Phylogenomics and adaptive evolution of hydrophytic umbellifers (tribe Oenantheae, Apioideae) revealed from chloroplast genomes

Jun Wen[1], Jun-Wen Zhu[1], Xu-Dong Ma[1], Hui-Min Li[1], Bao-Cheng Wu[1], Wei Zhou[1], Jia-Xin Yang[2], Chun-Feng Song[1,*]

[*]Correspondence: Chun-Feng Song cfsong79@cnbg.net

[1] Jiangsu Key Laboratory for the Research and Utilization of Plant Resources, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (Nanjing Botanical Garden Mem. Sun Yat-Sen), Nanjing, China, 210014

[2] Huaiyin Institute of Technology, School of Life Science and Food Engineering, Huaian, China, 223000

Abstract:

Background: Tribe Oenantheae consists mainly of aquatic species within the Apioideae. The unique morphology and habitat distinguish this group from other Apioideae groups. However, the genomic information of these group species has not been widely developed, and the molecular mechanisms of adaptive evolution remain unclear.

Results: We provide comparative analyses on 30 chloroplast genomes of this tribe representing five genera to explore the molecular variation response to plant adaptations. The Oenantheae chloroplast genomes presented typical quadripartite structures, with sizes ranging from 153,024 bp to 155,006 bp. Gene content and order were highly conserved with no significant expansion or contraction observed. Seven regions (*rps16* intron–*trnK*, *rpoB–trnC*, *trnE–trnT–psbD*, *petA–psbJ*, *ndhF–rpl32–trnL*, *ycf1a–rps15*, and *ycf1a* gene) were identified as remarkable candidate DNA markers for future studies on species identification, biogeography, and phylogeny of tribe Oenantheae. Our study elucidated the relationships among the genera of tribe Oenantheae and subdivided the genera of *Sium* and *Oenanthe*. However, relationships among the *Oenanthe* I clade remain to be further clarified. Eight positively selected genes (*accD*, *rbcL*, *rps8*, *ycf1a*, *ycf1b*, *ycf2*, *ndhF*, and *ndhK*) were persuasively detected under *site* models tests, and these genes might have played roles in Oenantheae species adaptation to the aquatic environments.

Conclusions: Our results provide sufficient molecular markers for the subsequent molecular studies of the tribe Oenantheae, and promote the understanding of the adaptation of the Oenantheae species to aquatic environments.

Keywords: Oenantheae, adaptation, phylogeny, positive selection, candidate DNA markers

Table S1. Voucher information for species newly sequenced in this study. NAS: Herbarium, Institute of Botany, Chinese Academy of Sciences, Jiangsu Province.

Taxon	Collection number	Collector name	Locality	Latitude Longitude	Altitude/m	Place of voucher deposition	Deposition number	Determiner
Cicuta virosa	LHM1252	Hui-Min Li	Arele town, Qinghe County, Xinjiang Province, China	N 46.860700, E 90.312274	1351	NAS	NAS00640404	Jun Wen
Cicuta virosa	WJ2368	Yong-Bo Yu	Jingyu County, Jiling Province, China	N 42.128859, E 126.972341	828	NAS	NAS00640405	Jun Wen
Cryptotaenia canadensis	WJ220908	Jun Wen	Nanjing Botanical Garden Mem.Sun Yat-Sen, jiangsu Province, China	N 32.055262, E 118.838222	47	NAS	NAS00640406	Jun Wen
Cryptotaenia japonica	WJ2375	Xu-Dong Ma and Jun Wen	Yuexi County, Anhui Province, China	N 30.987528, E 116.095014	1119	NAS	NAS00640407	Jun Wen
Cryptotaenia japonica	WJ2384	Jun Wen	Jinggang Mountain, Jiangxi Province, China	N 26.589113, E 114.136933	921	NAS	NAS00640408	Jun Wen
Oenanthe hookeri	WJ2334	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Shangri-la, Yunnan Province, China	N 27.372579, E 100.126096	2791	NAS	NAS00640409	Jun Wen
Oenanthe javanica	WJ2305_02	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Kunming, Yunnan Province, China	N 25.140806, E 102.740129	1989	NAS	NAS00640410	Jun Wen
Oenanthe javanica	WJ2320	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Jingdong County, Yunnan Province, China	N 24.540939, E 101.018529	2481	NAS	NAS00640411	Jun Wen
Oenanthe javanica	WJ2372_AH01	Xu-Dong Ma and Jun Wen	Yuexi County, Anhui Province, China	N 31.010486, E 116.174966	823	NAS	NAS00640412	Jun Wen

Oenanthe linearis	ZJW034	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Shangri-la, Yunnan Province, China	N 27.373053, E 100.171049	2121	NAS	NAS00640413	Jun Wen
Oenanthe linearis subsp. *rivularis*	NZD2301	Wei Zhou	Lancang County, Yunnan Province, China	N 22.576876, E 100.273407	1135	NAS	NAS00640414	Jun Wen
Oenanthe linearis subsp. *rivularis*	WJ2319	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Jingdong County, Yunnan Province, China	N 24.444515, E 100.974998	1485	NAS	NAS00640415	Jun Wen
Oenanthe thomsonii	WJ2321	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Jingdong County, Yunnan Province, China	N 24.540465, E 101.017887	2474	NAS	NAS00640416	Jun Wen
Oenanthe pimpinelloides	wj20230607_01	Jun Wen	Nanjing Botanical Garden Mem.Sun Yat-Sen, jiangsu Province, China	N 32.055262, E 118.838222	47	NAS	NAS00640417	Jun Wen
Sium serra	WJ2374	Xudong Ma and Jun Wen	Yuexi County, Anhui Province, China	N 30.987528, E 116.095014	1119	NAS	NAS00640418	Jun Wen
Sium suave	WBC2301	Bao-Cheng Wu	China National Botanical Garden, Beijing, China	N 40.007561, E 116.216186	81	NAS	NAS00640419	Jun Wen
Sium ventricosum	WJ2339	Jun-Wen Zhu, Xu-Dong Ma, and Jun Wen	Shangri-la, Yunnan Province, China	N 27.760711, E 99.939763	3471	NAS	NAS00640420	Jun Wen

Table S2. The junction positions of the four regions of Oenantheae chloroplast genomes. JLA (IRA-LSCjunction); JLB (LSC-IRB junction); JSB (IRB-SSC junction); JSA (SSC-IRA junction).

Taxon	JLA	JLB	JSB	JSA	GenBank accession No.
***Cicuta virosa* LHM1252**	*rpl2, trnH-GUG*	*rps19, rpl2*	*ycf1*	*ycf1a*	**PQ283862**
Cicuta virosa	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_037711
***Cicuta virosa* WJ2368**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283863**
Cryptotaenia canadensis	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283864**
***Cryptotaenia japonica* WJ2375**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283865**
***Cryptotaenia japonica* WJ2384**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283866**
Cryptotaenia japonica	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_046737
Oenanthe hookeri	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283867**
Oenanthe javanica	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_049874
***Oenanthe javanica* WJ2305_02**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283868**
***Oenanthe javanica* WJ2320**	*rpl2, trnH-GUG*	*rps19*	*ycf1*	*ycf1a*	**PQ283869**
***Oenanthe javanica* WJ2372_AH01**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283870**
***Oenanthe linearis* ZJW034**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283871**
***Oenanthe linearis* subsp. *rivularis* NZD2301**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283872**
***Oenanthe linearis* subsp. *rivularis* WJ2319**	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283873**
Oenanthe thomsonii	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283875**
Oenanthe virgata	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	KX832335
Oenanthe pimpinelloides	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283874**
Sium crispulifolium	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	OP234518
Sium medium	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_072108
Sium ninsi	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_072107
Sium serra	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283876**

Sium suave	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_071929
Sium suave	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	OP234519
Sium suave	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	**PQ283877**
Sium tenue	*rpl2, trnH-GUG*	*rps19*	*ycf1 & ndhF*	*ycf1a*	NC_072106
Sium ventricosum	*rpl2, trnH-GUG*	*rps19*	*ycf1*	*ycf1a*	NC_070095
Sium ventricosum	*rpl2, trnH-GUG*	*rps19*	*ycf1*	*ycf1a*	OP234514
Sium ventricosum	*rpl2, trnH-GUG*	*rps19*	*ycf1*	*ycf1a*	**PQ283878**
Tiedemannia filiformis subsp. greenmannii	*rpl2, trnH-GUG*	*rps19*	*ycf1*	*ycf1a*	HM596071

Table S3. Nucleotide diversity (Pi) of the Oenantheae chloroplast genomes under a slide window calculated in DnaSP v6. Regions with Pi> 0.02 were listed and tagged with genetic information.

