South African Journal of Botany 2003, 69(2): 151–157
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
ISSN 0254–6299

Fruit anatomy of the genus *Bupleurum* (Apiaceae) in northeastern China and notes on systematic implications

M (Rebecca) Liu[1], L Shi[2], B-E van Wyk[1] and PM Tilney[1]*

[1] *Department of Botany, Rand Afrikaans University, PO Box 524, Auckland Park, Johannesburg 2006, South Africa*

[2] *The Beijing Botanical Garden, Institute of Botany, The Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, China*

* *Corresponding author, e-mail: pmt@na.rau.ac.za*

Received 25 June 2002, accepted in revised form 7 November 2002

The fruit anatomy of all eight species of the genus *Bupleurum* L. mentioned in the most recent literature from northeastern China was studied for the first time. The eight species were divided into two groups based on the shape of the mericarps in transverse section. The first group (round mericarps without prominent ribs) includes *B. longiradiatum* and *B. komarovianum*, and the second group (angular mericarps with prominent ribs) includes *B. angustissimum*, *B. bicaule, B. chinense, B. euphorbioides, B. scorzonerifolium* and *B. sibiricum*. The second group can be further subdivided into group 2a (large vittae, very small vascular bundles) — *B. euphorbioides* and *B. sibiricum*, and group 2b (small vittae, large vascular bundles) — *B. angustissimum*, *B. bicaule, B. chinense* and *B. scorzonerifolium*. This result closely supports the traditional classification system which was based on gross morphology (plant height; leaf shape and size; involucre number, size and shape). The only exception is that *B. komarovianum* should not be close to the second group, but close to *B. longiradiatum.* Group 1 corresponds with *Bupleurum* sect. *Longifolia* and group 2 with sect. *Eubupleura*; group 2a with ser. *Ranunculoides* and group 2b with ser. *Falcata*. Fruit anatomical characters seem to have potential for evaluating infrageneric relationships in the genus *Bupleurum*.

Introduction

The genus *Bupleurum* consists mainly of herbs but a few species are woody. There are between 180 and 190 species worldwide (Pimenov and Leonov 1993) with about 36 species in China (Shan and Sheh 1979). According to Flora Plantarum Herbacearum Chinae Boreali-Orientalis (Anon. 1977) there are six species (*B. bicaule* Helm, *B. chinense* DC., *B. euphorbioides* Nakai, *B. longiradiatum* Turcz., *B. scorzonerifolium* Willd. and *B. sibiricum* Vest), four varieties and one form in northeastern China. In Clavis Plantarum Chinae Boreali-Orientalis (Fu 1995), however eight species (*B. angustissimum* (Franch.) Kitagawa, *B. bicaule, B. chinense, B. euphorbioides, B. komarovianum* Lincz., *B. longiradiatum, B. scorzonerifolium and B. sibiricum*), two varieties and four forms have been recorded. The main difference between the two publications is that *B. chinense* var. *komarovianum* (Lincz.) Liou *et* Huang and *B. scorzonerifolium* var. *angustissimum* (Franch.) Huang are treated as species (*B. angustissimum* and *B. komarovianum*) respectively in the latter.

Wolff (1910) divided *Bupleurum* into six sections based on leaf shape, leaf texture and shape of the base of the petiole. Only five of the eight species studied from China were included in his scheme. He placed these in two sections (*B. longiradiatum* in sect. *Longifolia* Wolff and *B. bicaule, B. chinense, B. scorzonerifolium* and *B. sibiricum* in sect. *Eubupleura* Brig.). In the latter section, *B. sibiricum* was placed in ser. *Ranunculoides* Wolff and *B. chinense, B. scorzonerifolium* and *B. sibiricum* in ser. *Falcata* Wolff.

