yellowish. Calyx tube 5, 4 mm long, lobes ca. 2 mm long, tomentose; Hypanthium cupular, tomentose; Sepals triangular, abaxially brown tomentose, adaxially glabrous, Petals suborbicular, 4 - 5 mm long, apex emarginated, villous at base; Sepals triangular, abaxially brown tomentose, adaxially glabrous, stamens 15 - 20, filament 3 mm long, anthers 1 mm long; Ovary pubescent apically; Styles 3 in number, connate and sparsely villous at base, 2 mm long. Fruit not seen.

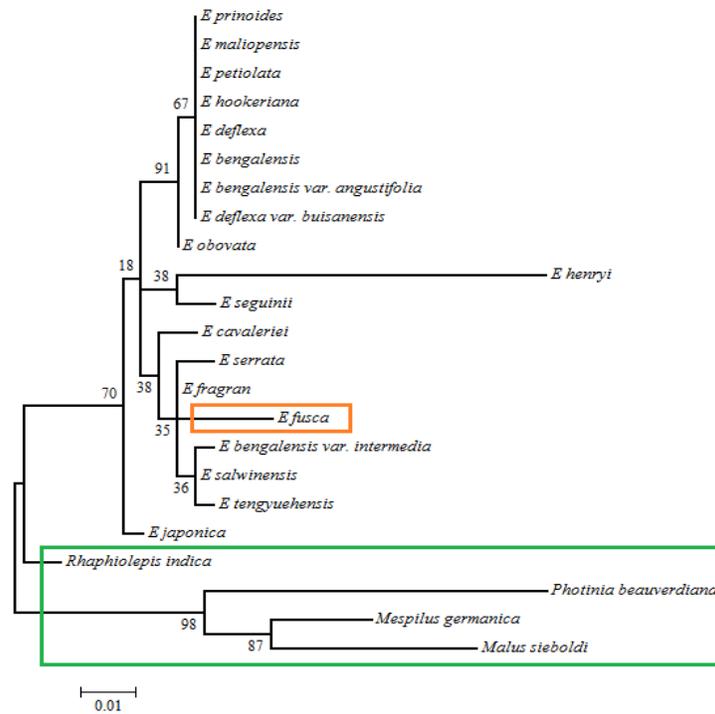


Fig. 1. Details of the Maximum Likelihood analysis derived from ITS sequences. Numbers in the branches are bootstrap percentages above 50%; New species sequence label with a red color; while the outgroup label with green color.

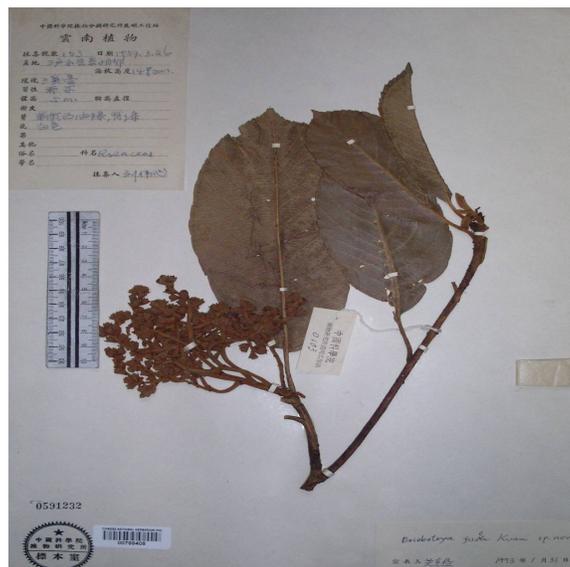


Fig. 2. Holotype of *Eriobotrya fusca* Kuan ex Gao & Idrees.

The new species is found along side stream or river at ca. 1450 m elev. SW Yunnan, China (Based on annotated specimens).

Many new species and hybrid of *Eriobotrya* have been reported in recent years based on morphological and molecular markers and this has dramatically increased the number of known *Eriobotrya* species (Yang *et al.* 2007, Li *et al.* 2012, Wang *et al.* 2014, Idrees *et al.* 2018) and hybrid species (Ding *et al.* 2015).

