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ORIGINAL ARTICLE

Fruit and seed morphology in some representative genera of tribe Rhinantheae *sensu lato* (Orobanchaceae) and related taxa

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Abstract We investigated fruit and seed morphology in 48 taxa of 22 genera in the tribe Rhinantheae s.l. and related genera of Orobanchaceae. Fruit and seed morphology are heterogeneous across the studied taxa. The most useful features for this study are the indumentum of the capsule, seed number per capsule, seed coat ornamentation, and secondary structure of the seed coat. On the basis of seed coat features, five major types are recognized, viz. reticulate, cristate-winged, sulcate, psilate and irregularly striate. In addition, phenetic analysis of 19 characters produced an unweighted pair-group method using arithmetic averages tree and displayed highly heterogeneity at infra- and inter-genera. The classification of Rhinantheae s.l. is discussed and keys to the genera considered in this work, based exclusively on fruit or seed characters, are provided.

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Introduction

The tribe Rhinantheae sense lato (s.l.) Benth. is often considered to represent a transition between the non-parasitic Scrophulariaceae and holoparasitic Orobanchaceae (Boeshore 1920; Hutchinson 1969; Cronquist 1981). As traditionally circumscribed (e.g., Bentham and Hooker 1876), the tribe includes 17 genera in subfamily Rhinanthoideae of Scrophulariaceae. These are primarily distributed in temperate areas of the Northern Hemisphere, with some widespread genera (*Pedicularis* L., *Euphrasia* L., *Bartsia* L., and *Melampyrum* L.), some concentrated in the New World (*Castilleja* Mutis ex L.f., *Orthocarpus* Nutt., *Cordylanthus* Nutt. ex Benth., *Schwalbea* L.), and some mainly in the Old World (*Bungea* C.A.Mey, *Monochasma* Maxim. ex Franch. & Sav., *Odontites* Ludw., *Omphalotrix* Maxim., *Phtheirospermum* Bunge ex Fisch. & C.Mey., *Pseudobartsia* D.Y. Hong, *Pterygiella* Oliv., *Rhinanthus* L., *Siphonostegia* Benth., *Xizangia* D.Y. Hong). The highest generic diversity, the highest endemicity, and also probably the most basally branching elements of the tribe are found in eastern Asia (Hong 1983). The tribe is characterized by bilaterally symmetrical flowers, a strongly bilabiate corolla, didynamous stamens, and especially, its hemiparasitic trait.

Recent results from molecular phylogenetics show that relationships in traditional Scrophulariaceae are complicated. Former tribes of Scrophulariaceae s.l. have been moved into Scrophulariaceae sensu stricto (s.s.), Veronicaceae, Orobanchaceae, Calceolariaceae, and Stilbaceae (Olmstead et al. 2001). Within these families, Orobanchaceae is strongly

supported as a monophyletic group, including holoparasitic traditional Orobanchaceae, hemiparasitic Rhinantheae s.l. and non-parasitic *Lindenbergia* Lehm. (Young et al. 1999; Olmstead et al. 2001; Bennett and Mathews 2006; McNeal et al. 2013). With relation to Rhinantheae s.l., Boeshore (1920) depicted a continuous and transitional change in parasitic trait from the hemiparasites through a progressive reduction to the holoparasitic habit. However, in the family Orobanchaceae, parasitism evolved multiple times without a transition series from hemi- to holoparasitism (dePamphilis et al. 1997; Young et al. 1999; Olmstead et al. 2001; Wolfe et al. 2005; McNeal et al. 2013) and the tribe is in any case polyphyletic (Olmstead and Reeves 1995; Young et al. 1999; Olmstead et al. 2001; Wolfe et al. 2005; Bennett and Mathews 2006).

Based on these detailed molecular-phylogenetic studies in Scrophulariaceae and Orobanchaceae, there is a clear need for a revision of classification in tribe Rhinantheae s.l. Hence, Fischer (2004) splits Rhinantheae s.l. into three tribes, viz. Cymbarieae D.Don, Castillejeae, and Rhinantheae s.s. Cymbarieae include six small genera: *Schwalbea* (1 species), *Cymbaria* (4 species), *Siphonostegia* (2 species), *Lesquereuxia* (1 species), *Bungea* (2 species), and *Monochasma* (4 species). These genera are identified by the presence of prophylls and rounded or mucronate anther thecae and have a disjunct distribution. Castillejeae includes *Castilleja* (200 species), *Orthocarpus* (9 species), *Triphysaria* (5 species), *Clevelandia* (1 species), *Gentrya* (1 species), *Cordylanthus* (18 species), and *Ophiocephalus* (1 species). In a revised classification of Castillejeae, Tank et al. (2009) moved *Clevelandia* and *Ophiocephalus* into *Castilleja*, and split *Cordylanthus* into three genera (*Chloropyron* Behr, *Dicranostegia* (A.Gray) Pennell and *Cordylanthus* Nutt. ex Benth.). This tribe is monophyletic and characterized by having anther sacs that are unequal in size and unequally attached (Tank and Olmstead 2008; Tank et al. 2009). Rhinantheae s.s. includes 21 genera and over 1,200 species. The plants are herbs to small shrubs, with prophylls absent or present, racemose inflorescences, upper lip fused into a helmet-like to rostrate galea, or expanded, thecae mucronate or rounded, and stigma capitate to clavate or bilobed. The absence of uniquely defining traits raises the possibility that the tribe Rhinantheae s.s. are not monophyletic, which has been stressed by molecular-phylogenetic analyses (Wolfe et al. 2005; Bennett and Mathews 2006; Těšitel et al. 2010; Scheunert et al. 2012). Recently, McNeal et al. (2013) recognized 19 genera within the tribe Rhinantheae s.s., delimited *Pedicularis* and *P. japonicum* with Castillejeae as Pedicularideae Duby, and moved *Conopholis* into Orobancheae Lam. & DC. With relation to molecular phylogeny, morphological studies are needed for describing the new clades.

Palynological characteristics have been extensively studied for generic delimitation in Rhinantheae s.l. (Musselman and Mann 1976; Lu et al. 2007). However, these characters display high variability among genera and species and exhibit no synapomorphies in the tribe. Corolla morphology has also been paid much attention for classification in the tribe, but this feature is convergent and its usefulness may have been overestimated (Scheunert et al. 2012). Fruit and seed morphology have also been considered to contain important information for classification in genera of the traditional Scrophulariaceae s.l. and Orobanchaceae (Thieret 1955; Tiagi 1966; Chuang and Heckard 1972; Juan et al. 2000; Dong et al. 2013; Liu et al. 2013). However, these morphological features have as yet been insufficiently described in Rhinantheae s.l.

In this paper, we describe fruit and seed characters in 22 representative genera of Rhinantheae s.l. and related genera. Our aims are to: (a) offer a general view on the morphology of fruit and seed; (b) provide fruit- and seed-based keys to aid identification; and (c) discuss the classification of genera based on these characters.

Materials and methods

Taxon sampling

In this study, the classification of Rhinantheae s.l. was based on the treatment of Fischer (2004). According to his treatment, Rhinantheae s.l. is divided into three tribes, viz. Cymbarieae, Castillejeae, and Rhinantheae s.s. In Cymbarieae, three out of six genera were studied. We also examined one specimen of *Schwalbea americana* L., which was found to have seed characters in accordance with the research of Musselman and Mann (1976). However, we excluded this species from the study due to lack of information on the collector and accession code. In Castillejeae, five out of seven genera were examined. In Rhinantheae s.s., seven out of 21 genera were examined. Seven genera of related taxa were also included. In total, 48 taxa in 22 genera were observed for this study ("Appendix 1").

Morphological observations

Mature fruits were selected from herbarium collections. The capsules and seeds were observed using light microscope. The selected samples were treated according to the method of Martínez-Ortega and Rico (2001) to remove impurity deposits from the surface of the capsules and seeds. Treated capsules and seeds were mounted on stubs, coated with gold in a sputter coater, and observed with a KYKY-1000B scanning electron microscope (SEM; Science Instrument Company, Beijing) at 5 kV.

Terminology for capsule and seed morphology is taken from Musselman and Mann (1976), Chuang and Heckard (1972, 1983), Juan et al. (2000), Zhang et al. (2005) and Oh et al. (2008).

Phenetic analysis

Characters were selected and coded on the strength of homology. Characters that varied within species were excluded on the basis that they were likely altered by mounting conditions in the herbarium. Invariable characters across all studied taxa were also excluded. Overall, 19 qualitative characters were selected and coded (Table 1, "Appendices 3, 4"). All character states were coded as unordered and unweighted.

In addition to the 48 taxa studied above, nine species in four genera of the *Pterygiella* complex (Dong et al. 2013) in Rhinantheae s.s. were also included in our phenetic analysis. In total, 57 taxa in 26 genera were analyzed using the 19-qualitative character dataset ("Appendix 4"). Phenetic analysis was executed in NTSYSpc version 2.11c (Rohlf 2000), to produce a phenogram using the unweighted pair-group method using arithmetic averages (UPGMA).

Results

General description of fruit and seed features

Fruit and seed characters were found to be highly diverse in the studied taxa ("Appendix 2," Figs. 1, 2, and 3, 4). Capsule size varied both among and within genera. Capsule shape was mainly ovoid or oblong. The capsule was found to be coriaceous in most taxa, but papyraceous capsules occurred in the genera *Euphrasia*, *Melampyrum,* and *Omphralotrix*. The capsule surface could be recognized as bearing eglandular hairs (Fig. 1a–f), aculeate hairs (Fig. 1g, h), or glabrous (Fig. 1i). Among the eglandular hairs, unicellular ones with enlarged bases were the most characteristic (e.g., Fig. 1e).

Seed characters were also highly variable. Seed size varied within genera. The number of seeds produced per capsule was defined as numerous (>100), many (≥10 and <100), or several (<10). Seed shape was also heterogeneous. In general, it can be recognized as elliptic, globose, fusiform, reniform, ovoid, or oblong. Seed color was yellow, black, brown, or white, varying from species to species.

The ornamentation of the seed coat was found to be relatively constant within genera (Figs. 2, 3), while secondary ornamentation of the seed coat varied among genera [identified as lineate (Fig. 4a–d), reticulate (Fig. 4e–f), hook-like (Fig. 4g–i), poroid-alveolate (Fig. 4j), and smooth (Fig. 4k–l)]. The outer seed coat was membranous and found to rupture at maturity in some taxa. The seed surface may be spinulose (Fig. 4a) or tuberculate (Fig. 4f).

Table 1 Capsule and seed characters and character states of Rhinantheae s.l. and related taxa

No.	Characters	Character states
Capsule		
1	Shape	(0) Ovoid; (1) oblong
2	Color	(0) Yellow; (1) brown
3	Surface	(0) Glabrous; (1) eglandular hairs; (2) aculeate
4	Type of hairs	(0) Pilose; (1) strigose; (2) branched hairs
5	Texture	(0) Coriaceous; (1) papyraceous
6	Apex	(0) Acuminate; (1) cuspidate; (2) emarginate
Seed		
7	Size (represented by seed length, mean value, mm)	(0) Dust-like (≤ 1 mm); (1) small (> 1 mm and < 2 mm); (2) Medium (≥2 mm and <3 mm); (3) large (≥3 mm)
8	Number per capsule	(0) Few (<10); (1) many (≥10 and <100); (2) numerous (>100)
9	Shape laterally	(0) Concave; (1) flattened
10	Wing	(0) Unwinged; (1) winged
11	Wing number	(0) One; (1) several
12	Projections on surface	(0) Smooth; (1) spinulose; (2) tuberculate
13	Testa	(0) Tight-fitting; (1) loose-fitting
14	Primary ornamentation	(0) Reticulate; (1) cristate-winged; (2) sulcate; (3) Psilate; (4) striate
15	Adjacent ridges of reticulum region	(0) Trough; (1) ridge
16	Lines of reticulum	(0) Obscurely; (1) normal; (2) deep
17	Lines of reticulum height	(0) Uniformly (1) unevenly
18	Shape of mesh	(0) Narrowly or shortly rectangle; (1) isodiametric polygon; (2) irregular polygon
19	Secondary ornamentation	(0) Smooth; (1) reticulate; (2) lineate; (3) poroid-alveolate; (4) hook-like

In the present study, five principal morphological types and three subtypes were distinguished based on the ornamentation of the seed coat.

Type I Reticulate (Figs. 2,3a–f) This type includes 19 taxa, viz. *Aeginetia*, *Agalinis*, *Boschniakia*, *Brandisia*, *Castilleja*, *Clevelandia*, *Conopholis*, *Cordylanthus ramosus,* *Cymbaria*, *Epifagus*, *Lindenbergia*, *Ophiocephalus*, *Orobanche*, *Pedicularis*, *Rhinanthus*, *Siphonostegia*, *Sopubia*, *Striga,* and *Triphysaria*.

