

基于分子和形态学证据确定传统中药白花蛇舌草及其替代品的学名

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摘要: 白花蛇舌草是我国重要的传统中药, 主要是指茜草科(Rubiaceae)钮扣草族(Spermacoceae)的 *Oldenlandia diffusa*, 但伞房花耳草(*O. corymbosa*)在民间或中药市场也常被作为替代品使用。由于长期以来 *Hedyotis-Oldenlandia* 复合群的分类存在许多争论, 因此白花蛇舌草有时被归入非洲耳草属(*Oldenlandia* L.), 有时又作为广义耳草属(*Hedyotis* L. s. l.)的成员。为了澄清白花蛇舌草命名上的问题, 基于 7 个叶绿体片段和 2 个核基因片段对钮扣草族 85 个分类群进行了系统发育分析。结果表明, 白花蛇舌草不属于以上两属中的任何一属, 而应为蛇舌草属[*Scleromitron* (Wight & Arn.) Meisn.]的成员。依此结果, 对 5 种植物进行了新组合, 并提供了白花蛇舌草和伞房花耳草的形态学比较, 以有助于在实践中更好地进行区分。

关键词: 白花蛇舌草; 分子系统学; 非洲蛇舌草属; 蛇舌草属; 中药

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Nomenclature Clarification of the Traditional Chinese Medicine Baihuasheshecao and Its Adulterants Based on Molecular and Morphological Evidence

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Abstract: Baihuasheshecao is one of the important traditional Chinese medicines and mainly refers to *Oldenlandia diffusa* but can be adulterated with *O. corymbosa* in commercial and folk herbal medicine. Because of the long-time taxonomic debate and confusion about the taxonomy of *Hedyotis-Oldenlandia* complex, the scientific names of Baihuasheshecao components were treated under the genus of either *Oldenlandia* or *Hedyotis* in practice. In order to clarify the nomenclatural confusion, a comprehensive molecular phylogenetic analysis was conducted on basis of seven plastid loci and two nuclear gene regions from the selected 85 taxa in the tribe Spermacoceae (Rubiaceae). The results demonstrated that *O. diffusa* should not be a member of *Oldenlandia* L. but *Scleromitron* (Wight & Arn.) Meisn. Five new combinations are accordingly made and a detailed comparison between the two species is provided for identification in future practice.

Key words: Baihuasheshecao; Molecular phylogeny; *Oldenlandia*; *Scleromitron*; Traditional Chinese medicine

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Baihuasheshecao is a popular traditional medicine for anti-inflammatory, hepatoprotective, neuroprotective, antioxidant, antipyretic, diuretic, blood-stimulant, anti-carbuncular effects and enormous potential in the therapy of cancer and tumor in many Asian countries^[1-3]. It is also an important ingredient of herbal tea for health maintenance in the Eastern and tropical Asia^[4]. According to *Pharmacopoeia of the People's Republic of China* (2005), Baihuasheshecao mainly refers to the species *Oldenlandia diffusa*, but can be substituted by and interchanged with *O. corymbosa* in the official medication^[5]. However, it is also usually adulterated with *O. pinifolia* and *O. verticillata* etc. in folk.

It was reported that *Oldenlandia corymbosa* has antipyretic purposes in traditional Thai medicine^[6] and hepatoprotective effect known as “Parppatakapullu” in India^[7]. And many recent studies revealed that *O. diffusa* and *O. corymbosa* had different chemical compositions and bioactivities^[4,8-9].

Hedyotis-Oldenlandia complex belongs to the tribe Spermacoaceae of the family Rubiaceae. The centuries' taxonomic debate and confusion about the congeneric or separate between *Hedyotis* L. and *Oldenlandia* L. bewildered not only the plant taxonomists for species description but the traditional Chinese physicians for medicinal practice. Consequently, most species described under *Hedyotis* almost certainly has a synonym under *Oldenlandia*, vice versa. This caused almost arbitrary or random nomenclature choices^[10]. Hao et al.^[11] doubted the unrelated phylogenetic relationship between *O. diffusa* and *O. corymbosa*, but they did not uncover it explicitly due to the lack of enough research materials at that time. Recent phylogenetic study based on large scale samples from Asian countries verified the previous doubt and revealed that these two species belonged to two different clades representing different evolutionary lineages^[10,12]. In the present study, a comprehensive molecular phylogenetic analysis was conducted on basis of seven plastid loci (*petD*, *rps16*, *trnL-trnF*, *atpB-rbcL*, *matK*, *rbcL*, *trnH-psbA*) and two nuclear gene regions (ETS, ITS) of 85 selected taxa from the newly defined tribe Spermacoaceae^[13]. This study

not only is an integration of previous series results but will present a deep analysis for this complicate complex to reveal the phylogenetic relationship of different components of Baihuasheshecao and clarify their taxonomic and nomenclature confusions.