Window	Midpoint	Pi	Sequence region
1-774	474	0.01913	*psbA-trnH*
1375-2097	1699	0.01887	*matK-psbA*
1575-2297	1992	0.02371	*matK-psbA*
1800-2497	2197	0.02075	*matK-psbA*
2098-2697	2397	0.01757	*matK*
2298-2897	2597	0.01531	*matK*
2498-3097	2797	0.01516	*matK*
2698-3297	2997	0.01898	*matK*
2898-3510	3197	0.01744	*matK*
3098-3716	3397	0.02149	*rps16-matK*
3298-3917	3616	0.01553	*rps16-matK*
3511-4133	3816	0.01606	*rps16-matK*
3918-4586	4233	0.01825	*rps16-matK*
4134-5016	4457	0.02202	*rps16-matK*
4341-5274	4741	0.03063	*rps16-trnK-UUU*
4587-5494	5164	0.02633	*rps16-trnK-UUU*
5017-5711	5394	0.02525	*rps16 intron-trnK-UUU*
5275-5950	5594	0.02064	*rps16 intron-trnK-UUU*
5495-6151	5820	0.02043	*rps16 intron*
5712-6378	6051	0.01714	*rps16 intron*
6152-7876	6478	0.01631	*rps16 intron-trnQ-UUG*
6379-8091	7762	0.02031	*psbK-trnQ-UUG-rps16*
6595-8301	7984	0.02211	*psbK-trnQ-UUG-rps16*
7877-8504	8201	0.01508	*rps16 intron-trnUUG-psbK*
8302-8912	8604	0.01706	*psbK-psbI*
8505-9125	8812	0.02082	*psbK-psbI-trnS-GCU*
8705-9332	9015	0.01867	*psbK-psbI-trnS-GCU*
8913-9589	9232	0.02093	*psbK-psbI-trnS-GCU-trnG-UCC*
9126-9867	9488	0.02715	*psbI-trnS-GCU-trnG-UCC*
9333-10083	9727	0.02596	*trnS-GCU-trnG-UCC*
9590-10295	9967	0.02259	*trnS-GCU-trnG-UCC*
9868-10522	10189	0.01766	*trnG-UCC*
10084-10743	10410	0.01732	*trnG-UCC*
10296-11127	10631	0.02137	*trnG-UCC-trnR-UCU-atpA*
10523-11330	11011	0.01783	*trnG-UCC-trnR-UCU-atpA*
10744-11530	11230	0.01588	*trnG-UCC-trnR-UCU-atpA*
12938-13557	13237	0.01577	*atpF intron*
13138-13779	13450	0.01629	*atpF intron*
13338-14000	13679	0.01525	*atpF intron*
13780-14476	14100	0.02046	*atpH-atpF intron*

14001-14679	14373	0.0214	*atpH-atpF*
14255-14895	14579	0.02036	*atpI-atpH-atpF*
14680-15662	15022	0.02017	*atpI-atpH*
14896-15891	15381	0.02525	*atpI-atpH*
15145-16091	15762	0.01634	*atpI-atpH*
16292-17185	16591	0.01766	*rps2-atpI*
16492-17385	16823	0.01842	*rps2-atpI*
16692-17585	17285	0.01718	*rps2-atpI*
17586-18225	17906	0.01525	*rpoC2-rps2*
17786-18425	18124	0.01639	*rpoC2-rps2*
28372-29155	28678	0.01657	*rpoB-trnC-GCA*
28572-29410	29000	0.02356	*rpoB-trnC-GCA*
28894-29653	29264	0.02893	*rpoB-trnC-GCA*
29156-29951	29520	0.03154	*rpoB-trnC-GCA*
29411-30180	29775	0.02566	*rpoB-trnC-GCA*
29654-30958	30074	0.02252	*rpoB-trnC-GCA-petN*
30181-31640	31066	0.01905	*trnC-GCA-petN-psbM*
30959-32063	31467	0.02094	*trnC-GCA-petN-psbM*
31167-32292	31916	0.02417	*petN-psbM*
31641-32503	32192	0.0175	*petN-psbM-trnD-GUC*
32064-32713	32402	0.0153	*petN-psbM-trnD-GUC*
32293-33152	32603	0.01715	*trnD-GUC-psbM*
32504-33374	33049	0.01713	*trnY-GUA-trnD-GUC-psbM*
32714-33585	33261	0.01672	*trnE-UUC-trnY-GUA-trnD-GUC-psbM*
33153-34703	33485	0.02318	*trnD-GUC-trnY-GUA-trnE-UUC-trnT-GGU*
33375-34955	33790	0.03363	*trnY-GUA-trnE-UUC-trnT-GGU*
33586-35176	34803	0.04315	*trnE-UUC-trnT-GGU-psbD*
34704-35468	35076	0.03724	*trnT-GGU-psbD*
34959-36236	35313	0.0265	*trnT-GGU-psbD*
35177-36453	35845	0.02086	*trnT-GGU-psbD*
35469-36661	36336	0.01542	*trnT-GGU-psbD*
39068-39844	39403	0.01984	*psbC-trnS-UGA-psbZ*
39518-40269	39944	0.01693	*trnS-UGA-psbZ-trnG-GCC*
40045-40761	40381	0.01511	*psbZ-trnG-GCC-trnfM-CAU*
46462-47250	46819	0.01552	*ycf3 intron-psaA*
46720-47458	47058	0.01702	*ycf3 intron*
48475-49162	48789	0.01652	*ycf3 intron-trnS*
48682-49399	49017	0.02322	*ycf3-trnS-GGA*
48899-49626	49267	0.02556	*ycf3-trnS-GGA*
49163-49865	49526	0.0242	*ycf3-trnS-GGA-rps4*
49404-50088	49740	0.02008	*ycf3-trnS-GGA-rps4*
49627-50288	49973	0.01503	*trnS-GGA-rps4*
50489-51209	50803	0.02015	*rps4-trnT-UGU-trnL-UAA*
50704-51736	51109	0.02774	*rps4-trnT-UGU-trnL-UAA*

50994-51944	51411	0.02565	*rps4-trnT-UGU-trnL-UAA*
51210-52195	51844	0.01783	*trnT-UGU-trnL-UAA*
52830-53559	53141	0.0221	*trnL-UAA-trnF-GAA-ndhJ*
53042-53759	53391	0.01734	*trnL-trnF-ndhJ*
55186-56135	55514	0.01525	*trnV-UAC-ndhC*
56565-57232	56919	0.01609	*atpE-trnM-CAU-trnV-UAC*
61232-61912	61538	0.01534	*rbcL-accD*
61432-62122	61781	0.01622	*rbcL-accD*
61913-62538	62222	0.02153	*rbcL-accD*
62123-62741	62432	0.02209	*accD*
62323-62941	62641	0.02231	*accD*
63142-64195	63441	0.01725	*accD-psaI*
63342-64414	63899	0.01712	*accD-psaI-ycf4*
63542-64637	64296	0.0187	*accD-psaI-ycf4*
65122-66313	65421	0.0198	*ycf4-cemA*
65322-66513	66175	0.01871	*ycf4-cemA*
65524-66722	66413	0.01685	*ycf4-cemA*
67991-68622	68290	0.01989	*petA-psbJ*
68191-68898	68513	0.02576	*petA-psbJ*
68414-69140	68777	0.03367	*psbJ-petA*
68623-69340	69033	0.02389	*petA-psbJ-psbL*
68899-69548	69240	0.01761	*petA-psbJ-psbL*
69949-70690	70331	0.01528	*psbE-petL*
70173-70922	70577	0.0229	*psbE-petL*
70442-71156	70805	0.02205	*psbE-petL*
70691-71356	71029	0.01979	*psbE-petL*
70923-71579	71256	0.01717	*psbE-petL-petG*
71157-71789	71479	0.01559	*psbE-petL-petG-trnW-CCA*
71357-72059	71686	0.01642	*petL-petG-trnW-CCA-trnP-UGG*
71580-72320	71900	0.01722	*petG-trnW-CCA-trnP-UGG-psaJ*
71790-72535	72165	0.01907	*trnW-CCA-trnP-UGG-psaJ*
72060-72735	72431	0.01827	*trnP-psaJ-rpl33*
72321-73002	72635	0.01603	*trnP-psaJ-rpl33*
73669-74311	73980	0.01705	*rps18-rpl20*
74994-75621	75317	0.01902	*clpP-rpl20*
75218-75821	75521	0.01516	*clpP-rpl20*
76697-77342	77021	0.01559	*clpP intron*
76905-77563	77233	0.02084	*clpP intron*
77127-77764	77462	0.01949	*clpP intron*
77343-78063	77663	0.02031	*clpP intron-psbB*
79267-79914	79566	0.0154	*psbB-psbT*
80549-81215	80897	0.01889	*psbH-petB intron*
80789-81426	81105	0.01617	*psbH-petB intron*
80998-81627	81326	0.01533	*petB intron*