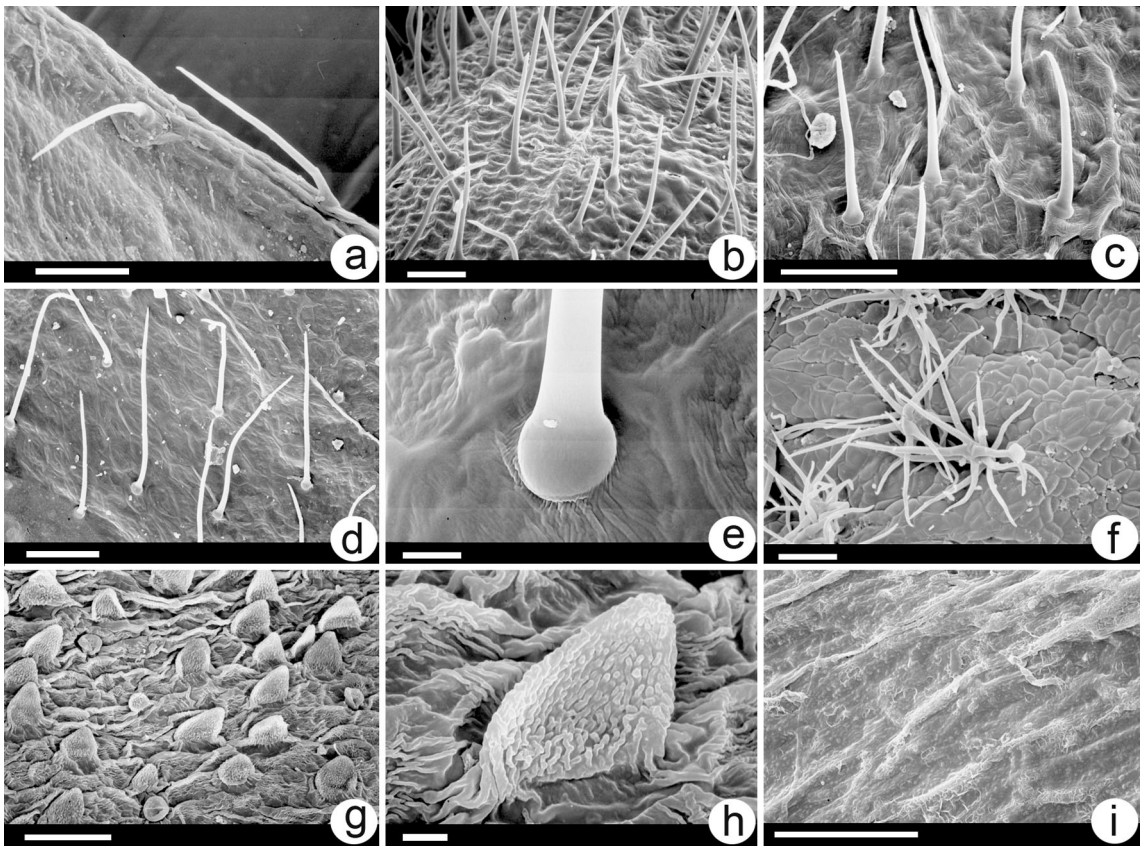

Fig. 1 Characteristic features of capsules. *Scale bars* 100 μm for whole capsule, 10 μm for higher magnification of capsule surface. **a**–**f** Eglandular hairs. **g**, **h** Aculeate hairs. **i** Smooth surface. **a** *Euphrasia regelii*, strigose. **b** *Lindenbergia philippnensis*, pilose. **c** *Odontites vulgaris*, strigose. **d**, **e** *Omphralotrix longipes*, pilose. **f** *Brandisia hancei*, branched hairs. **g**, **h** *Melampyrum nemorosum*, aculeate hairs. **i** *Pedicularis elwesii*, smooth

Subtype Ia Regular reticulate (Fig. 2) A reticulum is formed by the polygonal edges of the epidermal cells, in which the anticlinal cell walls are clearly or slightly raised above the periclinal cell walls: *Agalinis*, *Brandisia*, *Castilleja* (excluding *C. foliolosa* and *C. pilosa*), *Clevelandia*, *C. ramosus*, *Cymbaria*, *Epifagus*, *Lindenbergia*, *Orobanche*, *Pedicularis*, *Siphonostegia*, *Sopubia*, *Striga,* and *Triphysaria*.

In this group, the fruits are coriaceous, mostly with a glabrous capsule surface. The indumentum of *Lindenbergia* is unicellular pilose (Fig. 1b), and *Brandisia hancei* shows a very distinct hair type, with branched hairs (Fig. 1f). The seeds of this group are elliptic, ovoid, or globose. The hilum is terminal and convex in most taxa. The largest seed is found in *Pedicularis superba* (4.21 mm long) and the smallest in *Orobranche fasciculata* (0.24 mm long). Seed color varies within genera; for instance in *Castilleja,* it is yellow in *Castilleja attenuata*, *Castilleja densiflora,* and *Castilleja sulphurea*; brown in *Castilleja longiflora;* and black in *Castilleja tenuis*. The number of seeds produced per capsule is numerous in *Brandisia*, *Epifagus*, *Lindenbergia*, *Orobranche*, *Sopubia,* and *Striga*. The seed coat is membranous, and the periclinal wall partly or completely ruptured in *C. densiflora*, *C. longiflora*, *Castilleja miniata*, *C. sulphurea*, *C. tenuis*, *Clevelandia,* and *Triphysaria*. *Pedicularis* presents a unique secondary ornamentation of the seed coat, termed poroid-alveolate, which appears sponge-like (Fig. 4j). The projections of the seed surface may be spinulose (Fig. 4a) or tuberculate (Fig. 4f). Fibrils are discovered in *Cymbaria*, *Pedicularis,* and *Siphonostegia*.

Subtype Ib Obscurely reticulate (Fig. 3a, b) The lines and ridges of the reticulum are blurred: *Conopholis* and *Rhinanthus*.

The fruits of this group are ovoid, coriaceous, and glabrous, the seeds yellow. The hilum is lateral and slightly or distinctly convex. Dorsal wings were discovered in *Rhinanthus* (Fig. 3b).

Subtype Ic Deep reticulate (Fig. 3c, f) The anticlinal cell walls are extremely raised above the periclinal cell walls: *Aeginetia*, *Boschniakia*, *Castilleja foliolosa*, *Castilleja pilosa,* and *Ophiocephalus*.

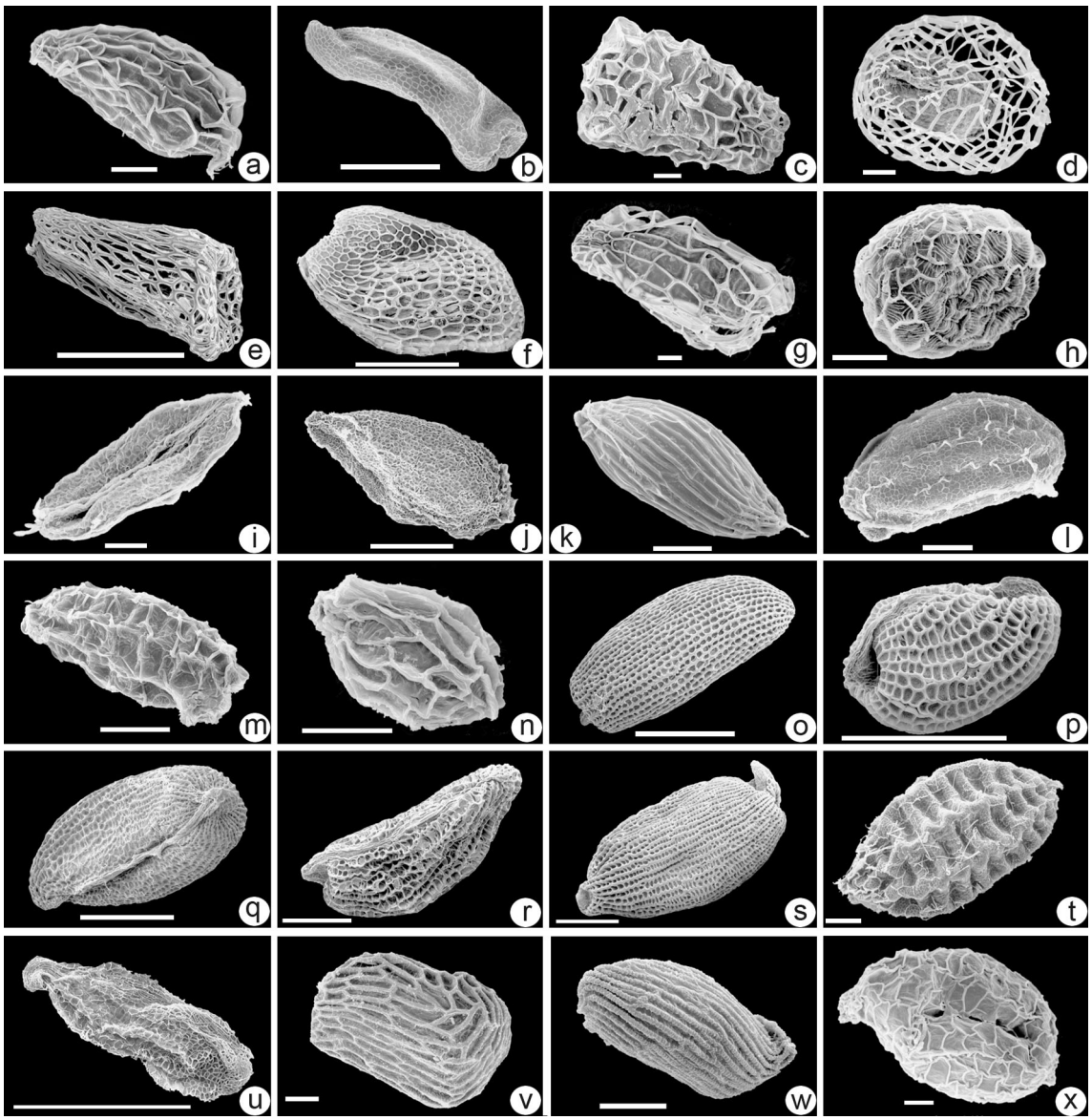

Fig. 2 Characteristic features of the regular reticulate seed coat (Type Ia). *Scale bars* 1 mm for **b**, **e**, **f**, **j**, **o**–**s**, **u**; 100 μm for all other seeds. **a** *Agalinis tenuifolia*. **b** *Brandisia hancei*. **c** *Castilleja attenuata*, partly ruptured. **d** *Castilleja densiflora,* completely ruptured. **e** *Castilleja longiflora*, completely ruptured. **f** *Castilleja sulphurea*, partly ruptured. **g** *Castilleja tenuis*, partly ruptured. **h** *Clevelandia beldingii*. **i** *Cordylanthus ramosus*. **j** *Cymbaria daurica*. **k** *Epifagus virginiana*. **l** *Lindenbergia indica*. **m** *Lidenbergia philippensis*. **n** *Orobanche fasciculata*. **o** *Pedicularis cephalantha*. **p** *Pedicularis densispica*. **q** *Pedicularis elwesii*. **r** *Pedicularis rupicola*. **s** *Pedicularis superba*. **t** *Siphonostegia chinensis*. **u** *Siphonostegia laeta*. **v** *Sopubia trifida*. **w** *Striga asiatica*. **x** *Triphysaria eriantha*, partly ruptured

The fruits of this group are ovoid and coriaceous without indumentum. The seeds are globose (elliptic in *Castilleja pilosa*). The seed coat is membranous and partly ruptured. The common secondary ornamentation of the seed coat is lineate but reticulate in *Boschniakia* (Fig. 4e).

Type II Cristate-winged (Fig. 3g–o) Three genera were delimited in this type: *Euphrasia*, *Odontites,* and *Omphalotrix*.

The fruits are oblong in *Euphrasia* and ovoid in *Odontites* and *Omphalotrix*; they are yellow in *Euphrasia* and *Omphalotrix* and brown in *Odontites*. This type shares an indumentum with eglandular hairs on the surface of capsule (Fig. 1a–e). The seeds are elliptic with many per capsule. The hilum is terminal, convex to varying extents. The seed coat exhibits longitudinal wings, and the seed surface is tuberculate.

Type III Sulcate (Fig. 3p) One genus: *Monochasma*.