1 Methods

1.1 Taxon sampling

Referring to the several recent publications^[10,12-14], 54 ingroup taxa related to *Oldenlandia diffusa* or *O. corymbosa*, as well as 31 associated taxa, were selected for the present phylogenetic analysis. The generic name *Oldenlandia*, rather than *Hedyotis*, was chosen for the ingroup taxa (if have) so as to keep consistency with the previous phylogenetic analysis. Detailed information about species names used currently and previously, vouchers, and GenBank accession No. is given in Table 1.

1.2 Pre-treatment for the taxon names used in this paper

Some scientific names of the selected taxa were pre-treated in order to address the taxonomic issues explicitly.

The name *Oldenlandia erecta* of Li Heng 11298 (A) from China and Neupane 2 (ODU) from Nepal^[10] was replaced by *O. corymbosa* var. *linearis* according to the treatments by Deb and Dutta^[15] and Dutta and Deb^[16].

Oldenlandia tenelliflora has ever been doubted to be possibly indistinct from *Scleromitron angustifolium* (Cham. & Schlecht.) Benth.^[17]. The detailed examination about the type materials of *O. tenelliflora* from Java showed that it has ovate and 1–1.7 cm wide (vs. linear or linear-lanceolate, 0.3–0.9 cm wide in *S. angustifolium*) leaves, 7–9-bristled (vs. 1–4-bristled in *S. angustifolium*) stipules, and septically from base to apex and then loculicidally (vs. loculicidally from apex only in *S. angustifolium*) dehiscent capsules. The Chinese samples are in agreement with the characters of *S. angustifolium*.

Moreover, the samples of YU05, YU06, and

Table 1 List of taxa sampled with taxonomic authorities, voucher information and GenBank accession No.

No.	Name used in this paper	Name used while sequenced*	Voucher (Herbarium)	ETS	ITS	petD	rps16	trnL-trnF	atpB-rbcL	matK	rbcL	trnH-psbA
1	<i>Agathisanthemum bojeri</i> Klotsch		Zambia: Dessein et al. 671 (BR)	/	AM939424	EU557678	EU543018	EU543077	EU542917	/	/	/
2	<i>Arcyophytium muticum</i> (Wedd.) Standl.		Colombia: Andersson et al. 2195 (GB)	/	AM939429	EU557682	AF002754	EU543081	EU542921	/	/	/
3	<i>Batopedina pulvinellata</i> Robb.		Zambia: Dessein et al. 264 (BR)	/	/	EU557684	EU543021	EU543083	EU542924	/	/	/
4	<i>Bouvardia glaberrima</i> Engelm.		Unknown: cult. Forbes s.n. (S)	AM932922	AM939432	EU557685	EU543022	EU543084	EU542925	/	/	/
5	<i>Carphalea madagascariensis</i> Lam.		Madagascar: Razafimandimbison 524 (UPS)	/	AM266995	/	/	AM266908	/	/	/	/
6	<i>Cordylostigma microcala</i> (Bremek.) Groeninckx & Dessein		Zambia: Dessein et al. 1149 (BR)	AM932962	AM939479	EU557725	EU543039	EU543121	EU542966	/	/	/
7	<i>C. virgata</i> (Willd.) Groeninckx & Dessein		Madagascar: De Block et al. 539 (BR)	AM932965	AM939483	EU557728	/	EU543124	EU542969	/	/	/
8	<i>Dentella repens</i> (L.) J. R. Forst. & G. Forst.		Australia: Andersson 2262 (GB)	AM932930	AM939440	EU557693	AF333370	EU543091	EU542932	/	/	/
9	<i>Dibrachionostylus kaessneri</i> (S. Moore) Bremek.		Kenya: Strid 2598 (GB)	AM932932	AM939442	EU557694	AF002761	/	EU542933	/	/	/
10	<i>Hedyotis ampliflora</i> Hance		China: Ruijiang Wang et al. 1147 (IBSC)	/	JX111198	JX111086	JX111242	JX111317	/	JX111051	JX111124	JX111161
11	<i>H. cantoniensis</i> How ex Ko		China: Ruijiang Wang et al. 1250 (IBSC)	/	JF699912	JF700061	JX111247	JX111322	/	JF699987	JF699836	JF699773
12	<i>H. hedyotideae</i> (DC.) Merr.		China: Ruijiang Wang et al. 1249 (IBSC)	/	JX111213	JX111099	JX111265	JX111338	/	JX111062	JX111136	JX111172
13	<i>H. koana</i> R. J. Wang		China: Ruijiang Wang et al. 978 (IBSC)	/	JX111215	JX111101	JX111267	JX111341	/	JX111064	JX111138	JX111174
14	<i>H. koana</i> R. J. Wang	<i>Oldenlandia herbacea</i> (L.) Roxb.	China: YU05 (CUHK)	/	HQ148809	/	HM752967	HM752882	/	HM753137	/	HM640367
15	<i>H. koana</i> R. J. Wang	<i>Oldenlandia herbacea</i> (L.) Roxb.	China: YU06 (CUHK)	/	HQ148810	/	HM752968	HM752883	/	HM753138	/	HM640368
16	<i>H. koana</i> R. J. Wang	<i>Oldenlandia herbacea</i> (L.) Roxb.	China: YU07 (CUHK)	/	HQ148811	/	HM752969	HM752884	/	HM753139	/	HM640369