82715-83368	83032	0.01531	*petD intron*
85429-86041	85741	0.01619	*infA-rpl36-rps11*
86251-86862	86550	0.01513	*rpl14-rps8*
87510-88205	87809	0.01611	*rpl16 intron*
87710-88412	88101	0.0202	*rpl16 intron*
87997-88671	88305	0.01881	*rpl16 intron*
88206-88928	88570	0.0204	*rps3-rpl16 intron*
88413-89128	88804	0.01624	*rps3-rpl16 intron*
115712-117664	117364	0.01811	*ycf1b*
115912-117870	117564	0.01648	*ycf1b-ndhF*
117465-118076	117770	0.01804	*ndhF*
117665-118276	117970	0.01615	*ndhF*
117871-118476	118176	0.01867	*ndhF*
119080-120240	119379	0.02372	*ndhF-rpl32*
119280-121149	119592	0.03547	*ndhF-rpl32*
119480-121583	121049	0.04089	*ndhF-rpl32-trnL-UAG*
120241-122263	121250	0.03678	*ndhF-rpl32-trnL-UAG*
121150-122549	122154	0.02817	*rpl32-trnL-UAG*
121584-122754	122449	0.02626	*rpl32-trnL-UAG-ccsA*
122264-122954	122654	0.02132	*rpl32-trnL-UAG-ccsA*
122550-123154	122854	0.02044	*trnL-UAG-ccsA*
122755-123354	123054	0.01775	*ccsA*
122955-123554	123254	0.01575	*ccsA*
123155-123774	123454	0.02726	*ccsA-ndhD*
123355-124003	123672	0.02872	*ccsA-ndhD*
123555-124203	123903	0.02874	*ccsA-ndhD*
123775-124403	124103	0.01546	*ccsA-ndhD*
125404-126210	125705	0.01771	*ndhE-psaC-ndhD*
125606-126410	125905	0.01673	*ndhE-psaC*
125806-126671	126310	0.01947	*ndhG-ndhE-psaC*
126411-127071	126771	0.01537	*ndhG-ndhE*
126872-127595	127171	0.01801	*ndhI-ndhG*
127072-127810	127411	0.02079	*ndhI-ndhG*
127276-128010	127710	0.01873	*ndhI-ndhG*
128618-129343	128917	0.01882	*ndhA intron*
128818-129544	129156	0.02234	*ndhA intron*
129032-129750	129444	0.02568	*ndhA intron*
129344-129963	129644	0.01985	*ndhA intron*
129545-130163	129851	0.01755	*ndhA intron*
131564-132178	131872	0.01587	*ycf1a-rps15-ndhH*
131773-132653	132072	0.0253	*ycf1a-rps15-ndhH*
131973-132859	132476	0.0256	*ycf1a-rps15*
132179-133074	132759	0.03038	*ycf1a-rps15*
132654-133289	132974	0.0341	*ycf1a-rps15*

132860-133489	133189	0.03162	*ycf1a*
133075-133701	133389	0.02739	*ycf1a*
133290-133907	133595	0.02333	*ycf1a*
133490-134131	133807	0.02993	*ycf1a*
133702-134349	134013	0.03059	*ycf1a*
133908-134549	134249	0.03517	*ycf1a*
134132-134782	134449	0.03349	*ycf1a*
134350-134989	134649	0.03608	*ycf1a*
134550-135201	134889	0.04038	*ycf1a*
134783-135466	135101	0.06267	*ycf1a*
134990-135666	135301	0.06629	*ycf1a*
135202-135866	135566	0.05688	*ycf1a*
135467-136075	135766	0.03532	*ycf1a*
135667-136275	135966	0.03269	*ycf1a*
135867-136484	136175	0.02725	*ycf1a*
136076-136700	136384	0.02126	*ycf1a*

Table S4 Summary of major characteristics of Oenantheae chloroplast protein-coding sequences (CDSs), including nucleotide diversity (Pi), non-synonymous substitutions (Ka), synonymous substitutions (Ks), and the average of the Ka/Ks ratio (ω).

CDS	Pi	Ka	Ks	Ka/Ks
accD	0.01778	0.01441	0.03232	0.49426
atpA	0.00643	0.00051	0.02493	0.06517
atpB	0.00603	0.00039	0.02356	0.05792
atpE	0.00603	0.00046	0.02450	0.36816
atpF	0.00675	0.00392	0.01651	0.29170
atpH	0.00224	0.00000	0.00823	0.00000
atpI	0.00466	0.00138	0.01491	0.16953
ccsA	0.01530	0.01307	0.02394	**0.60474**
cemA	0.00958	0.00470	0.02832	0.23884
clpP	0.00322	0.00000	0.01361	0.00000
infA	0.01269	0.00515	0.03911	0.18329
matK	0.01797	0.01752	0.02063	**0.94079**
ndhA	0.00763	0.00377	0.01959	0.22391
ndhB	0.00189	0.00132	0.00368	0.46239
ndhC	0.00397	0.00000	0.01711	0.00000
ndhD	0.01171	0.00461	0.03522	0.17590
ndhE	0.00964	0.00556	0.02349	0.34425
ndhF	0.01529	0.00933	0.03653	0.29190
ndhG	0.01051	0.00455	0.02987	0.25658
ndhH	0.00819	0.00199	0.03027	0.10142
ndhI	0.00650	0.00277	0.01964	0.23348
ndhJ	0.00286	0.00243	0.00443	0.31150
ndhK	0.00837	0.00589	0.01652	0.46243
petA	0.00843	0.00286	0.02674	0.13132
petB	0.00429	0.00049	0.01625	0.16385
petD	0.00602	0.00106	0.02152	0.16220
petG	0.00336	0.00000	0.01326	0.00000
petL	0.00974	0.00000	0.03613	0.00000
psaA	0.00416	0.00029	0.01698	0.04395
psaB	0.00459	0.00046	0.01878	0.03834
psaC	0.00882	0.00036	0.03838	0.12586
psaI	0.00283	0.00000	0.01259	0.00000
psaJ	0.00296	0.00398	0.00000	/
psbA	0.00459	0.00016	0.01923	0.10606
psbB	0.00702	0.00128	0.02583	0.06279
psbC	0.00536	0.00077	0.01980	0.06380
psbD	0.00380	0.00000	0.01644	0.00000
psbE	0.00027	0.00000	0.00116	0.00000
psbF	0.00468	0.00000	0.01801	0.00000
psbH	0.00551	0.00196	0.01640	0.28219

psbI	0.00234	0.00000	0.00962	0.00000
psbJ	0.00600	0.00276	0.01503	0.36624
psbK	0.00538	0.00528	0.00605	**0.56387**
psbL	0.00058	0.00000	0.00282	0.00000
psbM	0.01281	0.00865	0.02844	0.36401
psbT	0.00655	0.00089	0.02520	0.31019
psbZ	0.00136	0.00134	0.00144	0.33180
rbcL	0.00633	0.00442	0.01250	**0.55514**
rpl2	0.00199	0.00047	0.00656	0.16979
rpl14	0.00827	0.00181	0.02898	0.16040
rpl16	0.00872	0.00225	0.02838	0.13239
rpl20	0.00603	0.00617	0.00578	**0.60516**
rpl22	0.01236	0.00742	0.02973	0.29621
rpl23	0.00070	0.00060	0.00105	0.30128
rpl32	0.02210	0.01511	0.04961	0.31272
rpl33	0.00442	0.00173	0.01364	0.30772
rpl36	0.00120	0.00080	0.00250	0.31332
rpoA	0.01034	0.00961	0.01330	**0.83790**
rpoB	0.00529	0.00225	0.01545	0.15556
rpoC1	0.00631	0.00254	0.01892	0.16932
rpoC2	0.00918	0.00548	0.02186	0.27100
rps2	0.00735	0.00301	0.02264	0.17565
rps3	0.00779	0.00473	0.01883	0.29398
rps4	0.00674	0.00393	0.01591	0.30822
rps7	0.00014	0.00000	0.00059	0.00000
rps8	0.01184	0.00810	0.02388	0.46571
rps11	0.00908	0.00447	0.02263	0.34165
rps12	0.00296	0.00038	0.01022	0.33800
rps14	0.00644	0.00560	0.00942	**0.50679**
rps15	0.01957	0.01848	0.02519	**0.88107**
rps16	0.02135	0.00960	0.05941	0.21889
rps18	0.00521	0.00162	0.01716	0.17227
rps19	0.01184	0.00394	0.03992	0.20952
ycf1a	0.02602	0.02460	0.03425	**0.72298**
ycf1b	0.00650	0.00648	0.00674	**0.92539**
ycf2	0.00322	0.00312	0.00362	**1.01611**
ycf3	0.00377	0.00000	0.01642	0.00000
ycf4	0.00950	0.00426	0.02732	0.17746

Table S5 The nucleotide diversity (Pi) of 11 genes with introns, including Pi for genes, introns, and CDSs.