The fruit is ovoid, brown, coriaceous, and glabrous. The seeds are oblong and yellow, and there are many per

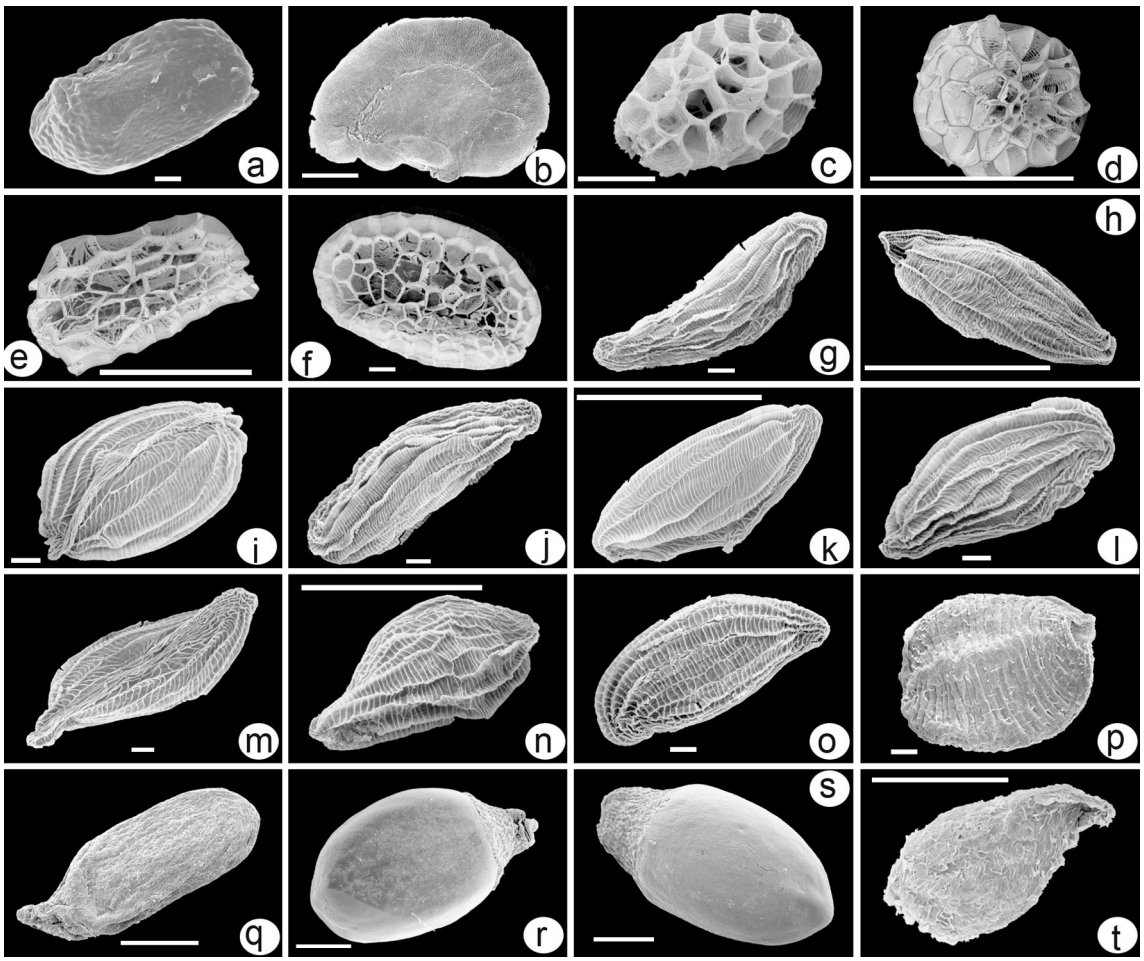

Fig. 3 Characteristic features of seed coat. *Scale bars* 1 mm for **b**, **d**, **e**, **h**, **k**, **n**, **q**–**t**; 100 μm for all other seeds. **a**, **b** Type Ib, obscurely reticulate. **g**, **f** Type Ic, deeply reticulate. **g**, **o** Type II, cristate-winged. **p** Type III, sulcate. **q**–**s** Type IV, psilate. **t** Type V, striate. **a** *Conopholis americana*. **b** *Rhinanthus glaber*, with dorsal wing. **c** *Aeginetia indica*, partly ruptured. **d** *Boschniakia himalaica*, partly ruptured. **e** *Castilleja pilosa*, partly ruptured. **f** *Ophiocephalus angustifolius*, partly ruptured. **g** *Euphrasia amurensis*. **h** *Euphrasia hirtella*. **i** *Euphraisa jaeschkei*. **j** *Euphrasia pectinata*. **k** *Euphrasia pectinata* subsp. *simplex*. **l** *Euphrasia regelii*. **m** *Euphrasia rostkoviana*. **n** *Odontites vulgaris*. **o** *Omphralotrix longipes*. **p** *Monochasma savatieri*. **q** *Melampyrum klebelsbergianum*. **r** *Melampyrum laxum*. **s** *Melampyrum nemorosum*. **t** *Cordylanthus rigidus*

capsule. The hilum is terminal and concave, and the surface of the seed is covered with fibrils.

Type IV Psilate (Fig. 3q–s) One genus: *Melampyrum*.

The fruit is ovoid, brown, and papyraceous. The indumentum of this genus, which is aculeate, is unique among the studied genera (Fig. 1g, h). The seed is ellipsoid, black, with several seeds per capsule. The hilum is terminal and distinctly convex.

Type V Irregularly striate (Fig. 3t) One species: *Cordylanthus rigidus*.

The fruit is ovoid, yellow, coriaceous, and glabrous. The seed is ovoid and yellow. The hilum is terminal and apparently convex.

Key to fruit and seed morphology of studied genera in Rhinantheae s.l. and related taxa

1. Seed coat reticulate	2
2. Seeds with dorsal wing	*Rhinanthus* L.
2. Seeds without wings	3
3. Capsules glabrous	4
4. Number of seeds per capsule fewer than 10	*Cymbaria* L.
4. Number of seeds per capsule more than 10	5
5. Seed coat loose-fitting and ruptured when mature	6
6. Secondary ornamentation of seed coat reticulate	*Boschniakia* C.A.Mey. ex Bong

6. Secondary ornamentation of seed coat smooth or of other types	7
7. Seeds dust-like and numerous (>100) per capsule	*Aeginetia* L.
7. Seeds relatively large and many (10–100) per capsule	8
8. Apex of capsule cuspidate	*Castilleja* Mutis ex L.f.
8. Apex of capsule not cuspidate	9
9. Surface of seeds tuberculate	*Ophiocephalus* Wiggins
9. Surface of seeds without projections	*Triphysaria* Fisch. & C.Mey.
5. Seed coat tight-fitting	10
10. Lines of reticulum obscure	*Conopholis* Wallr.
10. Lines of reticulum regular	11
11. Seeds relatively large (>2 mm long)	*Pedicularis* L.
11. Seeds small (≤2 mm long)	12
12. Seed numerous per capsule (>100)	13
13. Fruits brown	*Sopubia* Buch.-Ham. ex D.Don
13. Fruits yellow	14
14. Capsules oblong	*Striga* Lour.
14. Capsules ovoid	15
15. Capsules relatively large (around 10 mm)	*Orobanche* L.
15. Capsules relatively small (around 4 mm)	*Epifagus* Nutt.
12. Seeds many per capsule (10–100)	16
16. Surface of seeds with fibrils	*Siphonostegia* Benth.
16. Surface of seeds without fibrils	17
17. Secondary ornamentation of seed coat smooth	*Cordylanthus ramosus* Nutt. ex Benth.
17. Secondary ornamentation of seed coat lineate	18
18. Seed surface spinulose	*Clevelandia* Greene
18. Seed surface smooth	*Agalinis* Raf.
3. Capsule with hairs	19
19. Capsule provided with pilose; secondary ornamentation hook-like	*Lindenbergia* Lehm.
19. Capsule provided with branched hairs; secondary ornamentation smooth	*Brandisia* Hooker f. & Thomson
1. Seed coat not reticulate	20
20. Seed coat with cristate wings	21
21. Capsules coriaceous and cuspidate	*Odontites* Ludw.
21. Capsules papyraceous and emarginate	22
22. Capsules oblong and strigose	*Euphrasia* L.
22. Capsules ovoid and pilose	*Omphralotrix* Maxim.
20. Seed coat sulcate, psilate, or striate	23
23. Capsule surface aculeate and seed coat psilate	*Melampyrum* L.
23. Capsule surface glabrous and seed coat sulcate or striate	24
24. Secondary ornamentation lineate and spinulose	*Cordylanthus rigidus* Jeps.
24. Secondary ornamentation hook-like, tuberculate with fibrils	*Monochasma* Maxim. ex Franch. & Sav.

Systematic relationships within Rhinantheae s.l

The phenetic analysis produced a single tree based on the 19 qualitative characters of fruit and seed morphology (Table 1). Two major clusters were obtained: Group I and Group II (Fig. 5). Group I was very heterogeneous cluster, comprising members of eight different tribes: Cymbarieae, Castillejeae, Rhinantheae s.s., Gerardieae, Buchnereae, Orobancheae, Paulownieae, and Angelonieae. This group shared the common character of a reticulate seed coat. The other group, Group II, included all tribes in Rhinantheae s.l., viz. Cymbarieae, Castilljeae, and Rhinantheae s.s. This group can be distinguished by different types of seed coat.

Within Group I, two subgroups (Ia and Ib) were distinguished. Subgroup Ia included most studied taxa. *Pedicularis* and *Lindenbergia* were supported separately as monophyletic genera. *Pedicularis* was close to *Clevelandia*, *Cordylanthus ramosus*, *Conopholis, Cymbaria,* and *Siphonostegia chinensis*, probably due to its glabrous fruit and tight-fitting seed coat. The species of *Pedicularis* were distinguished from these taxa by the secondary ornamentation of the seed coat. *Brandisia*, *Xizangia*, *Phtheirospermum,* and *Pterygiella cylindrica* var. *suffruticosa* showed a close relationship with *Lindenbergia,* both having eglandular hairs on the surface of the capsule. Within the studied species of *Castilleja*, *Castilleja foliolosa*, *Castilleja miniata* and *C. longiflora* clustered with *Siphonostegia laeta*, *Aeginetia indica,* and *Boschniakia himalaica*, while the remaining species clustered with *Ophiocephalus angustifolius* and *Triphysaria eriantha*. These taxa shared the common characters of a loose-fitting seed coat, partly or completely ruptured in the mature seed. *Epifagus*, *Orobanche*, *Sopubia,* and *Striga* were clustered as a clade which can be recognized by having numerous seeds per capsule. The other subgroup, Ib, comprised the monophyletic *Rhinanthus*. Subgroup Ib can be distinguished from Ia mainly due to having dorsal wings on the seed.

Group II can also be separated into two subgroups (IIa and IIb). Subgroup IIa was formed by *Cordylanthus rigidus*, *Monochasma*, *Psuedobartsia*, *Pterygiella* (excluding *Pterygiella cylindrica* var. *suffruticosa*), and *Melampyrum*. Within these taxa, *Melampyrum* was distinct from the rest by having an aculeate surface to its capsule and psilate seed coat. *Pseudobartsia* was found to be close to *Pterygiella,* mainly due to the resemblance in their seed coat secondary ornamentation. *Cordylanthus rigidus* was sister to *Monochasma*. Within subgroup IIb, the genera of *Euphrasia*, *Omphalotrix,* and *Odontites* were clustered together by the possession of cristate wings along the seed.