续表(Continued)

No.	Name used in this paper	Name used while sequenced*	Voucher (Herbarium)	ETS	ITS	petD	rps16	trnL-trnF	atpB-rbcL	matK	rbcL	trnH-psbA
17	<i>H. merguensis</i> Hook. f.		China: Xing Guo & Ping Yang 22-1 (IBSC)	/	JX111218	JX111104	JX111270	JX111344	/	JX111067	JX111141	JX111177
18	<i>H. yangchunensis</i> Ko & Zhang		China: Ruijiang Wang 1270-1 (IBSC)	/	JF699972	JF700122	JX111290	JX111364	/	JF700046	JF699897	JF699821
19	<i>Houstonia caerulea</i> L.		USA: Vincent & Lammers s.n. (GB)	/	AM939464	EU557713	AF333379	EU543109	EU542953	/	/	/
20	<i>Kadua acuminata</i> Cham. & Schltdl.		Hawaii: cult. at BR	AM932952	AM939467	EU557714	/	EU543110	EU542955	/	/	/
21	<i>K. centranthoides</i> Hook. & Arn.		Hawaii: Skottsberg 6788 (S)	/	AM939468	EU557715	EU543033	EU543111	EU542956	/	/	/
22	<i>Kohautia coccinea</i> Royle		Zambia: Dessein et al. 751 (BR)	AM932959	AM939477	EU557723	EU543037	EU543119	EU542964	/	/	/
23	<i>K. cynanchica</i> DC.		Zambia: Dessein et al. 469 (BR)	AM932960	AM939477	EU557724	EU543038	EU543120	EU542965	/	/	/
24	<i>Lelya ostocarpa</i> Bremek.		Tanzania: Cereau 2513 (BR)	/	AM939485	EU557729	/	EU543125	EU542970	/	/	/
25	<i>Manettia lygistum</i> (L.) Sw.		Colombia: Andersson et al. 2128 (GB)	AM932967	AM939487	EU557730	AF002769	EU543126	EU542972	/	/	/
26	<i>Mitrasacmopsis quadrivalvis</i> Jovet		Zambia: Dessein et al. 1273 (BR)	AM932970	AM939490	EU557733	EU543045	EU543129	EU542976	/	/	/
27	<i>Neanotis wightiana</i> (Wall. ex Wight & Arn.) W. H. Lewis		China: Xing Guo 46 (IBSC)	/	JX111236	JX111118	JX111294	JX111368	/	JX111080	JX111155	JX111190
28	<i>Oldenlandia capensis</i> L. f.		Zambia: Dessein et al. 843 (BR)	AM932974	AM939496	EU557737	EU543048	EU543133	EU542980	/	/	/
29	<i>O. capensis</i> L. f. var. <i>pletiosepala</i> Bremek.		Tanzania: Kayombe et al. s.n. (BR)	AM932975	AM939497	EU557738	EU543049	EU543134	EU542981	/	/	/
30	<i>O. corymbosa</i> L.		Australia: Andersson 2260 (GB)	AM932977	AM939500	/	/	/	/	/	/	/
31	<i>O. corymbosa</i> L.		China: Macau MA25 (CUHK)	/	HQ148802	/	HM752960	HM752875	/	HM753130	HM753045	HM640360
32	<i>O. corymbosa</i> L.		China: Ruijiang Wang 1427 (IBSC)	/	JF699925	JF700074	/	/	/	JF699999	JF699849	JF699784
33	<i>O. corymbosa</i> L.		China: Taiwan TA01 (CUHK)	/	HQ148757	/	HM752915	HM752830	/	HM753085	HM753000	HM640315
34	<i>O. corymbosa</i> L.		China: Xing Guo 50 (IBSC)	/	JF699927	JF700076	JX111305	JX111379	/	JF700001	JF699851	JX111194
35	<i>O. corymbosa</i> L.		Gabon: Andersson & Nilsson 2263 (GB)	AM932978	AM939501	/	/	/	/	/	/	/

续表(Continued)