Gene name	Gene	Intron	CDS
atpF	0.00957	0.01172	0.00675
clpP	0.01249	0.01639	0.00322
ndhA	0.01448	0.02134	0.00763
ndhB	0.00177	0.00152	0.00189
petB	0.01044	0.01572	0.00429
petD	0.01004	0.01267	0.00602
rpl2	0.00288	0.00403	0.00199
rpl16	0.01811	0.02269	0.00872
rpoC1	0.00787	0.01228	0.00631
rps16	0.01782	0.01679	0.02135
ycf3	0.01163	0.01484	0.00377

Table S6 The nucleotide diversity (Pi), aligned length (bp), Variable sites, Parsimony-informative sits, and percent of Parsimony-informative of four fragments previously used and seven candidate barcode makers for phylogenetic analyses of Oenantheae.

DNAfragment	pi	Aligned length (bp)	Variable sits	Parsimony-informative sits	percent of Parsimony-informative
rps16 intron	0.01679	906	75	43	4.75
trnQ-trnK	0.02213	3618	382	231	6.38
psbI–trnK	0.02002	4761	466	283	5.94
rps16 intron-trnK	0.02214	2086	215	130	6.23
ndhF-rpl32-trnL	0.03625	2930	590	304	10.38
psbJ-petA	0.03438	726	83	63	8.68
rpoB-trnC	0.03049	799	80	57	7.13
rps16-trnK	0.02952	927	107	68	7.34
trnY-trnE-trnT-psbD	0.03757	2031	352	226	11.13
rps15-ycf1a	0.03331	1111	135	88	7.92
ycf1a	0.04115	2573	288	264	10.26

Table S7 The potential positive selection test on Oenantheae.

Gene	Null hypothesis (M7)		Alternative hypothesis (M8)		Significance test		
	lnL	np	lnL	np	Positively selected sites (BEB, *: PP > 95%; **: PP > 99%)	LRT P-value (M7 vs. M8)	LRT P-value (M1a vs. M2a)
accD	-2807.879949	61	-2795.571799	63	42 R 0.946,73 R 0.856,77 G 0.706,94 Y 0.814,97 I 0.565,98 E 0.684,99 N 0.851,**100 H 0.983***,108 H 0.910,119 Y 0.905,201 T 0.821,**207 K 0.954***,351 N 0.685	0.000004515	0.000000015
rbcL	-2338.212906	61	-2308.319122	63	**28 D 0.974*,91 A 1.000**,95 N 0.997**,97 Y 0.998**,225 I 0.997**,309 I 0.998**,328 S 1.000**,354 I 0.956*,355 A 0.972***,424 L 0.659,429 Q 0.600,**443 E 0.998****,461 V 0.510,470 Q 0.587	0.000000000	0.000000000
rps8	-806.420213	61	-769.258678	63	**57 D 1.000****	0.000000000	0.000000000

ycf1a	-12032.711716	61	-12008.804003	63	200 S 0.652,205 K 0.713,206 Y 0.779,217 S 0.649,270 V 0.887,**469 R 0.960***,540 R 0.929,673 L 0.930,694 F 0.938,703 K 0.701,707 V 0.885,716 V 0.780,738 Q 0.940,741 F 0.537,742 I 0.537,751 A 0.633,776 K 0.922,**795 V 0.982***,799 L 0.665,810 K 0.835,834 T 0.644,862 I 0.806,903 N 0.839,917 T 0.778,922 P 0.813,946 N 0.781,948 Q 0.589,949 A 0.566,950 I 0.541,960 E 0.532,962 T 0.553,963 K 0.745,964 K 0.940,966 I 0.520,989 S 0.628,1000 L 0.765,1005 K 0.926,1006 M 0.618,1011 Q 0.517,**1014 K 0.971***,1018 Y 0.870,1021 R 0.702,1032 M 0.507,1040 Y 0.579,1051 T 0.549,1063 S 0.561,1069 N 0.876,1072 N 0.716,1084 - 0.662,**1085 I 0.969***,**1092 K 1.000****,**1093 I 0.965***,**1094 L 0.986***,**1111 Y 0.962***,1115 K 0.566,1136 P 0.688,1139 L 0.654,1168 N 0.556,1193 R 0.924,1234 G 0.913,1239 L 0.612,1242 T 0.868,1251 G 0.666,1265 S 0.649,1303 D 0.862,1305 I 0.785,1320 Q 0.731,1393 Y 0.572,1397 L 0.806,1408 - 0.502,1410 H 0.560,1415 - 0.859,1419 G 0.681,1428 S 0.893,1475 A 0.575,1501 I 0.794,1511 E 0.516,1548 L 0.670,1551 L 0.656,1554 - 0.514,1560 H 0.628,1596 S 0.784,1622 I	0.000000000	0.000000000

					0.552,1710 D 0.717,1734 R 0.605,1750 L 0.750,1805 R 0.911,1806 V 0.552,1818 P 0.687		
ycf2	-9606.539455	61	-9577.122638	63	8 L 0.536,13 L 0.545,58 W 0.562,67 Q 0.545,**103 F 1.000****,189 R 0.532,196 G 0.545,213 S 0.563,257 G 0.541,271 Q 0.542,293 R 0.516,296 F 0.529,317 Q 0.947,368 L 0.557,387 P 0.534,430 D 0.937,438 A 0.566,**503 Q 0.996****,506 M 0.936,510 G 0.543,552 H 0.569,592 W 0.560,598 L 0.542,617 W 0.562,645 R 0.562,649 R 0.516,652 Q 0.522,**746 L 0.996****,821 R 0.947,**833 R 0.955***,863 Y 0.941,**915 P 0.950***,942 A 0.507,969 P 0.511,1022 E 0.525,**1025 Q 0.951***,1039 L 0.556,1061 L 0.546,1087 R 0.564,1167 L 0.554,1273 S 0.944,1303 R 0.515,1308 T 0.516,1317 R 0.515,1365 L 0.550,1525 E 0.527,1604 C 0.504,1666 Y	0.000000000	0.000000000

					0.510,1742 V 0.520,1840 W 0.566,1871 G 0.542,1881 L 0.555,1919 R 0.567,1931 E 0.945,1959 S 0.563,1974 R 0.947,2028 T 0.516,2089 G 0.542		
ycf1b	-1347.463536	61	-1333.528946	63	4 K 0.730,56 G 0.779,113 L 0.709,115 Y 0.741,121 R 0.747,198 N 0.713,**200 S 0.976*,205 K 0.994**,206 Y 0.980*,215 R 0.975*,217 S 0.975***,222 F 0.702,224 I 0.715,**244 N 0.971***,254 - 0.807,259 E 0.786,269 A 0.761,**270 K 1.000****,271 D 0.754	0.000000888	0.000000131
ndhF	-4463.537830	61	-4451.301461	63	67 S 0.820,220 N 0.734,230 L 0.688,306 F 0.775,428 L 0.544,463 N 0.617,**465 Q 0.988***,488 I 0.858,498 L 0.673,516 T 0.828,525 N 0.829,535 R 0.927,549 D 0.551,630 L 0.645,**655 N 0.952*,663 C 1.000****,733 L 0.744	0.000004851	0.000065086
ndhH	-1979.406149	61	-1973.758070	63	**198 I 0.960***,341 L 0.826	0.003524280	0.082768146
ndhK	-1154.319395	61	-1149.827692	63	3 Y 0.551,165 R 0.847,**181 F 0.964***,208 P 0.550,212 L 0.898,213 H 0.803,214 F 0.503,215 S 0.845,**218 K 0.957***,219 V 0.831	0.011201551	0.022716790
rps11	-693.771380	61	-689.366259	63	6 P 0.546,**14 G 0.993****	0.012214628	0.054106065
ndhG	-862.414305	61	-859.588574	63	17 S 0.533,49 V 0.847,102 V 0.821,124 I 0.780,131 Q 0.581,133 I 0.826	0.059265318	0.184379896