Bupleurum longiradiatum and *B. komarovianum* have large leaves with pinnate venation while *B. angustissimum*, *B. bicaule, B. chinense, B. euphorbioides, B. scorzonerifolium* and *B. sibiricum* have small leaves with parallel venation. The stem is hollow in the former group and solid in the latter (Liu *et al*. unpublished). The bracts of the involucels in *B. euphorbioides* and *B. sibiricum* are large with 5–10 veins in each and small with 1–3 (5) veins in *B. angustissimum*, *B. bicaule B. chinense* and *B. scorzonerifolium*. Based on gross morphology and leaf characters, *B. komarovianum* was placed close to *B. angustissimum*, *B. bicaule, B. chinense, B. euphorbioides, B. scorzonerifolium* and *B. sibiricum* (Anon. 1977, Fu 1995).

Historically, much emphasis has been placed on the habit, size, leaf shape and colour, size and number of involucral bracts of *Bupleurum* (Wolff 1910, Shan and Li 1974). In this study, fruit anatomy was used for the first time to evaluate possible relationships between the eight species of

Bupleurum presented by Fu (1995) and to see if the fruit structure can support the existing infrageneric classification system of Wolff (1910), Anon. (1977) and Fu (1995). This study is part of a broader investigation of fruit characters in the genus *Bupleurum* as a whole.

Materials and Methods

At least two mature fruits of each of the following eight species were studied. Herbarium accession numbers are given in brackets. *Bupleurum angustissimum* (IFP 366), *B. bicaule* (HANU 110292), *B. chinense* (IFP 3568), *B. euphorbioides* (IFP 9146), *B. komarovianum* (NEFI 6012), *B. longiradiatum* (IFP 630624), *B. scorzonerifolium* (IFP 6300) and *B. sibiricum* (HANU 110293). All materials were rehydrated and placed in FAA (formaldehyde-acetic acid-alcohol) for a minimum of 24h and then treated according to the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA). However, a minimum of 24h was used for the first two infiltrations in GMA and a minimum of five days for the third infiltration. The capsules containing the material and GMA were placed in an oven at 60°C for 24h. Transverse sections, about 5µm thick, were cut using a Porter-Blum ultramicrotome. The periodic acid-Schiff/toluidine blue staining method (Feder and O'Brien 1968) was used. Photographs were taken using a Leitz Wetzlar microscope and Ilford Pan F film. For SEM studies of fruits a JEOL JSM 5600 scanning electron microscope was used. Drawings were done with the aid of a *camera lucida*. Terminology is illustrated in Figure 3.

Results

Bupleurum fruits are oblong in shape (Figure 1) and have a smooth epidermal surface. The fruits may be without prominent ribs (Figures 2a–b and 3a–b) or have prominent ribs (Figures 2c–h and 3c–h). Vallecular and commissural vittae (oil ducts in the furrows between the ribs and in the commissure respectively) are present (Figure 3a) and crystals absent. The endocarp is soft (non-lignified). The carpophore (stalk supporting each mericarp) is free with the vascular bundles arranged dorsally (Figure 3a).

B. longiradiatum mericarps (Figures 1a, 2a and 3a) are narrowly oblong-elliptic (terminology according to Exell (1960)) in dorsal view, about 4.5mm long and 1.3mm wide, and round in transverse section. Ribs (one dorsal, two lateral and two marginal) are not prominent. Intrajugal oil ducts (present in the ribs) are absent, vallecular vittae 3–5 in each furrow and commissural vittae four. They are small (some of them may be nearly as wide as the pericarp but most of them are much smaller) and arranged regularly. There are no small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are small (less than the pericarp thickness) and transversely elliptic. The ventral endosperm is flat.

B. komarovianum mericarps (Figures 1b, 2b and 3b) are elliptic in dorsal view, about 3mm long and 1.3mm wide, and round in transverse section. Ribs are not prominent. Intrajugal oil ducts are present (but not each vascular bundle is associated with a vitta), vallecular vittae 1–3 in each furrow and commissural vittae two. They are small and arranged irregularly. There are some small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are small and transversely elliptic. The ventral endosperm is flat.

B. euphorbioides mericarps (Figures 1c, 2c and 3c) are narrowly oblong in dorsal view, about 3.1mm long and 1mm wide, and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are present (but not every vascular bundle is associated with a vitta), vallecular vittae 3–5 in each furrow and commissural vittae four. They are large (the largest ones nearly as wide as the pericarp width) and arranged regularly. There are no small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are very small (much less than the pericarp thickness) and round. The ventral endosperm is slightly concave.