No.	Name used in this paper	Name used while sequenced*	Voucher (Herbarium)	ETS	ITS	petD	rps16	tmL-trnF	atpB-rbcL	matK	rbcL	trnH-psbA
36	<i>O. corymbosa</i> L.		Singapore: Ruijiang Wang SIN02 (IBSC)	/	JX111239	JX111121	JX111306	JX111380	/	JX111083	JX111158	JX111193
37	<i>O. corymbosa</i> L.		Zambia: Dessein et al. 487 (BR)	AM932979	AM939502	EU557739	EU543050	EU543135	EU542982	/	/	/
38	<i>O. corymbosa</i> L. var. <i>linearis</i> (DC.) Verdc.	<i>Oldenlandia erecta</i> (Mamill. & Sivar.) R. R. Mill	China: Li Heng 11298 (A)	HE681554	HE657758	HE657643	HE649897	/	/	/	/	/
39	<i>O. corymbosa</i> L. var. <i>linearis</i> (DC.) Verdc.	<i>Oldenlandia erecta</i> (Mamill. & Sivar.) R. R. Mill	Nepal: S. Neupane 2 (ODU)	HE681541	HE657745	HE657630	HE649882	/	/	/	/	/
40	<i>Hedyotis corymbosa</i> L. var. <i>tereticaulis</i> Ko		China: Xing Guo et al. 16 (IBSC)	/	JF699926	JF700075	JX111307	JX111378	/	JF700000	JF699850	JX111195
41	<i>Oldenlandia densa</i> (ined.)		Zambia: Dessein et al. 346 (BR)	AM932980	AM939503	EU557751	EU543061	EU543147	/	/	/	/
42	<i>O. diffusa</i> (Willd.) Roxb.		China: Hong Kong TY01 (CUHK)	/	HQ148759	/	HM752917	HM752832	/	HM753087	HM753002	HM640317
43	<i>O. diffusa</i> (Willd.) Roxb.		China: Macau MA19 (CUHK)	/	HQ148796	/	HM752954	HM752869	/	HM753124	HM753039	HM640354
44	<i>O. diffusa</i> (Willd.) Roxb.		China: Ruijiang Wang 1449 (IBSC)	/	JF699931	JF700080	/	/	/	JF700005	JF699855	JF699788
45	<i>O. diffusa</i> (Willd.) Roxb.		China: Tan Ce-ming 95670 (UPS)	HE681539	HE657743	HE657628	HE649880	/	/	/	/	/
46	<i>O. diffusa</i> (Willd.) Roxb.		China: Xing Guo 51 (IBSC)	/	JF699932	JF700081	JX111308	JX111381	/	JF700006	JF699856	JF699789
47	<i>O. diffusa</i> (Willd.) Roxb.	<i>Oldenlandia brachypoda</i> DC.	Nepal: S. Neupane 88 (ODU)	/	HE657735	HE657620	HE649871	/	/	/	/	/
48	<i>O. duemmeri</i> S. Moore		Uganda: W. H. Lewis 6018 (GH)	HE681540	HE657744	HE657629	HE649881	/	/	/	/	/
49	<i>O. galioides</i> (F. Muell.) F. Muell.		Australia: Harwood 1511 (BR)	/	AM939507	EU557743	EU543053	EU543139	EU542986	/	/	/
50	<i>O. gracilipes</i> Craib		Thailand: J. F. Maxwell 01-591 (L)	HE681542	HE657746	/	HE649883	/	/	/	/	/
51	<i>O. herbacea</i> (L.) Roxb		China: Hong Kong Hu & But 22491 (A)	/	HE657749	HE657633	HE649886	/	/	/	/	/
52	<i>O. lancifolia</i> (Schumach.) DC.		Zambia: Dessein et al. 1256 (BR)	AM932976	AM939499	/	/	/	/	/	/	/
53	<i>O. lancifolia</i> (Schumach.) DC.		Zambia: Dessein et al. 1356 (BR)	AM932990	AM939512	/	EU543058	EU543144	EU542991	/	/	/

续表(Continued)