rpoC2	-7312.611360	61	-7310.120403	63	238 N 0.697,**239 I 0.969***,509 S 0.764,511 S 0.773,524 F 0.807,528 D 0.710,540 Y 0.755,764 T 0.817,796 Q 0.817,974 R 0.934,995 L 0.790,1040	0.082830660	0.347241769
matK	-3099.192238	61	-3096.756958	63	F 0.726,1356 F 0.805,1367 S 0.827,1369 N 0.697 39 R 0.642,76 F 0.665,78 I 0.824,87 I 0.629,129 H 0.558,190 R 0.599,233 S 0.544,238 T 0.909,287 S 0.668,356 A 0.658,358 L 0.901,406 V 0.544,410 G 0.733,428 T 0.566,442 R 0.646,460 D 0.726	0.087573223	0.185427557
rps19	-441.468244	61	-439.097584	63	17 R 0.945	0.093419049	0.392793598
rpl20	-608.635498	61	-606.296830	63	**12 R 0.964***,83 H 0.566,89 Q 0.558,105 K 0.825,112 S 0.597	0.096456032	0.117651549
rpl32	-350.197154	61	-348.220010	63	23 K 0.911,26 L 0.852,48 C 0.927,49 A 0.842,50 T 0.635	0.138464127	0.161286983
rpl22	-920.192400	61	-918.588586	63	5 K 0.787,126 L 0.725,131 I 0.896	0.201127951	0.261510198
petA	-1544.285542	61	-1542.682774	63	5 K 0.811,15 T 0.801	0.201338441	0.776470988
psbM	-185.617085	61	-184.086270	63	37 S 0.905,38 K 0.615	0.216359263	0.415684384
psbT	-148.916361	61	-147.428665	63	**33 K 0.972***	0.225892513	0.225960517
rpl16	-659.824215	61	-658.357974	63		0.230791402	0.957032431
rpoC1	-3257.765388	61	-3256.376813	63	442 P 0.818,635 S 0.791,651 S 0.774	0.249430490	0.976402871
ndhE	-483.182677	61	-481.805802	63	92 C 0.797	0.252365966	0.797258748
atpB	-2320.496553	61	-2319.146900	63	8 - 0.804	0.259330233	0.883818990
ycf4	-932.434559	61	-931.348889	63	50 S 0.784,152 I 0.753	0.337675468	0.710639507
ndhJ	-702.172305	61	-701.141703	63	46 Y 0.808	0.356792107	0.822973729
rpoA	-1424.098366	61	-1423.110963	63	68 E 0.774,167 Q 0.712,266 R 0.737,268 K 0.931,271 K 0.672	0.372542930	0.399142413
rps16	-447.853147	61	-446.929328	63	54 L 0.716,55 Y 0.521,65 G 0.683	0.396999999	0.828506994

ndhD	-2635.607342	61	-2634.697438	63	37 I 0.713, 212 I 0.708, 395 V 0.713, 449 A 0.735, 468 I 0.772	0.402562868	0.999937002
psaJ	-184.185810	61	-183.325378	63	**10 V 0.977*,14 L 0.977*,36 A 0.977***	0.422979316	0.423014425
ndhI	-792.471979	61	-793.312180	63		0.431623758	0.999943002
rps2	-1092.237722	61	-1091.516423	63	226 R 0.829	0.486120375	0.894594264
rps15	-524.481381	61	-523.915873	63	5 P 0.911,10 I 0.624	0.568071493	0.586282807
rps12	-697.476723	61	-696.916972	63	25 R 0.520,63 R 0.520	0.571351313	0.596940999
atpF	-898.063576	61	-897.627419	63	130 H 0.799	0.646516215	0.858517684
rpl36	-145.169081	61	-144.745738	63	15 R 0.531	0.654853980	0.987241094
infA	-389.600505	61	-389.229217	63	30 M 0.677	0.689845238	0.999687049
cemA	-1151.968800	61	-1151.598357	63	24 S 0.715, 80 H 0.711, 133 I 0.691	0.690428403	0.805069234
rps14	-473.337682	61	-472.994676	63	33 K 0.741	0.709633954	0.732919797
psbK	-279.386715	61	-279.113678	63	4 I 0.833,6 S 0.829,9 C 0.838,20 S 0.829,22 F 0.826,36 I 0.833	0.761064628	0.761085938
ccsA	-1796.358387	61	-1796.201534	63	31 I 0.675,57 V 0.742,62 Y 0.682,169 I 0.648,171 L 0.649,281 R 0.683,294 M 0.649	0.854829710	0.857317458
rps18	-442.187822	61	-442.304285	63		0.890063029	0.999993000
ndhA	-1741.995258	61	-1741.879124	63	290 Y 0.781	0.890355908	1.000000000
rpoB	-5066.312129	61	-5066.232909	63	113 S 0.693,803 T 0.704	0.923836658	0.999999000
psaC	-383.037984	61	-382.967272	63		0.931730192	0.968107640
rpl33	-303.802471	61	-303.871651	63		0.933158696	0.999998000
rpl2	-1145.077094	61	-1145.105836	63		0.971667122	0.999995000
psbH	-321.170633	61	-321.176627	63		0.994023928	0.999986000
psbA	-1583.765864	61	-1583.762377	63		0.996519073	0.999829015
rps4	-995.602728	61	-995.605847	63	39 G 0.709	0.996885859	0.999919003
psaB	-3325.314981	61	-3325.316754	63		0.998228571	0.999876008

psbJ	-156.066366	61	-156.068121	63		0.998246539	1.000000000
atpI	-1168.251501	61	-1168.253237	63		0.998265506	0.999979000
psbD	-1540.624846	61	-1540.625372	63		0.999474138	0.999474138
petD	-731.385686	61	-731.385168	63		0.999482134	0.999956001
clpP	-852.037339	61	-852.037854	63		0.999485133	0.949737166
petB	-960.354285	61	-960.353781	63		0.999496127	0.999988000
atpE	-613.557310	61	-613.556955	63		0.999645063	0.999992000
rps3	-1044.374085	61	-1044.374408	63	66 Q 0.736	0.999677052	1.000000000
psaA	-3421.244905	61	-3421.244683	63		0.999778025	0.999864009
atpA	-2339.733466	61	-2339.733649	63		0.999817017	0.999817017
ndhC	-500.271983	61	-500.272150	63		0.999833014	0.999763028
psbE	-337.581417	61	-337.581564	63		0.999853011	0.999887006
ndhB	-2141.438674	61	-2141.438796	63		0.999878007	0.999998000
petG	-155.688500	61	-155.688603	63		0.999897005	0.999896005
psbC	-2165.754258	61	-2165.754163	63		0.999905005	0.999762028
atpH	-324.714368	61	-324.714448	63		0.999920003	0.999921003
petL	-129.581838	61	-129.581905	63		0.999933002	0.985862881
psbB	-2388.667780	61	-2388.667829	63		0.999951001	0.999952001
psbF	-166.854517	61	-166.854566	63		0.999951001	0.999947001
psbI	-149.635607	61	-149.635648	63		0.999959001	0.975558648
psaI	-141.340114	61	-141.340146	63		0.999968001	0.999945002
rpl23	-379.261634	61	-379.261617	63	66 M 0.534	0.999983000	0.999080423
rpl14	-588.983752	61	-588.983762	63		0.999990000	0.999992000
psbZ	-241.697335	61	-241.697336	63		0.999999000	1.000000000

Fig. S1 GC contents of the Oenantheae whole genome, large single copy region (LSC), small single copy region (SSC), and inverted repeat regions (IRs).