B. sibiricum mericarps (Figures 1d, 2d and 3d) are narrowly oblong in dorsal view, about 3.2mm long and 0.9mm wide, and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are absent, vallecular vittae three in

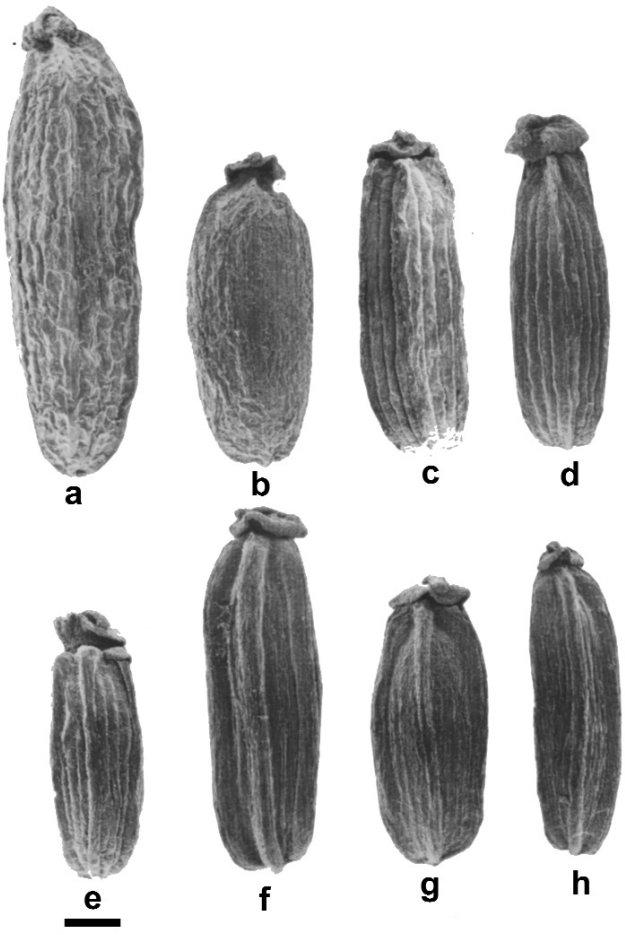

Figure 1: Mericarps (in dorsal view) of all eight species of *Bupleurum* indigenous to northeastern China. (a) *B. longiradiatum* (narrowly oblong-elliptic), (b) *B. komarovianum* (elliptic), (c) *B. euphorbioides* (narrowly oblong), (d) *B. sibiricum* (narrowly oblong), (e) *B. bicaule* (oblong), (f) *B. scorzonerifolium* (narrowly oblong), (g) *B. chinense* (elliptic-oblong), (h) *B. angustissimum* (narrowly oblong-elliptic). Scale bar = 500µm