No.	Name used in this paper	Name used while sequenced*	Voucher (Herbarium)	ETS	ITS	petD	rps16	tmL-trnF	atpB-rbcL	matK	rbcL	trnH-psbA
54	<i>O. nematocaulis</i> Bremek.		Zambia: Dessein et al. 924 (BR)	AM932994	AM939517	/	EU543060	/	EU542994	/	/	/
55	<i>O. ovatifolia</i> (Cav.) DC.	<i>Hedyotis ovatifolia</i> Cav.	China: Xing Guo et al. 20-1 (IBSC)	/	JF699940	JF700090	JX111309	JX111382	/	JF700015	JF699865	JF699795
56	<i>O. pinifolia</i> (Wall. ex G. Don) Kuntze	<i>Hedyotis pinifolia</i> Wall. ex G. Don	China: Hong Kong YU19 (CUHK)	/	HQ148821	/	HM752979	HM752894	/	HM753149	HM753064	HM640379
57	<i>O. pinifolia</i> (Wall. ex G. Don) Kuntze	<i>Hedyotis pinifolia</i> Wall. ex G. Don	China: Macau MA11 (CUHK)	/	HQ148788	/	HM752946	HM752861	/	HM753116	HM753031	HM640346
58	<i>O. pinifolia</i> (Wall. ex G. Don) Kuntze		China: Ruijiang Wang 1231 (IBSC)	/	JX111240	JX111122	JX111311	JX111384	/	JX111084	JX111159	JX111196
59	<i>O. stocksii</i> Hook. f.		India: Klackenber & Lundin 326 (S)	HE681558	HE657763	/	HE649901	/	/	/	/	/
60	<i>O. taborensis</i> Bremek.		Tanzania: Bidgood et al. 4015 (BR)	/	AM939522	EU557753	/	EU543149	EU542996	/	/	/
61	<i>O. umbellata</i> L.		India: S. Neupane 84 (ODU)	HE681565	HE657770	HE657653	HE649908	/	/	/	/	/
62	<i>O. umbellata</i> L.		Sri Lanka: F. Fagerind 3320 (S)	HE681469	HE657674	HE657569	HE649806	/	/	/	/	/
63	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Hong Kong YU12 (CUHK)	/	HQ148815	/	HM752973	HM752888	/	HM753143	HM753058	HM640373
64	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Macau MA14 (CUHK)	/	HQ148791	/	HM752949	HM752864	/	HM753119	HM753034	HM640349
65	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Ruijiang Wang 1438 (IBSC)	/	JF699965	JF700115	/	/	/	JF700040	JF699890	JF699816
66	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Ruijiang Wang 409 (IBSC)	/	JF699966	JF700116	JX111312	JX111385	/	/	JF699891	JF699817
67	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Xing Guo 66 (IBSC)	/	JF699969	JF700119	JX111313	JX111386	/	JF700043	JF699894	JF699819
68	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Xing Guo et al. 4-1 (IBSC)	/	JF699967	JF700117	/	/	/	JF700041	JF699892	/
69	<i>O. verticillata</i> L.	<i>Hedyotis verticillata</i> (L.) Lam.	China: Xing Guo et al. 4-2 (IBSC)	/	JF699968	JF700118	/	/	/	JF700042	JF699893	JF699818

续表(Continued)

No.	Name used in this paper	Name used while sequenced*	Voucher (Herbarium)	ETS	ITS	petD	rps16	tmL-trnF	atpB-rbcL	matK	rbcL	trnH-psbA
70	<i>O. wauensis</i> Schweinf. ex Hiern	<i>Thecorchus wauensis</i> (Schweinf. ex Hiern) Bremek.	Ethiopia: Friis et al. 2560 (UPS)	AM933018	AM939548	EU557774	EU543076	EU543168	EU543017	/	/	/
71	<i>O. wiedemannii</i> K. Schum.		Kenya: Luke & Luke 8362 (UPS)	AM933001	AM939525	EU557756	EU543063	EU543151	EU543000	/	/	/
72	<i>Paraknoxia parviflora</i> (Stapf ex Verde.) Verdc. ex Bremek.		Zambia: Dessein et al. 678 (BR)	/	/	EU557757	EU543064	EU543152	EU543001	/	/	/
73	<i>Pentodon pentandrus</i> (Schumach. & Thonn.) Vatke		Zambia: Dessein et al. 598 (BR)	AM933003	AM939528	EU557759	EU543066	EU543154	EU543002	/	/	/
74	<i>Scleromitron angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Hedyotis angustifolia</i> Cham. & Schltdl.	China: Shiu-Ying Hu 5401 (A)	HE681455	HE657662	/	HE649792	/	/	/	/	/
75	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Hong Kong (TM05)	/	HQ148762	/	HM752920	HM752835	/	HM753090	HM753005	HM640320
76	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Hong Kong (TM06)	/	HQ148763	/	HM752921	HM752836	/	HM753091	HM753006	HM640321
77	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Xing Guo et al. 12 (IBSC)	/	JF699957	JF700108	JX111297	JX111370	/	JF700033	JF699882	JF699810
78	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Xing Guo et al. 19 (IBSC)	/	JF699959	JF700109	/	/	/	JF700034	JF699883	/
79	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	Unknown: cult. at BR	AM932949	AM939451	EU557710	EU543062	EU543106	EU542997	/	/	/
80	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Liu Zhengyu 21840 (MO)	HE681560	HE657765	HE657648	HE649903	/	/	/	/	/
81	<i>S. angustifolium</i> (Cham. & Schlecht.) Benth.	<i>Oldenlandia tenelliflora</i> Blume	China: Ruijiang Wang 992 (IBSC)	/	JF699958	JF700107	JX111296	JX111371	/	JF700032	JF699884	JF699811
82	<i>Stenaria nigricans</i> (Lam.) Terrell		USA: Yatskievych 96-92 (MO)	/	AM939546	EU557772	AF333373	EU543166	EU543015	/	/	/
83	<i>Synaptantha tillaeacea</i> (F.Muell.) Hook. f.		Australia: Lazarides & Palmer 272 (K)	/	AM939547	EU557773	EU543075	EU543167	EU543016	/	/	/
84	<i>Thecagonum biflorum</i> (L.) Babu	<i>Hedyotis biflora</i> (L.) Lam.	China: Ruijiang Wang 1146 (IBSC)	/	JF699906	JF700055	JX111300	JX111374	/	JF699981	JF699830	JF699767
85	<i>T. pteritum</i> (Blume) Babu	<i>Hedyotis pterita</i> Blume	China: Ruijiang Wang 1423 (IBSC)	/	JF699942	JF700092	/	/	/	JF700017	JF699867	JF699797

*: The blank stands for that the name is the same as currently used; /: Missing sequences.