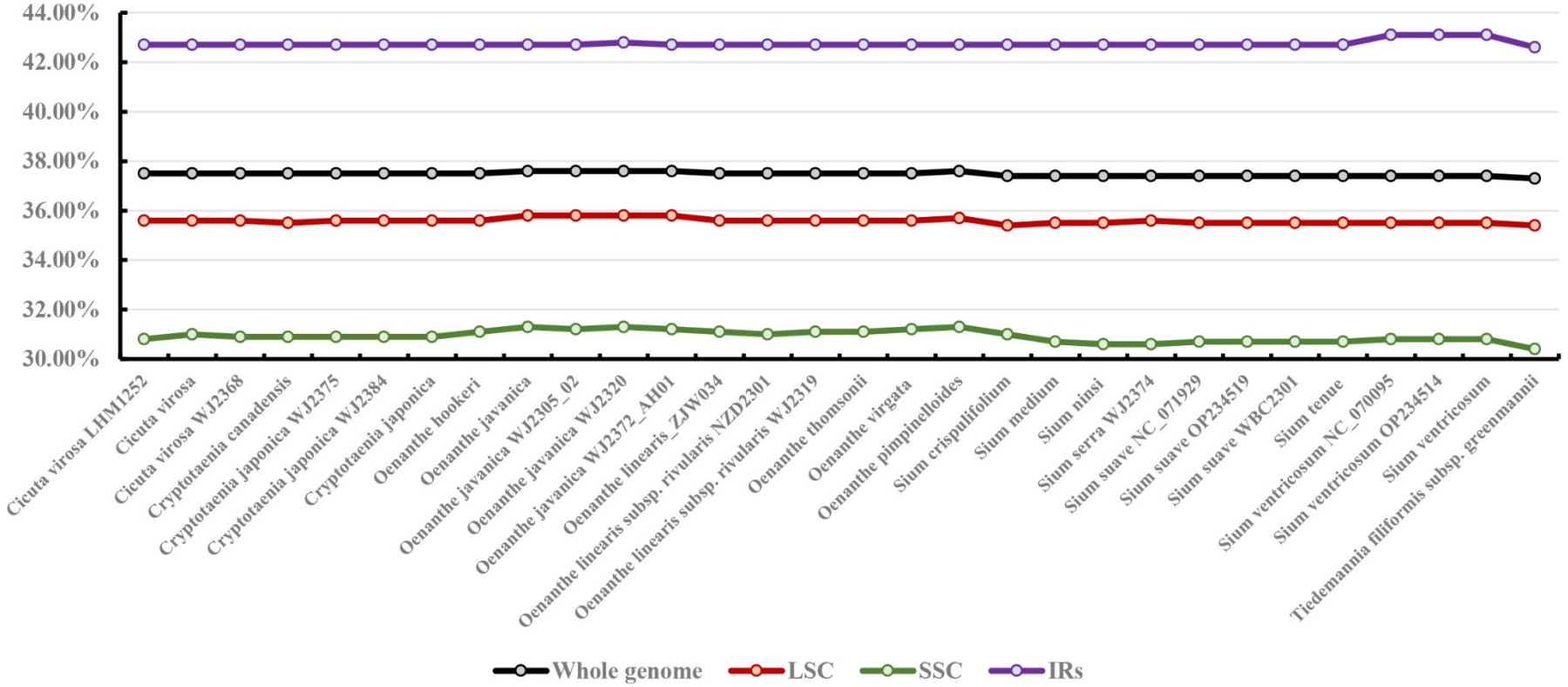


Fig. S2 MAUVE alignment of 30 Oenantheae chloroplast genomes using Geneious.

Fig. S3 The voucher specimens of 17 newly sequenced species were deposited in NAS (Herbarium, Institute of Botany, Chinese Academy of Sciences, Jiangsu Province).

1. NAS00640404: *Cicuta virosa* LHM1252

2. NAS00640405: *Cicuta virosa* WJ2368

3. NAS00640406: *Cryptotaenia canadensis*

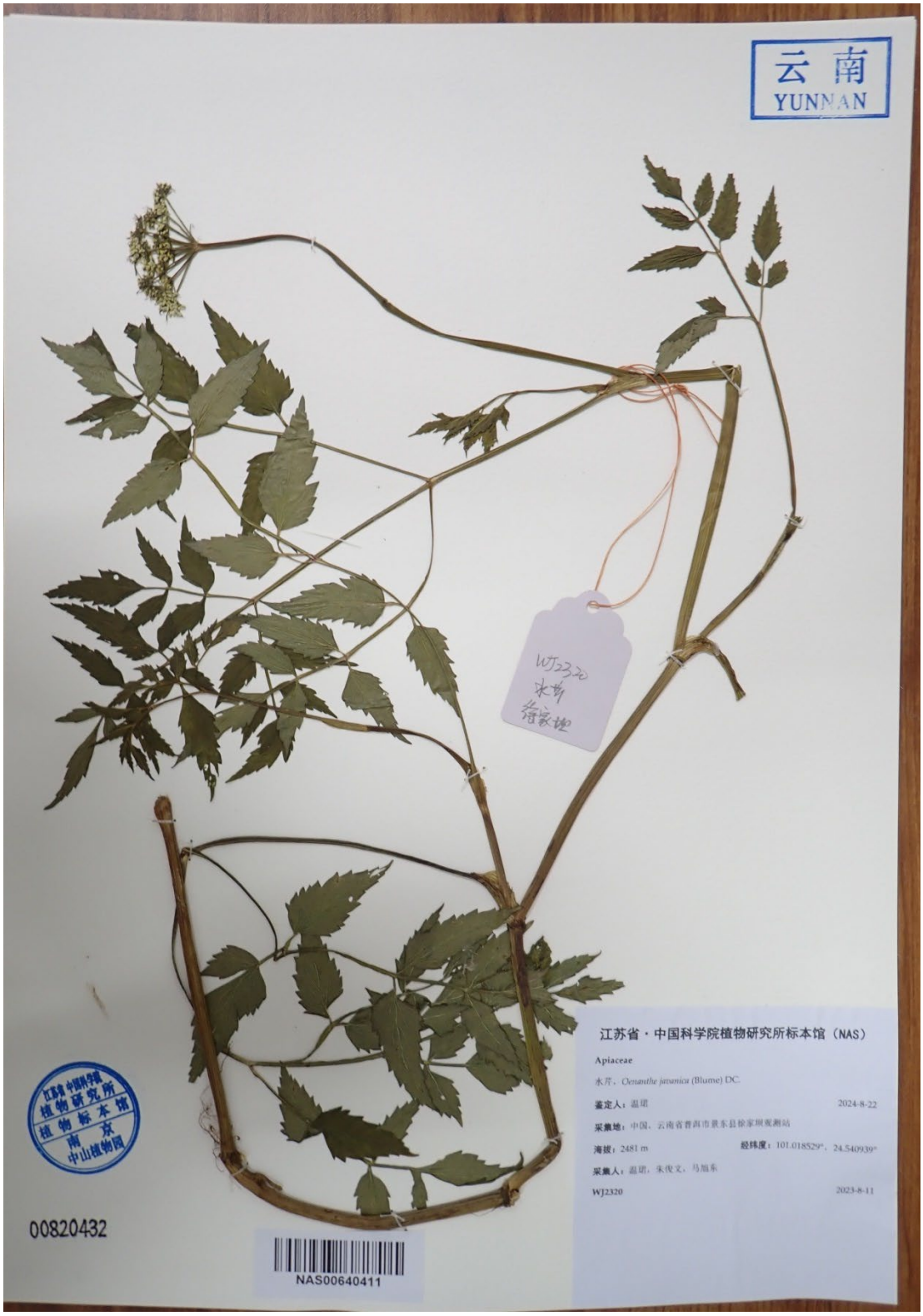
4. NAS00640407: *Cryptotaenia japonica* WJ2375

5. NAS00640408: *Cryptotaenia japonica* WJ2384

6. NAS00640409: *Oenanthe hookeri*

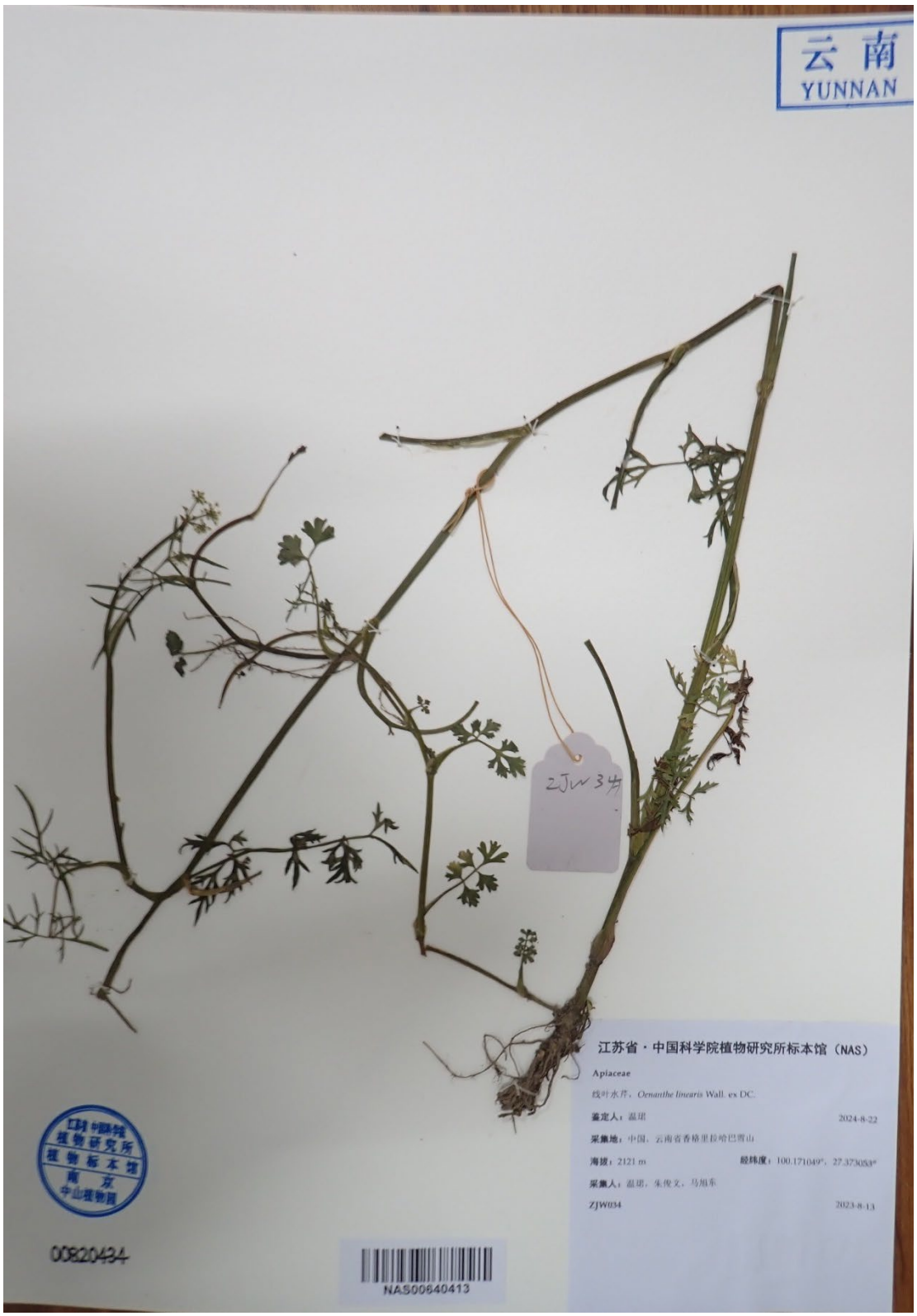
7. NAS00640410: Oenanthe javanica WJ2305_02