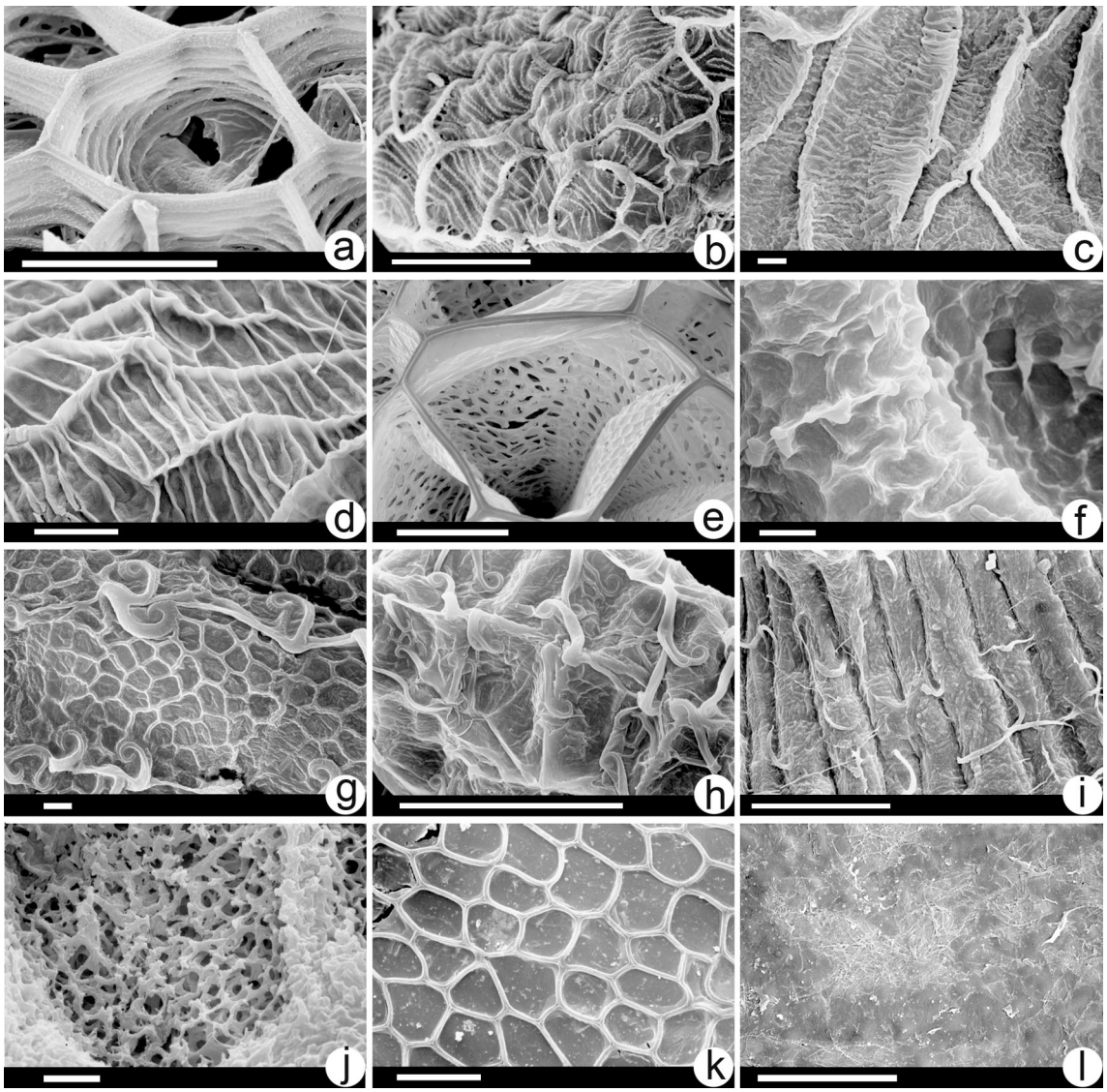

Fig. 4 Characteristic features of secondary ornamentation of seed coat. *Scale bars* 10 μm for **c**, **f**–**g**, **j**; 100 μm for all other images of seed coat. **a**, **b** lineate. **e**–**f** reticulate. **g**–**i** hook-like. **j** poroid-alveolate. **k**–**l** smooth. **a** *Castilleja sulphurea*, with spinulose projections. **b** *Clevelandia beldingii*. **c** *Cordylanthus rigidus*. **d** *Odontites vulgaris*. **e** *Boschniakia himalaica*. **f** *Siphonostegia chinensis*, with tuberculate projections. **g** *Lindenbergia indica*. **h** *Lindenbergia philippensis*. **i** *Monochasma savatieri*. **j** *Pedicularis densispica*. **k** *Brandisia hancei*. **l** *Melampyrum laxum*

Discussion

Fruit and seed were shown to be highly diverse in 48 species of 22 genera, especially within Rhinantheae s.l. It supports recent molecular-phylogenetic results in terms of the polyphyletic nature of Rhinantheae s.l. (Bennett and Mathews 2006; McNeal et al. 2013). In most studied taxa, common characters were found to be an ovoid, glabrous, coriaceous capsule and dust-like, numerous, reticulate seeds. Fruit and seed characters showed close relationships between Orobanchaceae and Rhinantheae s.l. and supported the transfer of *Lindenbergia* and *Brandisia* to Orobanchaceae (Olmstead et al. 2001; Bennett and Mathews 2006; McNeal et al. 2013).

The significance of fruit and seed features within Rhinantheae s.l.

Murley (1951) found that seed characters of the Cruciferae were in certain species constant and very reliable while in other species they may be highly variable. However, fruit and seed patterns are sufficiently diverse among the genera, sections, and species to furnish important features for classification, such as *Pedicularis* (Liu et al. 2013). In

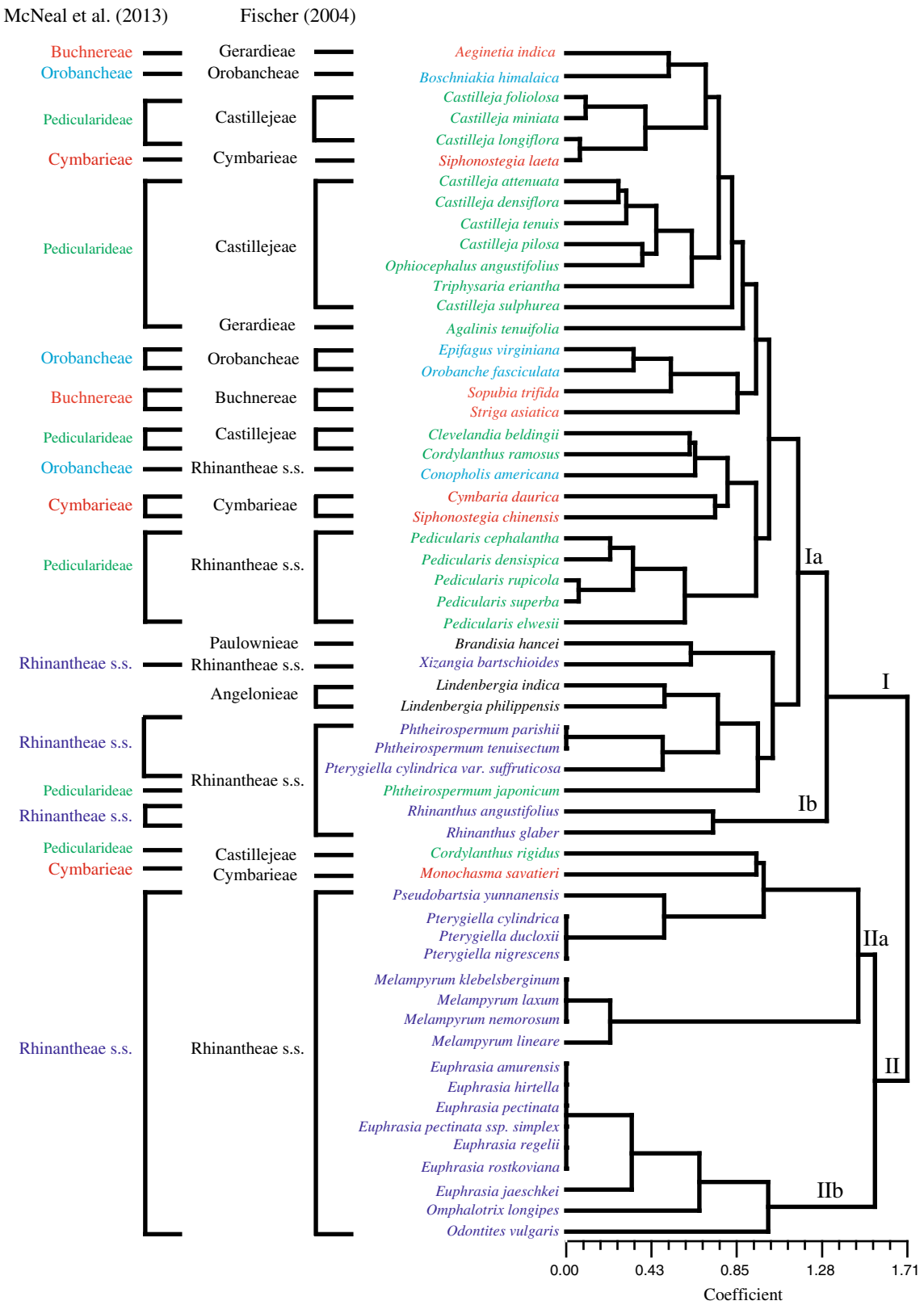

Fig. 5 UPGMA phenogram of Rhinantheae s.l. and related genera clustered on the basis of capsule and seed features

present study, capsule indumentum, seed number per capsule, seed size, seed color, and seed coat ornamentation may provide important information for classification at infra- and inter-generic level.

Previous studies have shown that hair features of the capsule may be useful in the identification of genera, species, and hybrids in the traditional family Scrophulariaceae (Raman 1991). In the tribe Rhinantheae, eglandular hairs were reported such as the genera of *Bartsia*, *Parentucellia*, *Phtheirospermum*, *Pterygiella*, *Pseudobartsia,* and *Xizangia* (Juan et al. 2000; Dong et al. 2013). This kind of hairs was mainly discovered and diverse among *Euphrasia* (Fig. 1a), *Odontites* (Fig. 1c), *Omphralotrix* (Fig. 1d, e), and *Lindenbergia* (Fig. 1b). The hairs of *Lindenbergia* and *Omphralotrix* were exhibited as pilose, while strigose in *Euphrasia* and *Odontites*. *Brandisia* was found to differ from the other taxa in its branched hairs. This hair type is also present in *Verbascum* L. (Juan et al. 2000) and some other genera of Scrophulariales (Cantino 1990). The most unique structure of the hair was discovered in *Melampyrum*, where it is aculeate (Fig. 1g) and covered by claviform tips (Fig. 1h). However, the sampling of this study is limited, glandular hairs were also discovered within the tribe, such as some species of *Bartsia* (Molau 1990). With relation to the hemiparasitic trait, the glandular hairs are mainly a means of secretory deposits for toxic secondary metabolites from the host plants (Press and Graves 1995). Thus, the hairs are matter of adaptation with limited information at taxonomy in this tribe.

Seed shape and size are very heterogeneous even within the same species (Chuang and Heckard 1983; Juan et al. 2000). However, seed size may still be used to identify different genera, because seed size is negatively correlated with seed number per capsule in Scrophulariaceae (Fischer 2004) and correlated with life history evolution in Rhinantheae s.l. (Těšitel et al. 2010). Most taxa in our study possessed dust-like seeds ($\leq$1 mm long) which were numerous per capsule (>100). *Euphrasia*, *Omphrasia*, *Cymbaria,* and *Odontites* had medium-sized seeds ($\geq$2 mm and <3 mm long) and possessed many seeds per capsule. *Pedicularis*, *Melampyrum,* and *Rhinanthus* had almost the biggest seeds ($\geq$3 mm long), and just several seeds per capsule. Seed color is diverse but constant within species. In *Castilleja*, *Euphrasia,* and *Pedicularis*, the color of seed may be used to distinguish different species.

Seed coat is constant and could provide systematic information in Scrophulariaceae and Orobanchaceae (Chuang and Heckard 1972; Musselman and Mann 1976; Molau 1990; Juan et al. 2000; Dong et al. 2013). The studied species of *Euphrasia* are restricted to the section *Semicalcaratae* Benth. (Hong et al. 1998). This section is supported by homologous fruit and seed characteristics (Type II): a strigose surface to the capsule, several seeds per capsule, and a seed coat with cristate wings. Cristate wings were also appeared in the species of genera *Bartsia* (Molau 1990), *Odontites,* and *Omphralotrix*. Cristate wings are an especially important feature for distinguishing these taxa from all others in Rhinantheae s.l. The seed data mentioned above reflect the close relationships among *Bartsia*, *Euphrasia*, *Odontites,* and *Omphralotrix* in the tribe Rhinantheae s.s.

The seed coat shows differences among closely related genera but similarities in divergent genera. A loose-fitting seed coat and terminal hilum have been treated as important characters to distinguish *Castilleja*, *Ophiocephalus*, *Triphysaria,* and *Clevelandia* from *Orthocarpus* and *Cordylanthus* (Chuang and Heckard 1991). Within *Castilleja*, species could be grouped into two clades (Fig. 5), supporting former taxonomies (Chuang and Heckard 1991; Tank and Olmstead 2008; Tank et al. 2009). One group comprises mainly subg. *Castilleja* (*Castilleja foliolosa*, *Castilleja miniata,* and *C. longiflora*). *Siphonostegia laeta* (Fig. 2u), *Aeginetia indica* (Fig. 3c), and *Boschniakia himalaica* (Fig. 3d) show a close relationship with this clade of *Castilleja* by also having a loose-fitting and reticulate seed coat. However, *Aeginetia indica* and *Boschniakia himalaica* are diverged from subg. *Castilleja* of *Castilleja* by morphology (Minkin and Eshbaugh 1989; Lu et al. 2007) and molecular analysis (Bennett and Mathews 2006). The other group comprises species in the subg. *Colacus* sect. *Oncorhynchus* (*C. attenuata*, *C. densiflora*, *C. tenuis*, *Castilleja pilosa,* and *C. sulphurea*) and is close to *Ophiocephalus* and *Triphysaria*. *Clevelandia beldingii* is sister to *Ophiocephalus angustifolius*, embedded into subg. *Castilleja*, and treated as *Castilleja beldingii* (Greene) Tank & J.M.Egger and *Castilleja ophiocephala* Tank & J.M.Egger, respectively, (Tank and Olmstead 2008; Tank et al. 2009). *Ophiocephalus angustifolius* is similar to *Castilleja* in having a seed coat with a deeply reticulate surface (Type Ic), which supports the movement of this monotypic genus into *Castilleja*. However, fruit and seed features suggest a close relationship between *Clevelandia beldingi* and *Cordylanthus ramosus* by having regular reticulate, tight-fitting seed coat. In genus *Cordylanthus*, two types of seed coat were discovered: regular reticulate (*Cordylanthus ramosus*) and irregularly striate (*Cordylanthus rigidus*), in accordance with Chuang and Heckard (1972). Although fruit and seed characters showed obvious divergence between *Cordylanthus ramosus* and *Cordylanthus rigidus*, these two species are placed in the same genus, *Cordylanthus*, with a close relationship between them (Tank and Olmstead 2008; Tank et al. 2009).