YU07 from Hong Kong, China, identified as “*Hedyotis herbacea*” in NCBI database and as “*Oldenlandia diffusa*” by Guo et al.^[12], should be *H. koana*, which has terminal and upper axillary inflorescence, on basis of our comprehensive voucher examination.

In addition, *Oldenlandia brachypoda* of Nepalese sample (Neupane 88, ODU) identified by Wikström et al.^[10], was merged under *O. diffusa*, following the treatment of Dutta and Deb^[16].

1.3 Sequence assembly, editing and phylogenetic analyses

Sequences for each region were prealigned with the Clustal X Version 2.1^[18] and ambiguously aligned regions were manually corrected using BioEdit Version 7.1.11^[19].

The Bayesian analyses (BI) were carried out using MrBayes version 3.2.1^[20]. Each of the nine regions was assigned its own best-fit nucleotide substitution model, as determined by the Akaike information criterion (AIC) in Modeltest Version 3.7^[21]. The best models were GTR+I+G (ITS), TVM+I+G (ETS, *atpB-rbcL*), TVM+G (*petD*, *trnH-psbA*, and *trnL-trnF*), GTR+G (*matK*, *rps16*), TrN+I (*rbcL*). Searches were based on 10000000 generations with four chains of the Markov Chain Monte Carlo (MCMC, one cold and three heated under default heating values) in each of two parallel runs, with each chain starting with a random tree. Trees were sampled and recorded every 1000 generations of the MCMC chain. Analyses were set to automatically stop when the average standard deviation of split frequencies under 0.01, which indicates the convergence of two runs. A 50% majority-rule consensus tree was constructed after removing the “burn-in period” samples (the first 25% of sampled trees).

2 Results

Phylogenetic relationships indicated by the MCMC analyses are summarized as a 50% majority-rule consensus tree in Figure 1 (CI=0.589, RI=0.794). The posterior probability values are indicated above each

node. Nodes with posterior probability values equal to or greater than 95% are considered well-supported^[22].

The topology of phylogenetic tree is almost correspondent to that revealed previously. All investigated species recognized under *Oldenlandia*, except *O. ovatifolia*, are resolved into two main monophyletic clades with both robust support values (BPP=100). And *O. corymbosa* and *O. diffusa* are included in the clade I and the clade II, respectively (Fig. 1).

The strongly supported clade I comprises 19 terminals, of which, eleven are *O. corymbosa* and its varieties from different localities. Except for the probably misidentified Zambian sample (Dessein et al. 487), all other *O. corymbosa* samples from pantropical areas of Africa, Asia and Australia are grouped in the monophyletic lineage. In addition, *O. corymbosa* var. *linearis* and *H. corymbosa* var. *tereticaulis* were also included in the subclade IA with robust support too (BPP=100). Other accompanied eight taxa are from either Africa or South Asia.

The second clade II with robust support includes nine samples under the name *O. diffusa* from Asia and 26 samples representing other nine species from Asia and Africa (only *O. lancifolia*). However, *O. herbaceae* on the basis of the collection Hu & But 22491 (A) from Hong Kong, China^[10], is nested within the subclade IIA of *O. diffusa* from China and Nepal.

3 Discussion

3.1 *Oldenlandia diffusa* and *O. corymbosa* belong to two different clades, representing two different genera

The phylogenetic tree involving the two main components of Baihuasheshecao indicated that *O. diffusa* and *O. corymbosa* are actually two species belonging to two different lineages with robust support values (Fig. 1). As suggested previously^[10,12-13,23], these two lineages actually represent two different genera, *Oldenlandia* L. (clade I) and *Scleromitron* (Wight & Arn.) Meisn. (clade II). Morphologically, they can be distinguished by inflorescence and flower