8. NAS00640411: *Oenanthe javanica* WJ2320

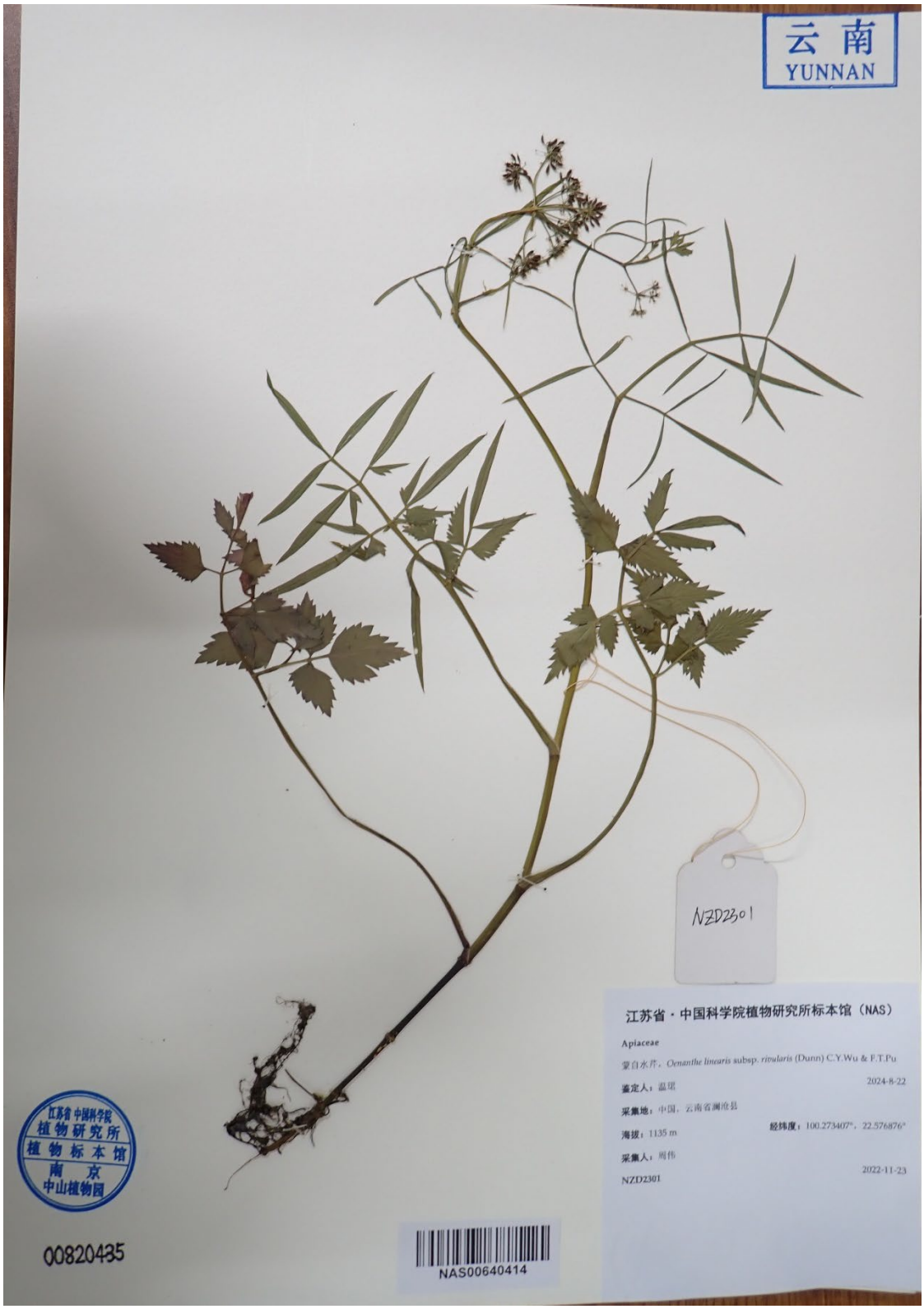


9. NAS00640412: *Oenanthe javanica* WJ2372_AH01

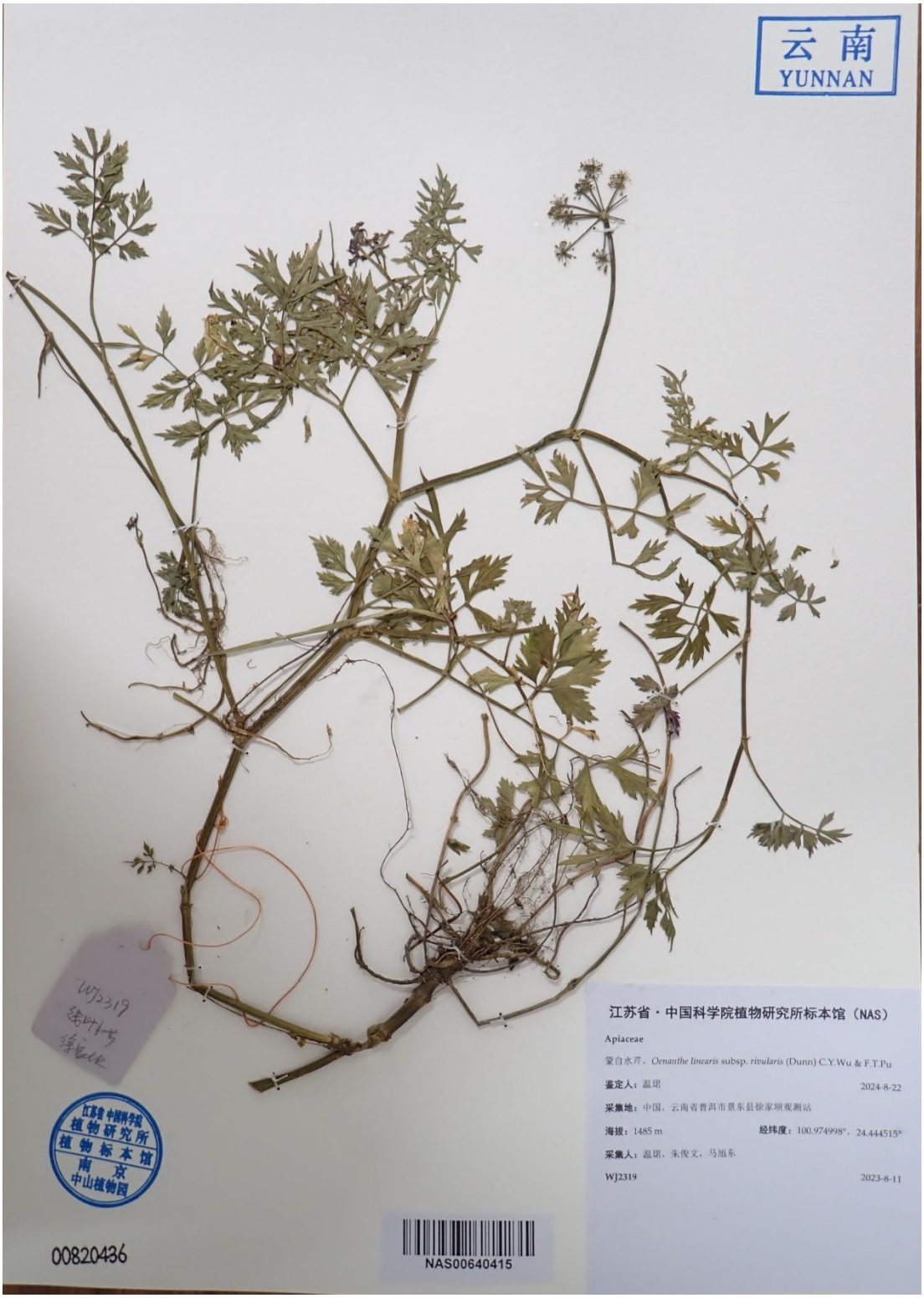
10. NAS00640413: *Oenanthe linearis* ZJW034