It is notable that fruit and seed features are relatively stable in external morphology but probably imply

significant evolutionary mechanisms in adaptation for successful dispersal and production. Most taxa studied have dust-like and reticulate seeds, which may be adapted to wind- or water-dispersal by possessing a multiform outer seed coat (Pennell 1935; Kuijt 1969). It is worthy to notice that minute reticulate seeds have a long dispersal distance depending on two steps, especially in moist, high alpine habitats (Molau 1990; Press and Graves 1995). Larger, heavier seeds, and relatively large and conspicuously protrudent hilum, as in *Melampyrum* and *Pedicularis,* may be transported by animal vectors (Berg 1954; Van de Pijl 1982). Meanwhile, large seed size appears generally advantageous for seedling size and competitive ability compared to small (Těšitel et al. 2010). The character state of having glandular/eglandular hairs of capsule is a matter of life history strategies between annual (r-strategists) and long-lived perennials (K-strategists). Thus, careful analyses are necessary to promote a greater use of fruit and seed characters for comparative and taxonomic purposes.

Comments on classification within Rhinantheae s.l.

Bentham and Hooker (1876) proposed a radically different basis for the division of Rhinanthideae on the basis of whether the plants were parasitic or not. The tribe Euphrasieae (=Rhinantheae s.l.) contained the species with hemi-parasitic characteristics. However, the tribe Rhinantheae s.l. has been shown by molecular systematics (Young et al. 1999; Wolfe et al. 2005; Bennett and Mathews 2006; McNeal et al. 2013) and pollen morphology (Lu et al. 2007) to be a polyphyletic group. According to Fischer (2004), the traditional Rhinantheae s.l. can be divided into three tribes: Cymbarieae, Castillejeae, and Rhinantheae s.s., but uniquely defining traits for these tribes are absent. Although the sampling of this study is limited, our results allow some taxonomic suggestions regarding the delimitation of three tribes.

Cymbarieae This tribe includes *Schwalbea*, *Cymbaria*, *Siphonostegia*, *Lesquereuxia*, *Bungea,* and *Monochasma*. Their close relationship is supported by morphology (Bentham and Hooker 1876) and their distribution (Hong 1983). The results of molecular-phylogenetic analyses by Bennett and Mathews (2006) and McNeal et al. (2013) showed a close relationship among *Siphonostegia*, *Schwalbea*, *Monochasma*, *Cymbaria,* and *Bungea*. Pollen features also exhibit similarities among *Cymbaria*, *Siphonostegia,* and *Monochasma*: All have a regular retipilate exine sculpture and pollen greater than 27 μm in size.

Common fruit and seed characters in this tribe include a glabrous capsule and reticulate seed in *Cymbaria* and *Siphonostegia*. The most-derived seed coat type was discovered in *Monochasma savatieri,* with sulcate seeds. Seed coat morphology has previously indicated a close relationship among *Cymbaria*, *Siphonostegia,* and *Schwalbea* (Musselman and Mann 1976). *Siphonostegia* includes four species, and two of them are distributed in China. In our study, these species of *Siphonostegia* can be diverged into two groups (Fig. 5): Tight-fitting seed coat (*Siphonostegia chinensis*) show similarity with seeds of *Cymbaria daurica* and loose-fitting seed coat (*Siphonostegia laeta*) similarity with seeds of *Castilleja*. *Siphonostegia* can easily be distinguished from *Cymbaria daurica* and *C. longiflora* by the morphology of their stem, leaf, and flower. The morphology of fruit and seed shows more information in adaptation to habitat by the stress of convergent evolution.

Castillejeae This tribe is defined as including *Castilleja*, *Orthocarpus*, *Triphysaria*, *Clevelandia*, *Gentrya*, *Cordylanthus,* and *Ophiocephalus* (Fischer 2004). The monophyly of the tribe is supported by molecular-phylogenetic analyses (Tank and Olmstead 2008; Tank et al. 2009). A proposed classification suggested that *Clevelandia* and *Ophiocephalus* should be moved into *Castilleja* and the biphyletic genus of *Cordylanthus* split into three genera (*Chloropyron*, *Dicranostegia,* and *Cordylanthus*; Tank et al. 2009). These genera are easily distinguished by theirs anther sacs, which are unequal in size and unequally attached (Bentham and Hooker 1876; Tank and Olmstead 2008; Tank et al. 2009).

Compared to the diverse pollen morphology in this tribe (Minkin and Eshbaugh 1989; Lu et al. 2007), fruit and seed characters are relatively consistent, with glabrous capsules, many seeds per capsule, and reticulate seeds. However, it is difficult to provide a synapomorphic fruit and seed character for distinguishing the tribe Castillejeae. Glabrous capsules are exhibited in most taxa of traditional tribe Rhinantheae s.l., and many seeds were also found in Rhinantheae s.s. (e.g., *Euphrasia* and *Odontites*). The reticulate seed coat is a plesiomorphic trait (Tank et al. 2009), and the depth of reticulations is not a clear distinction (Chuang and Heckard 1976) for the tribe. A derived seed coat character—irregularly striate—was found in *Cordylanthus rigidus*. The irregularly striate seed coat is an effective diagnostic feature for genus *Cordylanthus*, because it is not found in any other group of Castillejeae (Chuang and Heckard 1976; 1986).

While, the tribe Castillejeae is a paraphyletic group unless including *Agalinis* Raf., *Aureolaria* Raf., *Brachystigma* Pennell, *Dasistoma* Raf., *Esterhazya* Mikan, *Lamourouxia* Kunth, *Macranthera* Nutt. ex Benth., *Pedicularis* L., *Seymeria* Pursh, and *Phtheirospermum japonicum*,

which is definition as Pedicularideae Duby (McNeal et al. 2013). Liu et al. (2013) also discovered the similarity between *Pedicularis* and Fischer's (2004) Castillejeae (e.g., *Orthocarpus*, *Agalinis*, *Aureolaria*, and *Cordylanthus*). In our study, the regular reticulate secondary thickening and poroid-alveolate inner tangential wall ornamentation (Fig. 4f) of *Pedicularis* show similarity with seeds of *Agalinis*, *Castilleja*, *Cordylanthus,* and *Clevelandia* and clustered in Group Ia with the genera from Castillejeae (Fig. 5). Besides, the reticulate secondary thickening of seeds in *P. japonicum* is divergent from the other species in the genus *Phtheirospermum*, which is similar with *Boschniakia himalaica* and *Pedicularis* (Liu et al. 2013). Based on these differences in seed coat, *Pedicularis* and *Phtheirospermum japonicum* should be excluded from Rhinantheae s.s. and moved into Fischer's (2004) Castillejeae.

Rhinantheae s.s As Fischer's (2004) circumscribed, this tribe comprises ca. 1,200 species in 21 genera, e.g., *Bartsia*, *Odontites*, *Euphrasia*, *Omphalotrix*, *Pterygiella*, *Phtheirospermum*, *Pedicularis*, *Rhinanthus*, *Melampyrum,* and *Conopholis* et al. Relationships among taxa of the tribe inferred from analyses of cpDNA (Young et al. 1999), ITS (Wolfe et al. 2005), and PHYA (Bennett and Mathews 2006) indicate that it is polyphyletic (Tank et al. 2006; McNeal et al. 2013). The morphologies of inflorescence, corolla, anthers, and stigma are diverse and overlapping with other tribes in the family Orobanchaceae. The genera of the tribe lack generative or vegetative synapomorphies.

Xizangia, *Phtheirospermum*, and *Pterygiella cylindrica* var. *suffruticosa* were clustered with *Brandisia* and *Lindenbergia* in clade I, owing to the indumentum on the surface of fruit and reticulate seeds. *Pterygiella cylindrica* var. *suffruticosa* is differentiated from *Pterygiella cylindrica* by its shrub habit according to Hong (1996). Although the reticulate seed provided obviously divergent characters from the remains in the genus, *Pterygiella* is a monophyletic group and closed to *Xizangia*, *Phtheirospermum* (excluding *P. japonicum*), *Melampyrum,* and *Rhinanthus* (Dong et al. 2013; McNeal et al. 2013). Meanwhile, *Conopholis* is sister to *Epifagus* based on molecular-phylogenetic trees (Bennett and Mathews 2006) and moved into Orobancheae (McNeal et al. 2013), the fruit and seed morphology are differential between these genera. In this case, the fruit and seed characters are homoplasy and cannot imply the systematic information among these genera.

Euphrasia, *Omphalotrix,* and *Odontites* were clustered together in clade II by having indumentum and cristate-winged seed. The seed morphology is very similar to *Bartsia,* and the main differences are the apical part of capsule is never emarginated in the latter (Molau 1990). Recently, *Bartsia* is merged into *Hedbergia*, *Parentucellia,* and *Odontites,* respectively, leaving *B. alpine* only in *Bartsia* (Scheunert et al. 2012). However, the cristate-winged seed is only found in these genera of the tribe Rhinantheae s.s. Meanwhile, the seed character supported the close relationship among *Rhinanthus*, *Bartsia*, and *Euphrasia* by having dorsal wing. The wing of the seed surface may be synapomorphic character in these genera, which need confirmed by expending the sampling in future researches.

Melampyrum is an unusual member of the tribe by having aculeate hairs on the surface of capsule (Fig. 1g, h) and pislate seeds (Fig. 3q–s). While the phenetic analysis of fruit and seed morphology is coincide with recently molecular-phylogenetic researches that *Melampyrum* is close to *Pterygiella*, *Euphrasia*, *Omphlotrix*, and *Odontites* (Scheunert et al. 2012; McNeal et al. 2013). It is worth noting that Musselman and Mann (1976) documented the genus as having a reticulate seed including *Melampyrum lineare*, while Arekal (1963) described the seed coat of *Melampyrum lineare* as smooth. After carefully observing four species of the genus (*Melampyrum klebelsberginum*, *Melampyrum laxum*, *Melampyrum nemorosum,* and *Melampyrum lineare*) from specimens, our results are consistent with Arekal (1963). Further investigation is still required to clarify the seed coat structure of *Melampyrum* by collection of fresh fruit.

On the whole, the fruit and seed features of Rhinantheae s.s should be easily used to distinguished different genera but contain restricted systematic information in different taxa. Relationships among the tribe still require more detailed morphological studies with exhausting sample.

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Appendix 1

See Table 2.