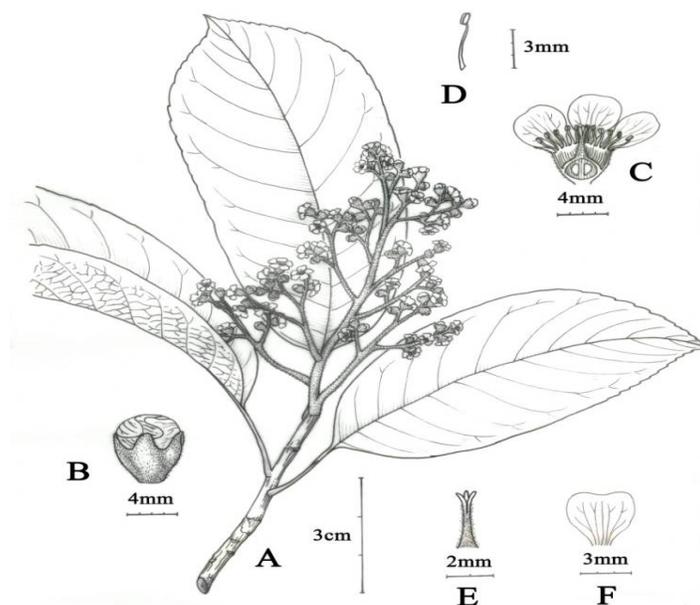


Fig. 3. *Eriobotrya fusca* Kuan ex Gao & Idrees, A. Habit, branch tip with leaves and inflorescence, B. Flower bud showing lobes calyx, C. Longitudinal section through Flower, D. Stamen, E. Style, F. Petal. Illustration by Jing Tian from the holotype.

Table 2. Comparison of morphological characters of new species with *E. salwinensis*.

Characters	<i>E. fusca</i>	<i>E. salwinensis</i> ^{1,2}
Leaf blade	Elliptic-oblong or oblong-ovate	Obovate-lanceolate, rarely elliptic
Blade length × width (cm)	11 - 20 × 6.5 - 8	10 - 20 × 2.5 - 6.5
Petiole length (cm)	2.5 - 4.0	2 - 3
Lateral vein (pairs)	12 - 15	15 - 20
Pedicel (mm)	Sessile	2 - 3
Peduncle (mm)	10	Short or nearly absent
Tomentose on pedicel & peduncle	Densely brown	Densely rusty
Petals (mm)	Suborbicular, yellow, 4 - 5, apex emarginated	Obovate, yellow, 5, apex rounded
Sepals (mm)	Triangular, 2 - 4, apex acute	Ovate, 5 mm, apex obtuse
Style number and size (mm)	3, connate, 2 and villous at base	2-3, free, 2, villous at base

⁽¹⁾ From the original protologue (Hand.-Mazz., 1929). Type: China, Handel-Mazzetti 9573 (WU!). ⁽²⁾ From the description (Gu Cuizhi and Spongberg SA (2003).

The new species morphologically closely resembles to *E. salwinensis* Hand.-Mazz. (leaf dimension, leaf margins and size of inflorescence) but differs from it by having elliptic-oblong or oblong-ovate leaves, less pairs of lateral veins (12 - 15), bracteoles lanceolate, pedicels sessile and peduncle 10 mm long, both densely brown tomentose, suborbicular petals with emarginate apices, triangular sepals with acute apices, and style that are connate at their base. In contrast, *E. salwinensis* has obovate-lanceolate leaves, more pairs of lateral veins (15 - 20), bracteoles ovate, pedicel 2 - 3 mm and peduncles short or nearly absent, both densely rusty tomentose, obovate petals with rounded apices, ovate sepal with obtuse apices, and style that are free at their base.