11. NAS00640414: *Oenanthe linearis* subsp. *rivularis* NZD2301

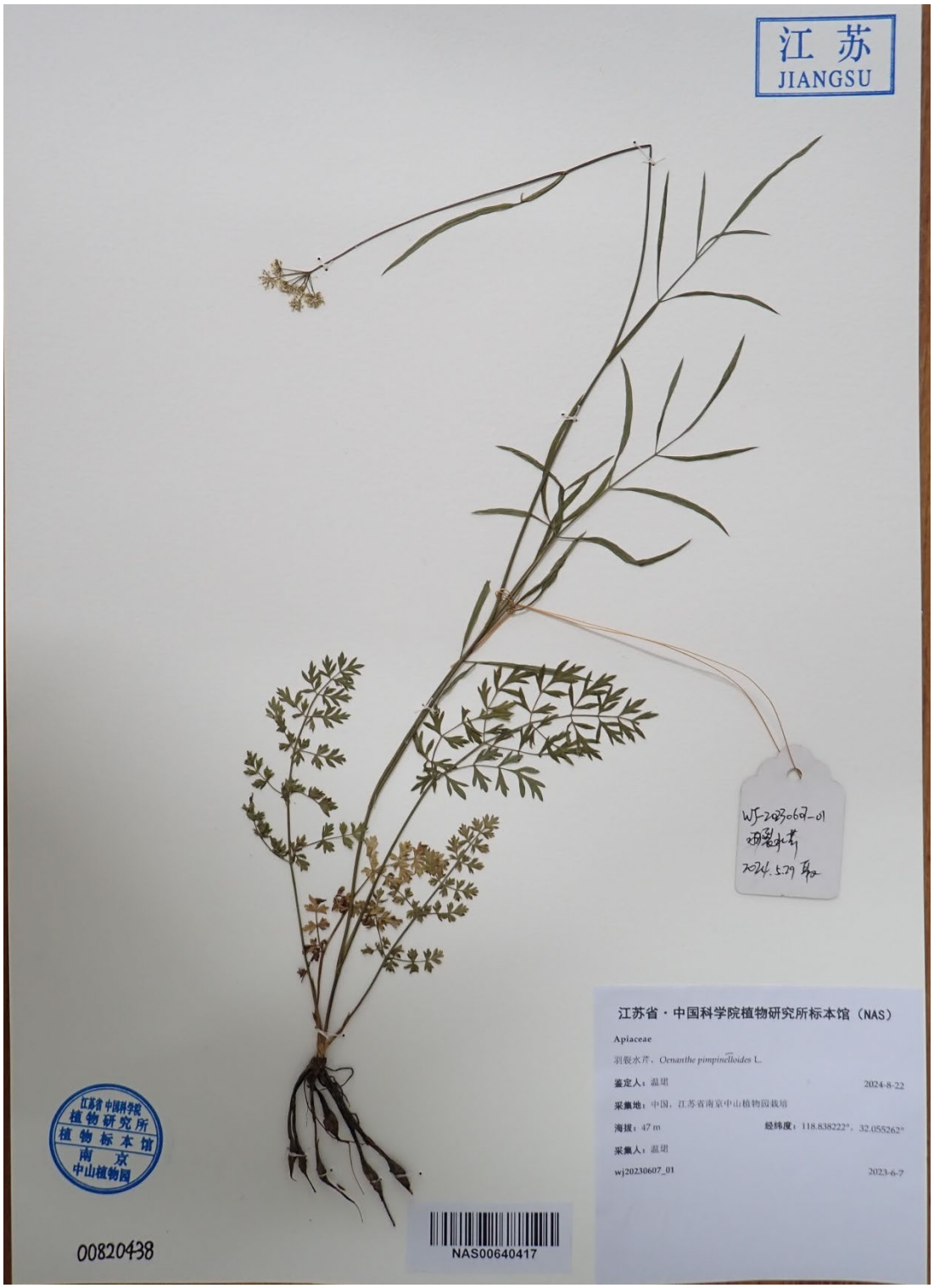


12. NAS00640415: *Oenanthe linearis* subsp. *rivularis* WJ2319



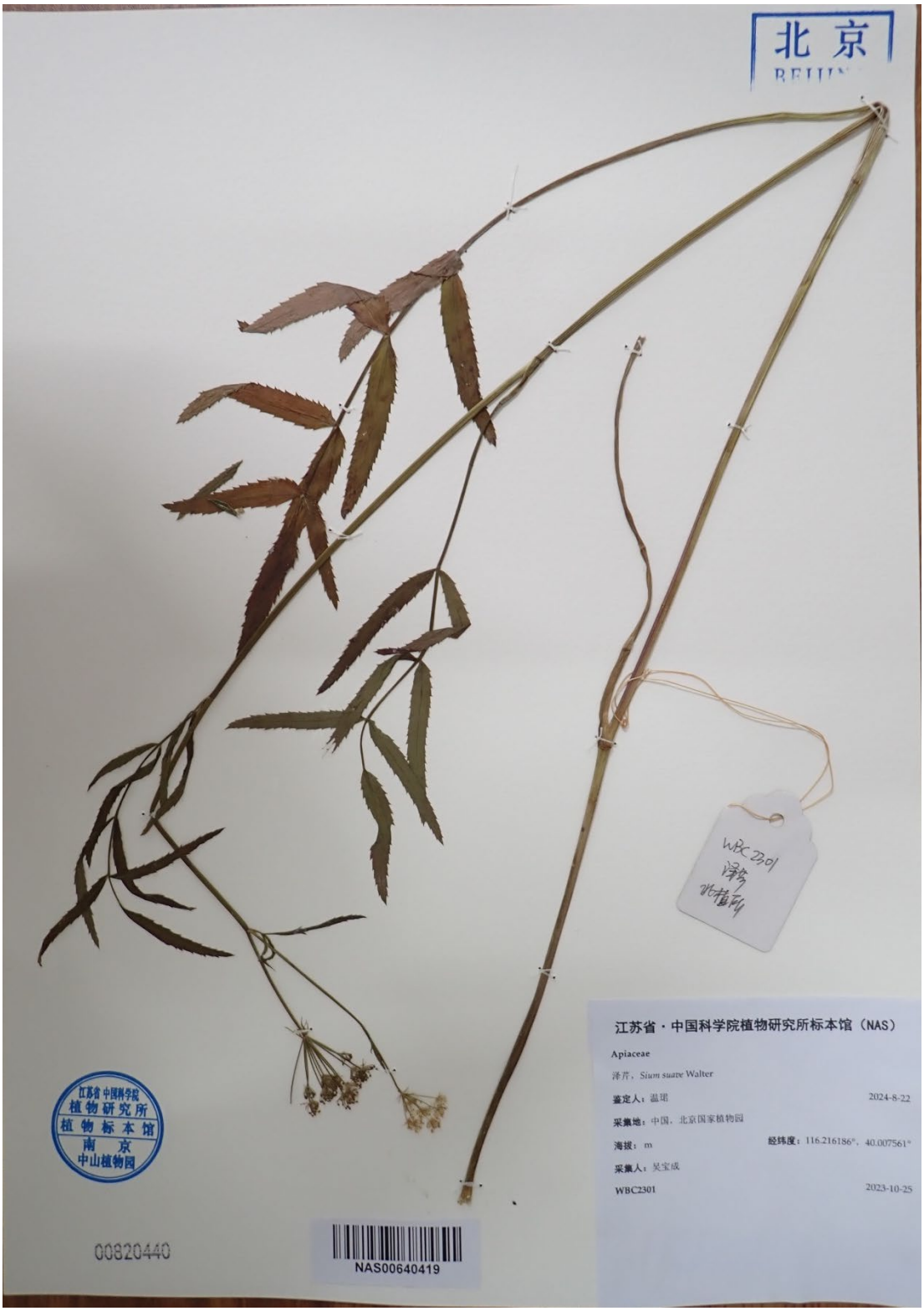
13. NAS00640416: ***Oenanthe thomsonii***

14. NAS00640417: ***Oenanthe pimpinelloides***



15. NAS00640418: ***Sium serra***

16. NAS00640419: Sium suave



17. NAS00640420: ***Sium ventricosum***