Table 2 Studied taxa of Rhinantheae *s.l.* and related taxa

Taxon	Collector, Accession code, Herbarium acronym	Classification			Figure	Type
		Bentham and Hooker (1876)	Fischer (2004)	McNeal et al. (2013)		
Aeginetia indica L.	Y.H. Li 4442 [KUN]	Orobanchaceae	Orobanchaceae, Gerardieae	Orobanchaceae, Buchnereae	Fig. 3c	Ic
Agalinis tenuifolia Raf.	L.K.Henry *s.n.* [KUN]	–	Orobanchaceae, Gerardieae	Orobanchaceae, Pedicularideae	Figs. 2a	Ia
Boschniakia himalaica Hook.f. & Thomson	Zhangdian Expedition 63-3968 [KUN]	Orobanchaceae	Orobanchaceae, Orobancheae	Orobanchaceae, Orobancheae	Figs. 3d, 4e	Ic
Brandisia hancei Hook.f.	H. Li 4 [KUN]	Scrophulariaceae, Cheloneae	Paulowniaceae, Paulownieae	Orobanchaceae, –	Figs. 1f, 2b, 4k	Ia
Castilleja attenuata (A.Gray) T.I.Chuang & Heckard	B. Bartholomew 4 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2c	Ia
Castilleja densiflora (Benth.) T.I.Chuang & Heckard	B. Bartholomew 1402 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2d	Ia
Castilleja foliolosa Hook. & Arn.	B. Bartholomew & B. Anderson 3991B [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	–	Ic
Castilleja longiflora Kunze	B. Bartholomew, L.R. Landrum, H.W. Li, T.S. Ying & O.R. Dorado R. 3067 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2e	Ia
Castilleja miniata Douglas ex Hook.	E. MeClintock et al. *s.n.* [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	–	Ia
Castilleja pilosa Rydb.	J.T. Howell 11993 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 3e	Ic
Castilleja sulphurea Rydb.	A.R. Kruckeberg 2870 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2f, 4a	Ia
Castilleja tenuis (A.Heller) T.I.Chuang & Heckard	A. Eastwood & J.T. Howell 2826 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2g	Ia
Clevelandia beldingii Greene	I.L. Wiggins 5617 [CAS]	–	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Figs. 2h, 4b	Ia
Conopholis americana (L.) Wallr.	D.E. Baufford 21117 [KUN]	Orobanchaceae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Orobancheae	Fig. 3a	Ib
Cordylanthus ramosus Nutt.	A. Tiehm & J. Nachlinger 10908 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2i	Ia
Cordylanthus rigidus Jeps.	H.M. Pollard 9th 9/62 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Figs. 3t, 4c	V
Cymbaria daurica L.	S.E. Liu 3199 [PE]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Cymbarieae	Orobanchaceae, Cymbarieae	Fig. 2j	Ia
Epifagus virginiana (L.) W.P.C.Barton	F.H. Utech 10 [KUN]	Orobanchaceae	Orobanchaceae, Orobancheae	Orobanchaceae, Orobancheae	Fig. 2 k	Ia
Euphrasia amurensis Freyn	S.E. Liu 8238 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3g	II
Euphrasia hirtella Jord. ex Reut.	Xingjiang Expedition 8456 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3h	II
Euphrasia jaeschkei Wettst.	Qinhai-Tibet Expedition 6325 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3i	II
Euphrasia pectinata Ten.	P.C.Tsoong 3018 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3j	II

Table 2 continued

Taxon	Collector, Accession code, Herbarium acronym	Classification			Figure	Type
		Bentham and Hooker (1876)	Fischer (2004)	McNeal et al. (2013)		
Euphrasia pectinata Ten. subsp. *simplex* (Freyn) D.Y. Hong	T. Yamazaki 4790 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3k	II
Euphrasia regelii Wettst.	Zhong Dian Expedition 3573 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 1a, 3l	II
Euphrasia rostkoviana Hayne	German-Chinese Expedition 35.542 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3m	II
Lindenbergia indica (L.) Vatke	E.D. Liu 304 [KUN]	Scrophulariaceae, Gratioleae	Veronicaceae, Angelonieae	Orobanchaceae, Lindenbergia	Fig. 2l, 4g	Ia
Lindenbergia philippinensis Benth.	W.Q. Yin 2008 [KUN]	Scrophulariaceae, Gratioleae	Veronicaceae, Angelonieae	Orobanchaceae, Lindenbergia	Figs. 1b, 2m, 4h	Ia
Melampyrum klebelsbergianum Soó	Sino-American Expedition to Yunnan 0153 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3q	IV
Melampyrum laxum Miq.	T. Yamazaki 1789 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3r, 4l	IV
Melampyrum lineare Lam.	F.H. Vetch 80-137 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	–	IV
Melampyrum nemorosum L.	H. Li 89-026 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 1g, h, 3s	IV
Monochasma savatieri Franch. ex Maxim.	M.X. Nie & S.K. Lai 2216 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Cymbarieae	Orobanchaceae, Cymbarieae	Figs. 3p, 4i	III
Odontites vulgaris Moench	S.E. Liu et al. 8480 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Figs. 1c, 3n, 4d	II
Omphalotrix longipes Maxim.	C.S. Wang 1 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Figs. 1d, e, 3o	II
Ophiocephalus angustifolius Wiggins	I. L. Wiggins 9072 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 3f	Ic
Orobanche fasciculata Nutt.	J.T. Howell 48015 [KUN]	Orobanchaceae	Orobanchaceae, Orobancheae	Orobanchaceae, Orobancheae	Fig. 2n	Ia
Pedicularis cephalantha Franch. ex Maxim.	H. Wang, J. Cai, W.L. Li & X. Yang 102 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Pedicularideae	Fig. 2o	Ia
Pedicularis densispica Franch. ex Maxim.	N. Fujü, T. Ohi & Y. Lu F00153 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Pedicularideae	Figs. 2p, 4j	Ia
Pedicularis elwesii Hook.f.	Qingzang Expedition 73-1307 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Pedicularideae	Figs. 1i, 2q	Ia
Pedicularis rupicola Franch.	L.M. Gao 105801 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Pedicularideae	Fig. 2r	Ia
Pedicularis superba Franch. ex Maxim.	Qingzang Expedition 12729 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Pedicularideae	Fig. 2s	Ia

Table 2 continued

Taxon	Collector, Accession code, Herbarium acronym	Classification			Figure	Type
		Bentham and Hooker (1876)	Fischer (2004)	McNeal et al. (2013)		
Rhinanthus angustifolius C.C.Gmel.	German-Chinese Expedition 35.575 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	–	Ib
Rhinanthus glaber Lam.	R.C. Qing 1549 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Rhinantheae s.s.	Orobanchaceae, Rhinantheae	Fig. 3b	Ib
Siphonostegia chinensis Benth.	S.G. Wu 214 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Cymbarieae	Orobanchaceae, Cymbarieae	Figs. 2t, 4f	Ia
Siphonostegia laeta S.Moore	Fudan University Mingxi Expedition 93012 [KUN]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Cymbarieae	Orobanchaceae, Cymbarieae	Fig. 2u	Ia
Sopubia trifida Buch.-Ham. ex D.Don	R.C. Qing 23528 [KUN]	Scrophulariaceae, Gerardieae	Orobanchaceae, Buchnereae	Orobanchaceae, Buchnereae	Fig. 2w	Ia
Striga asiatica (L.) Kuntz	L. Deng 2521 [KUN]	Scrophulariaceae, Gerardieae	Orobanchaceae, Buchnereae	Orobanchaceae, Buchnereae	Fig. 2v	Ia
Triphysaria eriantha (Benth.) T.I.Chuang & Heckard	H.I. Stevens & D. Christopher 293 [CAS]	Scrophulariaceae, Euphrasieae	Orobanchaceae, Castillejeae	Orobanchaceae, Pedicularideae	Fig. 2x	Ia

Appendix 2

See Table 3.

Appendix 3: Descriptions of characters and character states

Capsule:

(1) Shape. Two character states are described: ovoid (0) and oblong (1). Some capsules are narrowly or broadly ovoid, these are included in ovoid (0) as a qualitative description.
(2) Color. Two character states are described: yellow (0) and brown (1).
(3) Surface. Three character states are described: glabrous (0), eglandular hairs (1), and aculeate (2). Glabrous indicates no extra structures on the surface. Eglandular hairs are the most characteristic trait, and the hairs usually display an enlarged base. Aculeate indicates triangular protruberances covered by claviform tips.
(4) Type of indumentum. Three character states are described: pilose (0), strigose (1), and branched hairs (2). Pilose indicates long and erect hairs. Strigose indicates short and inclined hairs. Branched hair indicates several hairs on a single stem.
(5) Texture. Two character states are described: coriaceous (0) and papyraceous (1). Coriaceous is defined as tough and pliable, papyraceous as soft and fragile.
(6) Apex. Three character states are described: acuminate (0), cuspidate (1), and emarginate (2). Acuminate indicates a sharp point at the apex. Cuspidate indicates a narrowing to a point. Emarginated indicates a notch at the apex.

Seed:

(7) Size. Four character states are described: dust-like (0), small (1), medium (2), and large (3). These character states are divided based on the scatter of the mean seed length.
(8) Number. Three character states are described: several (0), many (1), and numerous (2). Several are defined as fewer than 10 seeds per capsule. Numerous is defined as above 100 seeds. Many are defined as between 10 and 100 seeds.
(9) Lateral shape. Two character states are described: concave (0) and flattened (1). Concave indicates depressed on the seed surface to varying degrees. Flattened displays a smooth plane on the surface.

Table 3 Summary of fruit and seed characteristics in representative genera within tribe Rhinantheae s.l. and related taxa

Taxon	Fruit						Seed						
	Shape	Color	Size (length × width, in mm)	Surface	Texture	Apex	Shape	Color	Size (length × width, in mm) & number	Hilum	Primary ornamentation	Secondary ornamentation	Other features
Aeginetia indica	Ovoid	Brown	20.79 × 14.76	Glabrous	Coriaceous	Acuminate	Ovoid	Yellow	0.28 × 0.18 > 100	Inconspicuous	Deeply reticulate, membranous	Lineate	Partly ruptured, tuberculate
Agalinis tenuifolia	Oblong	yellow	3.17 × 3.21	Glabrous	Coriaceous	Cuspidate	Ovoid	Yellow	0.50 × 0.24 ≥ 10 and < 100	Terminal, slightly convex	Reticulate, membranous	Lineate	
Boschniakia himalaica	Ovoid	Brown	13.13 × 7.00	Glabrous	Coriaceous	Acuminate	Globose	Brown	0.82 × 0.83 > 100	Inconspicuous	Deeply reticulate, membranous	Reticulate	Partly ruptured
Brandisia hancei	Ovoid	Brown	10.15 × 8.00	Branched hairs	Coriaceous	Acuminate	Elliptic	Yellow	2.44 × 0.58 > 100	Inconspicuous	Reticulate, membranous	Smooth	
Castilleja attenuata	Ovoid	Yellow	6.40 × 3.03	Glabrous	Coriaceous	Cuspidate	Oblong	Yellow	0.73 × 0.41 ≥ 10 and < 100	Inconspicuous	Reticulate	Smooth	Tuberculate
C. densiflora	Ovoid	Yellow	5.40 × 2.44	Glabrous	Coriaceous	Cuspidate	Globose	Yellow	0.67 × 0.40 ≥ 10 and < 100	Terminal distinctly convex	Reticulate, membranous	Smooth	Completely ruptured, spinulose
C. foliolosa	Ovoid	Brown	12.89 × 5.62	Glabrous	Coriaceous	Cuspidate	Elliptic	Black	2.24 × 1.38 ≥ 10 and < 100	Inconspicuous	Deeply reticulate, membranous	Lineate	
C. longiflora	Ovoid	Brown	10.66 × 5.83	Glabrous	Coriaceous	Cuspidate	Elliptic	Brown	1.95 × 1.00 ≥ 10 and < 100	Terminal, slightly concave	Reticulate	Smooth	Completely ruptured
C. miniata	Ovoid	Brown	14.12 × 6.80	Glabrous	Coriaceous	Cuspidate	Elliptic	Brown	2.75 × 1.03 ≥ 10 and < 100	Terminal, slightly concave	Reticulate, membranous	Lineate	Partly ruptured
C. pilosa	Ovoid	Yellow	11.25 × 4.76	Glabrous	Coriaceous	Cuspidate	Elliptic	Black	1.75 × 0.75 ≥ 10 and < 100	Inconspicuous	Deeply reticulate, membranous	Lineate	Partly ruptured, tuberculate
C. sulphurea	Ovoid	Yellow	10.99 × 5.98	Glabrous	Coriaceous	Cuspidate	ovoid	Yellow	2.12 × 1.46 ≥ 10 and < 100	Terminal, slightly concave	Reticulate, membranous	Lineate	Partly ruptured, spinulose
C. tenuis	Ovoid	Yellow	7.16 × 2.61	Glabrous	Coriaceous	Cuspidate	Elliptic	Black	1.17 × 0.54 ≥ 10 and < 100	Terminal, slightly convex	Reticulate, membranous	Smooth	Partly ruptured, tuberculate
Clevelandia beldingii	Ovoid	Yellow	4.82 × 1.90	Glabrous	Coriaceous	Cuspidate	Elliptic	Yellow	0.34 × 0.28 ≥ 10 and < 100	Inconspicuous	Reticulate	Lineate	Spinulose
Conopholis americana	Ovoid	Yellow	9.59 × 7.08	Glabrous	Coriaceous	Acuminate	Elliptic	Yellow	0.85 × 0.47 > 100	Lateral, slightly concave	Obscurely reticulate	Smooth	
Cordylanthus ramosus	Ovoid	Brown	4.66 × 1.70	Glabrous	Coriaceous	Cuspidate	Elliptic	–	0.64 × 0.20 ≥ 10 and < 100	Terminal, slightly convex	Reticulate	Smooth	