Present phylogenetic analysis resolved *E. fusca* sp. nov. in a separate clade with long branch indicating its isolated phylogenetic position in the genus. The phylogenetic analysis, based on nrITS cladistic analysis, demonstrated that the new species is sister to clade with *E. fragrans* Champion ex Bentham (1852: 80) and *E. serrata* J. E. Vidal (1965: 558) but morphologically, it can be distinguished by the former by leaf blades 11 - 20 × 6.5 - 8 cm, more pair of lateral veins (12 - 15), bracteoles lanceolate, pedicels sessile; suborbicular petals, 8 × 5 mm, triangular sepals and styles 3. In contrast *E. fragrans* has leaf blades 7 - 18 × 3 - 5.5 cm, less pair of lateral veins (10), bracteoles not seen, pedicel 3 - 5 mm, elliptic or broadly ovate petals, 8 × 5 mm, triangular-ovate sepal, and styles 4 or 5. The new species can be distinguished by the latter by leaf blades elliptic-oblong or oblong-ovate, 11 - 20 × 6.5 - 8 cm, leaf base cuneate, leaf apex acute or shortly acuminate, leaf margins remotely serrate apically, tomentose; bracteoles lanceolate, pedicels and peduncle densely brown tomentose; petals suborbicular, 4 - 5 mm; triangular sepals, 2 - 4 mm, apex acute. In contrast, *E. serrata* has leaf blades oblanceolate or obovate, 20 - 30 × 5 - 10 (-13) cm, leaf base attenuate, leaf apex obtuse or acute leaf margins sharply incurved-serrate, glabrous; bracteoles not seen, pedicels and peduncle densely yellow tomentose; obovate petals, 3 - 3.5 mm; ovate sepals, 2 cm, apex obtuse.

Specimens studied: *Eriobotrya fusca* sp. nov.: **SW. China**, Yunnan Province, Lushui Xian, Yanzijiao village, streamside or along river side, Alt. 1450 m. Liu Wei-Xin 103 (holotype: PE 00799408!; Isotype: PE 0079940!; KUN 0652504! and KUN 0313289!).

Eriobotrya salwinensis Hand.-Mazz.: **NW. China**, Yunnan Province, found mixed broad-leaved forests at alt. 1600 - 1700 m. Handel-Mazetti, H. R. E. von, 9573 (holotype: WU 0059392!; Isotype: A 00026480!). Anon. 8773, 23 May, 1960 (PE 00799705!), Feng, K. M. 6967, 26 Aug., 1940 (PE 00799700!), Feng, K. M. 7038, 28 Aug., 1940 (PE 00799703!), Feng, K. M. 7374, 4 Sept., 1940 (PE 00799708!), Handel-Mazetti 9107 (WU 0059393!), Qinghai-Tibet Vegetation Group 4455, 30 June, 1975 (PE 00051918!), Qingzang Team 8136, 18 July, 1982 (PE 01147727!), Qingzang Team 7390, 23 July, 1982 (PE 01147725!), Yu, T. T. 19180, 25 June, 1938 (PE 00799704!), Zhongdian team 63-4234, 19 Aug., 1963 (LBG 00065279!). **MYANMAR:** Kingdon-Ward, F. 20699 (E!; MB!), Li, H., Ji, Y. H. & Li, H. 14212, 13 April, 2002 (CAS 325545!), Li, H., Dao, Z., Ji, Y. H., Jin, X. H. G., Peter, F., Simon, C., Jin, H. P. & Catherine, B. 33001, 11 Aug., 2006 (CAS 325546!). **THAILAND:** Maxwell, J. F. 06-116, 3 Feb., 2006 (Fl. Thailand), Maxwell, J. F. 05-680, 3 Nov., 2005 (Fl. Thailand).

Eriobotrya fragrans Champion ex Bentham: **Hong Kong**, found in stream side, dry forest ground or woody species of low area or in a ravine on Mount Victoria at alt. 800 - 900 m. Champion s.n. (holotype: K 000758384!), Anon. 397(K 000758383!), Ford s. n., 2 June, 1885 (K 000758385!), Hu, S. Y. 11618, 23 March, 1972 (PE 00799397!), Hu, S. Y. 13559, 28 March, 1975 (PE 00799370!), Hu, S. Y. 13209, 31 March, 1973 (PE 00799395!), Hu, S. Y. 13238, 7 April, 1973 (PE 00799394!), Hu, S. Y. 10750, 30 July, 1970 (PE 00799399!), Hu, S. Y. 11255, 20 Nov., 1971 (PE 00799396!), Tsiang, Y. 242, 18 April, 1928 (PE 00799368!, PE 00799386!). **CHINA, Fujian:** Fujian Compl. Exped. 2297, 25 April, 1965 (NAS 00349792). **Guangdong:** Chen, N. Q.