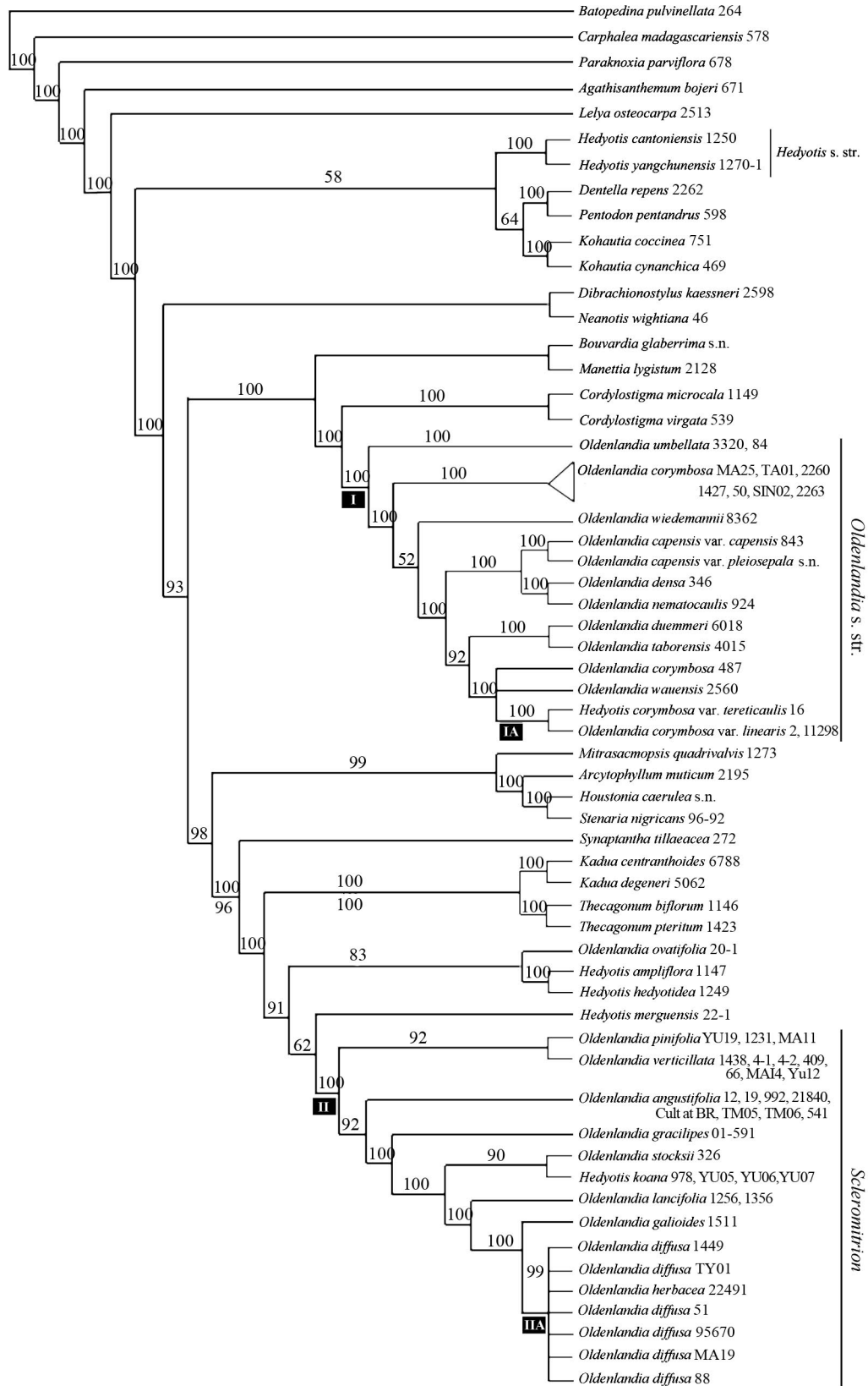


Fig. 1 Phylogenetic relationships of Baihuasheshecao, *Oldenlandia diffusa* and its adulterant *O. corymbosa*, revealing that they belong to two distinct clades in the tribe Spermacoceae. The tree is a 50% majority-rule consensus tree from a Bayesian Markov Chain Monte Carlo (MCMC) analysis of a combined dataset of seven plastid (*petD*, *rps16*, *trnL-trnF*, *atpB-rbcL*, *matK*, *rbcL*, *trnH-psbA*) and two nuclear (ETS, ITS) data. Bayesian Posterior Probability (BPP) is indicated above branches. The number following the species name refers to the collection number of each sample listed in Table 1.

characters. *Oldenlandia* usually has small erect or prostrate stems, terminal or axillary panicles with obvious or very short peduncles and pedicelled flowers, loculicidally dehiscent capsules and trigonous seeds. On the contrary, *Scleromitron* has erect stems, either axillary clusters of sessile flowers or a single flower with a long and slim pedicel, apically and loculicidally dehiscent capsules, and many obconic seeds. And the homostylous androecia and gynoecia are usually inserted within the corolla tube in *Oldenlandia*, but exerted in *Scleromitron*^[12].

3.2 *Oldenlandia* clade including the type *O. corymbosa*

Oldenlandia corymbosa is a common weed in tropical continents and the western Pacific Islands and was reported to origin from Africa^[24]. Hitchcock^[25] designated *O. corymbosa* as the lectotype of the genus, although it was usually treated as a member of *Hedyotis* for a very long time. Moreover, the close relationship between *O. corymbosa* and *O. umbellata* has also gotten support due to their similarity of pollen characters^[26]. Besides, *Hedyotis corymbosa* var. *tereticaulis* is involved in this *Oldenlandia* clade and should be transferred accordingly.

Oldenlandia corymbosa L. var. *tereticaulis* (Ko) R. J. Wang, **comb. nov.**

Basionym: *Hedyotis corymbosa* (L.) Lam. var. *tereticaulis* Ko, Fl. Hainan. **3**: 308, 580, 1974.