Table 3 continued

Taxon	Fruit						Seed						
	Shape	Color	Size (length × width, in mm)	Surface	Texture	Apex	Shape	Color	Size (length × width, in mm) & number	Hilum	Primary ornamentation	Secondary ornamentation	Other features
C. rigidus	Ovoid	Yellow	7.84 × 3.95	Glabrous	Coriaceous	Cuspidate	Ovoid	Yellow	0.17 × 0.10 ≥ 10 and < 100	Terminal, apparently convex	Irregularly striate	Lineate	Spinulose
Cymbaria daurica	Ovoid	borwn	1.15 × 8.24	Glabrous	Coriaceous	Acuminate	Ovoid	Brown	2.63 × 1.18 < 10	Inconspicuous	Reticulate	Smooth	Fibrils
Epifagus virginiana	Ovoid	Yellow	3.78 × 3.21	Glabrous	Coriaceous	Acuminate	Elliptic	Yellow	0.47 × 0.18 > 100	Terminal, slightly convex	Reticulate	Smooth	Tuberculate
Euphrasia amurensis	Oblong	Yellow	5.00 × 1.89	Strigose	Papyraceous	Emarginate	Elliptic	Yellow	1.18 × 0.25 ≥ 10 and < 100	Terminal, slightly convex	Cristate-winged, membranous	Lineate	Tuberculate
E. hirtella	Oblong	Yellow	5.44 × 2.01	Strigose	Papyraceous	Emarginate	Elliptic	White	1.16 × 0.76 ≥ 10 and < 100	Terminal, apparently convex	Cristate-winged, membranous	Lineate	Tuberculate
E. jaeschkei	Oblong	Yellow	5.17 × 1.98	Strigose	Papyraceous	Emarginate	Elliptic	White	0.92 × 0.52 ≥ 10 and < 100	Terminal, apparently convex	Cristate-winged, membranous	Lineate	Spinulose
E. pectinata	Oblong	Yellow	5.94 × 1.89	Strigose	Papyraceous	Emarginate	Elliptic	White	1.27 × 0.37 ≥ 10 and < 100	Terminal, apparently convex	Cristate-winged, membranous	Lineate	Tuberculate
E. pectinata subsp. *simplex*	Oblong	Yellow	6.55 × 2.35	Strigose	Papyraceous	Emarginate	Elliptic	White	1.44 × 0.47 ≥ 10 and < 100	Terminal, apparently convex	Cristate-winged, membranous	Lineate	Tuberculate
E. regelii	Oblong	Yellow	5.69 × 1.78	Strigose	Papyraceous	Emarginate	Elliptic	Yellow	1.13 × 0.31 ≥ 10 and < 100	Terminal, slightly convex	Cristate-winged, membranous	Lineate	Tuberculate
E. rostkoviana	Oblong	Yellow	4.31 × 1.81	Strigose	Papyraceous	Emarginate	Elliptic	White	1.27 × 0.41 ≥ 10 and < 100	Terminal apparently convex	Cristate-winged, membranous	Lineate	Tuberculate
Lindenbergia indica	Ovoid	Brown	6.51 × 2.35	Pilose	Coriaceous	Acuminate	Reniform	Yellow	0.52 × 0.25 > 100	Terminal slightly convex	Reticulate	Hook-like	
L. philippinensis	Ovoid	Brown	6.06 × 3.02	Pilose	Coriaceous	Acuminate	Elliptic	Yellow	0.29 × 0.17 > 100	Terminal, slightly convex	Reticulate	Hook-like	
Melampyrum klebelsbergianum	Ovoid	Brown	7.25 × 3.36	Aculeate	Papyraceous	Acuminate	Elliptic	Black	3.97 × 1.09 < 10	Terminal, distinctly convex	Psilate	Smooth	
M. laxum	Ovoid	Brown	6.92 × 3.97	Aculeate	Papyraceous	Acuminate	Elliptic	Black	4.61 × 2.51 < 10	Terminal, distinctly convex	Psilate	Smooth	

Table 3 continued

Taxon	Fruit						Seed						
	Shape	Color	Size (length × width, in mm)	Surface	Texture	Apex	Shape	Color	Size (length × width, in mm) & number	Hilum	Primary ornamentation	Secondary ornamentation	Other features
M. lineare	Ovoid	Brown	6.64 × 2.6	Aculeate	Papyraceous	Acuminate	Elliptic	Black	2.68 × 1.10 < 10	Terminal, distinctly convex	Psilate	Smooth	
M. nemorosum	Ovoid	Brown	5.97 × 3.06	Aculeate	Papyraceous	Acuminate	Elliptic	black	4.79 × 2.17 < 10	Terminal, distinctly convex	Psilate	Smooth	
Monochasma savatieri	Ovoid	Brown	8.4 × 4.42	Glabrous	Coriaceous	Acuminate	Oblong	Yellow	0.92 × 0.52 ≥ 10 and < 100	Terminal, apparently concave	Sulcate	Hook-like	Tuerculate, fibrils
Odontites vulgaris	Ovoid	Brown	6.20 × 3.16	Strigose	Coriaceous	Cuspidate	Fusiform	Brown	1.40 × 0.59 ≥ 10 and < 100	Terminal, convex	Cristate-winged, membranous	Lineate	Spinulose
Omphralotrix longipes	Ovoid	Yellow	4.36 × 2.11	Pilose	Papyraceous	Emarginate	Elliptic	White	0.93 × 0.34 ≥ 10 and < 100	Terminal, apparently convex	Cristate-winged, membranous	Lineate	Tuberculate
Ophiocephalus angustifolius	Ovoid	Yellow	5.99 × 2.28	Glabrous	Coriaceous	Emarginate	Elliptic	Brown	1.00 × 0.53 ≥ 10 and < 100	Inconspicuous	Deeply reticulate, membranous	Lineate	Partly ruptured, tuberculate
Orobranche fasciculata	Ovoid	Yellow	10.00 × 3.09	Glabrous	Coriaceous	Cuspidate	Elliptic	–	0.24 × 0.15 > 100	Terminal, slightly convex	Reticulate	Smooth	Tuberculate
Pedicularis cephalantha	Ovoid	Brown	14.76 × 4.52	Glabrous	Coriaceous	Acuminate	Elliptic	Yellow	2.75 × 1.19 ≥ 10 and < 100	Terminal, distinctly convex	Reticulate	Poroid-alveolate	Fibrils
P. densispica	Ovoid	Brown	7.43 × 3.45	Glabrous	Coriaceous	Acuminate	Ovoid	Yellow	2.75 × 1.19 ≥ 10 and < 100	Terminal, distinctly convex	Reticulate	Poroid-alveolate	Fibrils
P. elwesii	Ovoid	Brown	17.53 × 7.04	Glabrous	Coriaceous	Acuminate	Elliptic	White	2.97 × 1.49 ≥ 10 and < 100	Tezrminal, distinctly convex	Reticulate	Poroid-alveolate	Fibrils
P. rupicola	Ovoid	Brown	17.11 × 5.64	Glabrous	Coriaceous	Acuminate	Ovoid	Yellow	3.28 × 1.39 ≥ 10 and < 100	Terminal, distinctly convex	Reticulate	Poroid-alveolate	Fibrils
P. superba	Ovoid	Brown	23.29 × 8.41	Glabrous	Coriaceous	Acuminate	Elliptic	Brown	4.21 × 1.89 ≥ 10 and < 100	Terminal, distinctly convex	Reticulate	Poroid-alveolate	Fibrils
Rhinanthus angustifolius	Ovoid	Brown	11.49 × 11.30	Glabrous	Coriaceous	Cuspidate	Reniform	Yellow	2.53 × 1.98 < 10	Lateral, distinctly convex	Obscurely reticulate	Smooth	Dorsal wing
R. glaber	Ovoid	Brown	9.70 × 8.72	Glabrous	Coraceous	Cuspidate	Reniform	Yellow	3.79 × 2.80 < 10	Lateral, distinctly convex	Obscurely reticulate	Smooth	Dorsal wing

Table 3 continued

Taxon	Fruit					Seed					
	Shape	Color	Size (length × width, in mm)	Surface	Texture	Apex	Shape	Color	Size (length × width, in mm) & number	Hilum	Primary ornamentation	Secondary ornamentation	Other features
Siphonostegia chinensis	Ovoid	Brown	14.07 × 2.63	Glabrous	Coriaceous	Cuspidate	Elliptic	Black	0.69 × 0.39 ≥ 10 and < 100	Terminal, slightly convex	Reticulate	Reticulate	Tuberculate, fibrils
S. laeta	Ovoid	Brown	10.58 × 2.96	Glabrous	Coriaceous	Cuspidate	Elliptic	Yellow	1.38 × 0.48 ≥ 10 and < 100	Terminal, apparently convex	Reticulate	Smooth	Fibrils
Sopubia trifida	Ovoid	Brown	4.52 × 5.59	Glabrous,	Coriaceous	Acuminate	Oblong	Black	0.58 × 0.37 > 100	Inconspicuous	Reticulate	Smooth	Tuberculate
Striga asiatica	Oblong	Yellow	3.49 × 2.15	Glabrous	Coriaceous	Emarginate	Elliptic	–	0.33 × 0.16 > 100	Terminal, slightly convex	Reticulate	Smooth	Tuberculate
Triphysaria eriantha	Ovoid	Yellow	6.61 × 3.72	Glabrous	Coriaceous	Acuminate	Ovoid	Black	0.91 × 0.61 ≥ 10 and < 100	Terminal, apparently convex	Reticulate	Smooth	Partly ruptured

(10) Wing. Two character states are described: unwinged (0) and winged (1). The seed has a membranous protrusion along its longitude (1) or not (0).

(11) Wing number. Two character states are described: one (0) and several (1).

(12) Surface projections. Three character states are described: smooth (0), spinulose (1), and tuberculate (2). Smooth indicates no projections on the seed surface. Spinulose indicates tiny and protruding pinpoints. Tuberculate indicates small rounded protuberances.

(13) Testa. Two character states are described: tightly-fitting (0) and loosely-fitting (1). The testa of the seed is separated from the inner structure when mature (1) or not (0).

(14) Primary ornamentation. Five character states are described: reticulate (0), cristate-winged (1), sulcate (2), psilate (3), and irregularly striate (4). Reticulate indicates a network-like pattern. Cristate-winged indicates several protrusions along longitude of epidermal cells. Sulcate indicates paralleled lineation through the seed surface. Psilate indicates smooth surface. Irregularly striate indicates short and disorderly lines on the surface.

(15) Adjacent ridges of reticulum region. Two character states are described: trough (0) and ridge (1). For reticulate seeds, there may be a slight sunken region (0) or raised region (1) between the adjacent ridges of the reticulum.

(16) Reticulum lines. Three character states are described: obscure (0), normal (1), and deep (2). Obscure indicates blurred lines in the reticulum. Normal indicates clear lines to the reticulum. Deep indicates strongly uplifted lines in the reticulum.

(17) Reticulum line height. Two character states are described: uniform (0) and uneven (1). The lines of the reticulum are lengthened equally (0) or not (1).

(18) Mesh shape. Three character states are described: narrowly or shortly rectangular (0), isodiametrically polygonal (1), and irregularly polygonal (2).

(19) Secondary ornamentation. Four character states are described: smooth (0), reticulate (1), lineate (2) poroid-alveolate (3), and hook-like (4). Frequently, the seeds are reinforced with secondary thickening of various types on the epidermal cell wall.

Appendix 4

See Table 4.