41087, 24 May, 1930 (PE 00799369!), Chen, N. Q. 41316, 22 July, 1930 (PE 00799371!), Chen, N. Q. 41674, 25 Aug., 1930 (PE 00799401!), Chu, T. S. 642, 20 June, 1951 (IBSC 0299146!), Deng, L. 2311, 15 Aug., 1956 (PE 00799388!), Deng, L. 1070, 28 Aug., 1956 (PE 00799372!), Deng, L. 8669, 29 Nov., 1958 (PE 00799387!), Fu, H. T. 701136 (IBSC 0299185!), Gao, X. P. 51322, 15 April, 1931 (PE 00799380!), Guangdong Wood Survey Group 769 (IBSC 0299167!), Huang, Z. 32283, 30 April, 1932 (PE 00799402!), Huang, Z. 30935, 15 July, 1931 (PE 00799398!), Taam, Y. W. 114, 31 Nov 1937 (IBSC 0299133!), Tan, P. X. 58735, 10 July 1958 (PE 00799389!), Tan P. X. 58945, 11 Aug., 1958 (PE 00799377!), Tsang, W. T. 21587, 26 April, 1932 (NAS 00349807!), Tsang, W. T. 20479, 15 May, 1932 (P02140138!, PE 00799367!), Tsang, W. T. 20479, 15 May, 1930 (PE 00799400!), Tsang, W. T. 20573, 26 May, 1932 (PE 00799366!, PE 00799393!), Tsang, W. T. 28674, (IBSC 0299155!), Tsui, T. M. 814, July-Sept., 1932 (P02140137!, PE 00799384!), Wei, Z. F. 121006, 2 July, 1958 (PE 00799383!), Wei, Z. F. 121617 (NAS 00349799!), Wei, Z. F. 121617, 5 Sept., 1958 (PE 00799379!). **Guangsi:** Chen, S. Q. 4637, 4 March 1944 (IBSC 0299191!), Lau, S. K. 123-A, 19 June, 1932 (P 03240136!), Qin, R. C. 8074, 21 Oct., 1928 (PE 00799403!), Xin, Z. Z. 9047 (IBSC 0299187!), Zhang, H. 12860 (IBK 000060984!). **Jiangxi:** Deng, L. 4104, 6 April, 1957 (PE 00799373!).

Eriobotrya serrata J. E. Vidal: **Laos** (Xiang Khouang), found in forest on slope at alt. 1100–1900 m Poilane, E. 2345 (Holotype: P 02143235!) Isotypes: (P 02143236!; P 02143237!). **CHINA, Yunnan:** Ching, R. C. 50097, 1952 (PE 00799723!), Feng, G. M. 14488, 3 Feb 1952 (PE 00799750!), Feng, G. M. 14288, 22 Dec., 1951 (PE 00799754!), Liu, W. X. 604, 11 Aug 1953 (PE 00799742!), Sino-Russ. Exped. Yunnan Team 5220, 25 Feb. 1957 (KUN 0116176!, PE 00799743!), Sino-Russ. Exped. Yunnan Team 3137, 21 May 1956 (PE 00799748!), Tax, Y. M. 003915, 1 Nov., 1933 (PE 00799720!), Tsiang, Y. 13506, 1933 (PE 00799724!), Wang, C. W. 75373, June, 1936 (PE 00799751!), Wang, C. W. 74188, May, 1936 (PE 00799722!), Wang, C. W. & Liu, Y. 88511, 14 April, 1940 (PE 00799746!), 68-Wenshan formation 307, 11 Nov., 1965 (PE 00799744!).

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