3.3 The species in the clade II are accommodated by the genus *Scleromitron*

The monophyletic clade II including *O. diffusa* represented the resurrected genus *Scleromitron* with the generic type *S. angustifolium*^[12]. Wikström et al.^[10] indicated that the Chinese *O. herbacea* (Hu & But 22491, A) that is nested within the subclade IIA is clearly a representative of some other species because the true African *O. herbacea* was proved to be sisterhood to the genus *Conostomium* (Stapf) Cufod. with strong support^[23].

In addition, the mixture of *O. brachypoda* (Neupane 88, ODU) with all *O. diffusa* samples is in agreement with their merger^[16], given that the former was correctly

identified.

Taxonomically, the name transfer from *Hedyotis* or *Oldenlandia* to *Scleromitron* leads to the following four new combinations.

(1) *Scleromitron diffusum* (Willd.) R. J. Wang, **comb. nov.**

Basionym: *Hedyotis diffusa* Willd., Sp. Pl. **1**: 566, 1797.

(2) *Scleromitron koanum* (R. J. Wang) R. J. Wang, **comb. nov.**

Basionym: *Hedyotis koana* R. J. Wang, Acta Phytotax. Sin. **45**: 696, 2007.

(3) *Scleromitron pinifolium* (Wall. ex G. Don) R. J. Wang, **comb. nov.**

Basionym: *Hedyotis pinifolia* Wall. ex G. Don, Gen. Hist. **3**: 526, 1834.

(4) *Scleromitron verticillatum* (L.) R. J. Wang, **comb. nov.**

Basionym: *Oldenlandia verticillata* L., Mant. Pl. **1**: 40, 1767.

Sivarajan and Biju^[27] proposed the concept of “*Hedyotis corymbosa*-*diffusa* complex” because of their morphological similarity. In order to give a clear elucidation between these two species, a comparison was prepared on basis of their morphology, chromosome numbers, palynology, chemical constituents, and their bioactivity (Table 2).

In summary, this study clarified the traditionally misapplied scientific name of Baihuasheshecao based on the molecular phylogenetic analysis. The results demonstrated that the two main mixed components of the herbal Baihuasheshecao, *Oldenlandia diffusa* and its adulterant *O. corymbosa*, phylogenetically belonged to two separate lineages that representing two respective genera. We then suggested the application of *Scleromitron diffusum* to replace the misapplied name *Hedyotis diffusa* or *Oldenlandia diffusa* for future scientific research and medicinal practice. The proper application of the scientific name for traditional Chinese medicine not only can promote the quality of the Chinese medicinal material, but can enhance the standardization and utilization of genuine traditional Chinese herbs during the internationalization and

Table 2 Comparison of *Oldenlandia diffusa* and *O. corymbosa*

	<i>Oldenlandia diffusa</i>	<i>Oldenlandia corymbosa</i>	References
Habit	Annual herbs	Perennial herbs	
Branch	Diffuse at base, erect or decumbent	Erect or prostrate	
Stem transverse section	Subrounded; covered with unicellular cell of mastoid process	Quadrangular; covered with unicellular cell of mastoid process	[28]
Leaf petiole	Sessile or subsessile	Sessile	
Leaf shape	Elliptic lanceolate, linear-lanceolate or linear	Linear to narrowly elliptic	
Stipule	With 2–3 fimbriate at apex	With 2–5 fimbriate at apex	
Inflorescence	Solitary, rarely 2–3 flowered	Corymbs, umbels or racemose cymes, 2–5 flowers	
Inflorescence position	Axillary	Axillary	
Peduncle	Present rarely	Always present	
Flower	Homostylous	Homo- or heterostylous	
Stamens and style	Exserted	Included	
Capsule shape	Subglobose, truncate or flat at top	Globose or ellipsoid; truncate or slightly raised at top	
Capsule dehiscence	Apically and loculicidally dehiscent	Loculicidally dehiscent	
Epidermal cell of pericarp	Anticlinal wall straight	Anticlinal wall undulated	[28]
Fruit septal cell	Protuberance	Strip-shaped, arranged in inlaid shape	[28]
Seed	Trigonous, narrowly winged, reticulated	Trigonous, reticulated	[29]
Seed germination period	Most seeds within two weeks and all by 21 weeks	Most seeds within 36 weeks	[30]
Chromosomal number	$n=27$	$n=9$ $2n=18, 36, 54$	[31] [32–36]
Pollen aperture	3–(4)-colporate	3–(4–5)-colporate	[26,29]
Chemical constituent	2-Hydroxy-3-methyl anthraquinone	Furanocoumarins hedyotiscones A	[37]
Biological activity	Immunomodulating activity; inhibiting the growth of cancer cell lines and inducing apoptosis; antitumor activities	Significant hepatoprotective effects	[38] [7] [8]

modernization of Chinese herbology.

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