Table 4 Matrix of coded capsule and seed character states for the studied taxa

Taxon	Character																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Aeginetia indica	0	1	0	–	0	0	0	2	1	0	–	2	1	0	0	2	0	2	2
Agalinis tenuifolia	1	0	0	–	0	1	0	1	1	0	–	0	1	0	0	1	0	0	2
Boschniakia himalaica	0	1	0	–	0	0	0	2	1	0	–	0	1	0	0	2	0	2	1
Brandisia hancei	0	1	1	2	0	0	2	2	0	0	–	0	1	0	0	1	0	1	0
Castilleja attenuata	0	0	0	–	0	1	0	1	1	0	–	2	1	0	0	1	0	2	0
Castilleja densiflora	0	0	0	–	0	1	0	1	1	0	–	1	1	0	1	1	0	1	0
Castilleja foliolosa	0	1	0	–	0	1	2	1	1	0	–	0	1	0	0	2	0	1	2
Castilleja longiflora	0	1	0	–	0	1	1	1	1	0	–	0	1	0	0	1	0	1	0
Castilleja miniata	0	1	0	–	0	1	2	1	1	0	–	0	1	0	0	1	0	2	2
Castilleja pilosa	0	0	0	–	0	1	1	1	1	0	–	2	1	0	0	2	0	2	2
Castilleja sulphurea	0	0	0	–	0	1	2	1	0	0	–	1	1	0	0	1	0	1	2
Castilleja tenuis	0	0	0	–	0	1	1	1	1	0	–	2	1	0	1	1	0	0	0
Clevelandia beldingii	0	0	0	–	0	1	0	1	0	0	–	1	0	0	0	1	0	2	2
Conopholis americana	0	0	0	–	0	0	0	2	0	0	–	0	0	0	1	0	1	2	0
Cordylanthus ramosus	0	1	0	–	0	1	0	1	0	0	–	0	0	0	0	1	0	2	0
Cordylanthus rigidus	0	0	0	–	0	1	0	1	1	0	–	1	0	4	–	–	–	–	2
Cymbaria daurica	0	1	0	–	0	0	2	0	0	0	–	0	0	0	0	1	0	1	0
Epifagus virginiana	0	0	0	–	0	0	0	2	1	0	–	2	0	0	0	1	0	0	0
Euphrasia amurensis	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Euphrasia hirtella	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Euphrasia jaeschkei	1	0	1	1	1	2	0	1	1	1	1	1	0	1	–	–	–	–	2
Euphrasia pectinata	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Euphrasia pectinata ssp. *simplex*	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Euphrasia regelii	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Euphrasia rostkoviana	1	0	1	1	1	2	1	1	1	1	1	2	0	1	–	–	–	–	2
Lindenbergia indica	0	1	1	0	0	0	0	2	0	0	–	0	0	0	1	1	0	1	4
Lindenbergia philippensis	0	1	1	0	0	0	0	2	0	0	–	2	0	0	1	1	0	0	4
Melampyrum klebelsberginum	0	1	2	–	1	0	3	0	1	0	–	0	0	3	–	–	–	–	0
Melampyrum laxum	0	1	2	–	1	0	3	0	1	0	–	0	0	3	–	–	–	–	0
Melampyrum lineare	0	1	2	–	1	0	2	0	1	0	–	0	0	3	–	–	–	–	0
Melampyrum nemorosum	0	1	2	–	1	0	3	0	1	0	–	0	0	3	–	–	–	–	0
Monochasma savatieri	0	1	0	–	0	0	0	1	0	0	–	2	0	2	–	–	–	–	4
Odontites vulgaris	0	1	1	1	0	1	1	1	1	1	1	1	0	1	–	–	–	–	2
Omphalotrix longipes	0	0	1	0	1	2	0	1	1	1	1	2	0	1	–	–	–	–	2
Ophiocephalus angustifolius	0	0	0	–	0	2	0	1	1	0	–	2	1	0	0	2	0	1	2
Orobanche fasciculata	0	0	0	–	0	1	0	2	1	0	–	2	0	0	1	1	0	2	0
Pedicularis cephalantha	0	1	0	–	0	0	2	1	1	0	–	0	0	0	0	1	1	0	3
Pedicularis densispica	0	1	0	–	0	0	1	1	1	0	–	0	0	0	0	1	1	0	3
Pedicularis elwesii	0	1	0	–	0	0	2	1	0	0	–	0	0	0	1	1	1	0	3
Pedicularis rupicola	0	1	0	–	0	0	3	1	1	0	–	0	0	0	0	1	0	0	3
Pedicularis superba	0	1	0	–	0	0	3	1	1	0	–	0	0	0	0	1	1	0	3
Phtheirospermum japonicum†	0	1	1	0	0	0	0	2	1	1	1	1	0	0	1	1	1	0	1
Phtheirospermum parishii†	0	1	1	0	0	0	0	2	1	0	–	2	0	0	1	1	0	0	1
Phtheirospermum tenuisectum†	0	1	1	0	0	0	0	2	1	0	–	2	0	0	1	1	0	0	1
Pseudobartsia yunnanensis†	0	1	1	1	0	0	0	2	1	0	–	0	0	4	–	–	–	–	4
Pterygiella cylindrica†	0	1	1	0	0	0	0	2	1	0	–	2	0	4	–	–	–	–	4
Pterygiella cylindrica var. suffruticosa†	0	1	1	0	0	0	0	2	1	0	–	2	0	0	1	1	0	0	4

Table 4 continued

Taxon	Character																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Pterygiellla duclouxii[†]	0	1	1	0	0	0	0	2	1	0	–	2	0	4	–	–	–	–	4
Pterygiella nigrescens[†]	0	1	1	0	0	0	0	2	1	0	–	2	0	4	–	–	–	–	4
Rhinanthus angustifolius	0	1	0	–	0	1	2	0	1	1	0	0	0	0	1	0	1	2	0
Rhinanthus glaber	0	1	0	–	0	1	3	0	1	1	0	0	0	0	1	0	1	2	0
Siphonostegia chinensis	0	1	0	–	0	1	0	1	0	0	–	2	0	0	1	1	0	0	1
Siphonostegia laeta	0	1	0	–	0	1	1	1	1	0	–	0	1	0	0	1	0	2	0
Sopubia trifida	0	1	0	–	0	0	0	2	1	0	–	2	0	0	1	1	0	2	0
Striga asiatica	1	0	0	–	0	2	0	2	1	0	–	2	0	0	1	1	0	0	0
Triphysaria eriantha	0	0	0	–	0	0	0	1	1	0	–	0	1	0	1	1	0	2	0
Xizangia bartschioides[†]	0	1	1	1	0	1	1	2	0	0	–	2	1	0	1	1	0	2	0

[†] Details shown in Dong et al. (2013)

References

Arekal GD (1963) Embryological studies in Canadian representatives of the tribe Rhinantheae, Scrophulariaceae. Can J Bot 41:267–303

Bennett JR, Mathews S (2006) Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. Am J Bot 93:1036–1051

Bentham G, Hooker JD (1876) Scrophulariaceae. Genera Plantarum Reeve & Co., London, pp 913–980

Berg RY (1954) Development and dispersal of the seed of Pedicularis sylvatica. Nytt Mag Bot 2:1–60

Boeshore I (1920) The morphological continuity of Scrophulariaceae and Orobanchaceae. Contr Bot Lab Univ Penn 5:139–197

Cantino PD (1990) The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae. J Arnold Arbor 71:323–370

Chuang TI, Heckard LR (1972) Seed-coat morphology in *Cordylanthus* (Scrophulariaceae) and its taxonomic significance. Am J Bot 59:258–265

Chuang TI, Heckard LR (1976) Morphology, evolution, and taxonomic significance of the inflorescence in *Cordylanthus* (Scrophulariaceae). Am J Bot 63:272–282

Chuang TI, Heckard LR (1983) Systematic significance of seed-surface features in *Orthocarpus* (Scrophulariaceae-Subtribe Castillejinae). Am J Bot 70:877–890

Chuang TI, Heckard LR (1986) Systematics and evolution of Cordylanthus (Scrophulariaceae-Pedicularieae). Syst Bot Monogr 10:1–105

Chuang TI, Heckard LR (1991) Generic realignment and synopsis of subtribe Castillejinae (Scrophulariaceae-tribe Pedicularieae). Syst Bot 16:644–666

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York

dePamphilis CW, Young ND, Wolfe AD (1997) Evolution of plastid gene *rps2* in a lineage of hemiparasitic and holoparasitic plants: many losses of photosynthesis and complex patterns of rate variation. Proc Natl Acad Sci USA 94:7367–7372

Dong LN, Wang H, Wortley AH, Lu L, Li DZ (2013) Phylogenetic relationships in the *Pterygiella* complex (Orobanchaceae) inferred from molecular and morphological evidence. Bot J Linn Soc 171:491–507

Fischer E (2004) Scrophulariaceae. In: Kadeceit JW (ed) The families and genera of vascular plants, vol 7. Springer Press, Berlia, pp 333–432

Hong DY (1983) The distribution of Scrophulariaceae in the Holarctic with special reference to the floristic relationships between Eastern Asia and Eastern North America. Ann Missouri Bot Gard 70:701–712

Hong DY (1996) Additional notes on the Scrophulariaceae of China. Novon 6:372–374

Hong DY, Yang HB, Jin CL, Holmgren NH (1998) Scrophulariaceae In: Wu ZY, Raven PH (ed) Flora of China, vol 18. Science Press/ Missouri Botanical Garden Press, Beijing/St Louis, pp 98–101

Hutchinson J (1969) Evolution and phylogeny of flowering plants. Academic Press, New York

Juan R, Pastor J, Fernández I (2000) SEM and light microscope observations on fruit and seeds in Scrophulariaceae from southwest Spain and their systematic significance. Ann Bot 86:323–338

Kuijt J (1969) The biology of parasitic flowering plants. University of California Press, Berkeley

Liu ML, Yu WB, Li DZ, Mill RR, Wang H (2013) Seed morphological diversity of Pedicularis (Orobanchaceae) and its taxonomic significance. Plant Syst Evol 299:1645–1657

Lu L, Wang H, Blackmore S, Li DZ, Dong LN (2007) Pollen morphology of the tribe Rhinantheae (Orobanchaceae) and its systematic significances. Plant Syst Evol 268:177–198

Martínez-Ortega MM, Rico E (2001) Seed morphology ant its systematic significance in some *Veronica* species (Scrophulariaceae) mainly from the western Mediterranean. Plant Syst Evol 228:15–32

McNeal JR, Bennett JR, Wolfe AD, Mathews S (2013) Phylogeny and origins of holoparasitism in Orobanchaceae. Am J Bot 100:971–983

Minkin JP, Eshbaugh WH (1989) Pollen morphology of the Orobanchaceae and rhinanthoid Scrophulariaceae. Grana 28:1–18

Molau V (1990) The genus *Bartsia* (Scrophulariaceae-Rhinanthoideae). Opera Bot 102:1–99

Murley MR (1951) Seeds of the Cruciferae of Northeastern North America. Am Midl Nat 46:1–81

Musselman LJ, Mann WF (1976) A survey of surface characteristics of seeds of Scrophulariaceae and Orobanchaceae using scanning electron microscopy. Phytomorphology 26:370–378

Oh IC, Anderberg AL, Schönenberger J, Anderberg AA (2008) Comparative seed morphology and character evolution in the genus *Lysimachia* (Myrsinaceae) and related taxa. Plant Syst Evol 271:177–197

Olmstead RG, Reeves PA (1995) Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbc*L and *ndh*F sequences. Ann Missouri Bot Gard 82:176–193

Olmstead RG, dePamphilis CW, Wolfe AD, Young ND, Elisons WJ, Reeves PA (2001) Disintegration of the Scrophulariaceae. Am J Bot 88:348–361

Pennell FW (1935) The Scrophulariaceae of Eastern temperate North America. Monographs 1. Academy of Natural Sciences, Philadelphia, pp 1–650

Press MC, Graves JD (1995) Parasitic plants. Chapman and Hall, London, pp 167–173

Raman S (1991) The trichomes on the corolla of the Scrophulariaceae. X: Taxa of uncertain relationship to, or within, the family. Beitr Biol Pfl 66:127–143

Rohlf FJ (2000) NTSYS-pc: numerical taxonomy and multivariate analysis system. Exerter Publishing Ltd, New York

Scheunert A, Fleischmann A, Olano-Marín C, Bräuchler C, Heubl G (2012) Phylogeny of tribe Rhinantheae (Orobanchaceae) with a focus on biogeography, cytology and re-examination of generic concepts. Taxon 61:1269–1285

Tank DC, Olmstead RG (2008) From annuals to perennials: phylogeny of subtribe Castillejinae (Orobanchaceae). Am J Bot 95:608–625

Tank DC, Beardsley PM, Olmstead RG (2006) Review of the systematics of Scrophulariaceae s.l. and their current disposition. Aust Syst Bot 19:289–307

Tank DC, Egger JM, Olmstead RG (2009) Phylogenetic classification of subtribe Castillejinae (Orobanchaceae). Syst Bot 34:182–197

Těšitel J, Říha P, Malinová T, Svobodová Š, Štech M (2010) Phylogeny, life history evolution and biogeography of the Rhinanthoid Orobanchaceae. Folia Geobot 45:347–367

Thieret JW (1955) The seeds of *Veronica* and allied genera. Lloydia 18:37–45

Tiagi B (1966) Development of the seed and fruit in *Rhinanthus major* and *R. serotinus*. Am J Bot 53:645–651

Van de Pijl L (1982) Principles of dispersal in higher plants, 3rd edn. Springer, Berlin

Wolfe AD, Randle CP, Liu L, Steiner KE (2005) Phylogeny and biogeography of Orobanchaceae. Folia Geobot 40:115–134

Young ND, Steiner KE, dePamphilis CW (1999) The evolution of parasitism in Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series. Ann Missouri Bot Gard 86:876–893

Zhang ZY, Yang DZ, Lu AM, Knapp S (2005) Seed morphology of the tribe Hyoscyamea (Solanaceae). Taxon 54:71–83