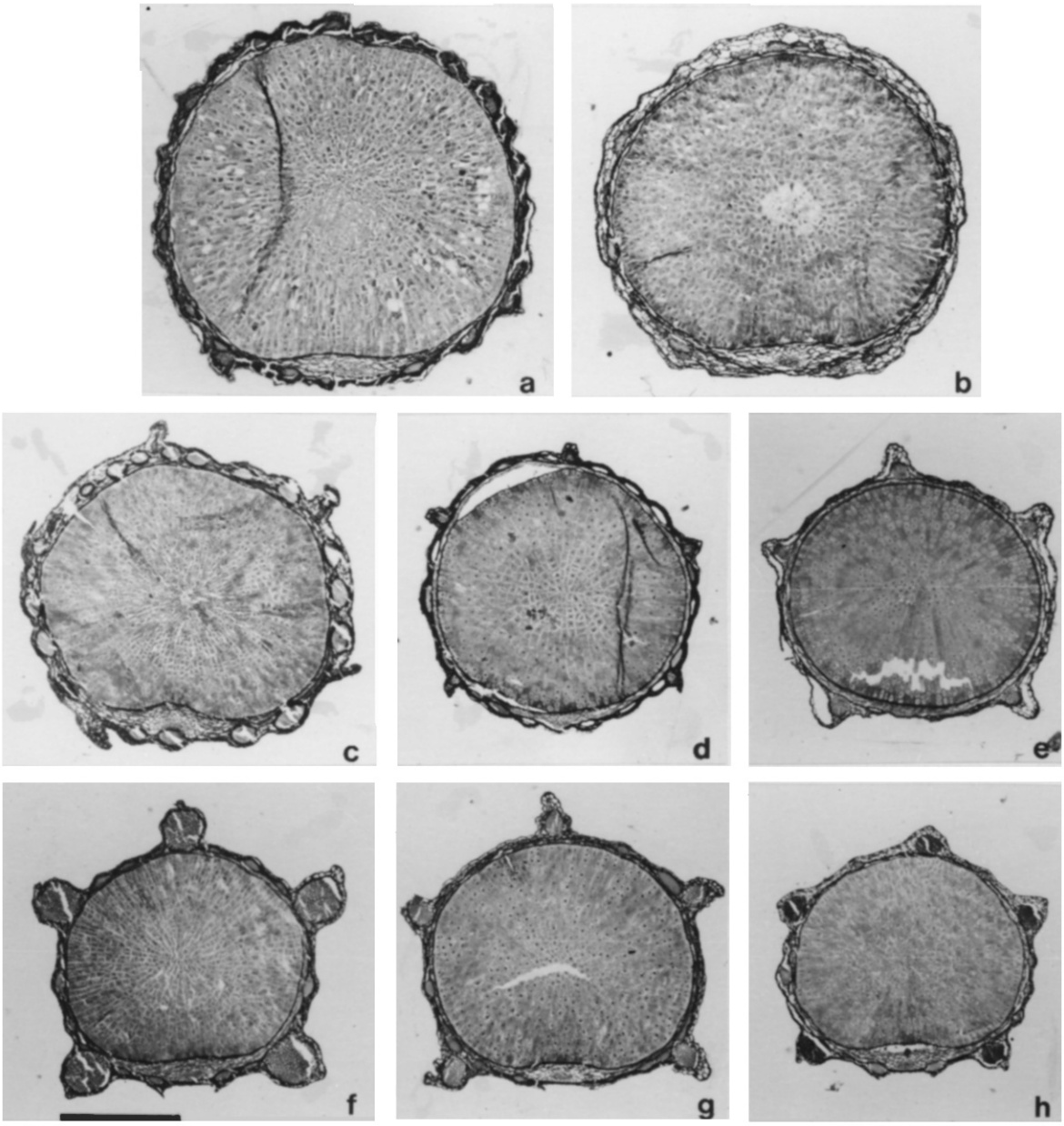

Figure 2: Mericarp structure of all eight species of *Bupleurum* indigenous to northeastern China as seen in transverse section. Note differences in mericarp shape and general structure. Details are shown in Figure 3. (a) *B. longiradiatum*, (b) *B. komarovianum,* (c) *B. euphorbioides*, (d) *B. sibiricum*, (e) *B. bicaule*, (f) *B. scorzonerifolium*, (g) *B. chinense*, (h) *B. angustissimum*. Scale bar = 0.7mm

each furrow and commissural vittae six. They are large and arranged regularly. There are no small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are very small and round. The ventral endosperm is convex.

B. bicaule mericarps (Figures 1e, 2e and 3e) are oblong in dorsal view, about 2.5mm long and 0.8mm wide, and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are absent, vallecular vittae three in each furrow and commissural vittae three or four. They are small and arranged regularly. There are no small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are large (less than three times the pericarp thickness) and elliptic. The ventral endosperm is convex.

B. scorzonerifolium mericarps (Figures 1f, 2f and 3f) are narrowly oblong in dorsal view, about 3.4mm long and 1mm wide, and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are absent, vallecular vittae three in each furrow and commissural vittae four. They are small and arranged regularly. There are some small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are very large (more than four times the pericarp thickness) and round. The ventral endosperm is flat.

B. chinense mericarps (Figures 1g, 2g and 3g) are elliptic-oblong in dorsal view, about 2.7mm long and 1.1mm wide,

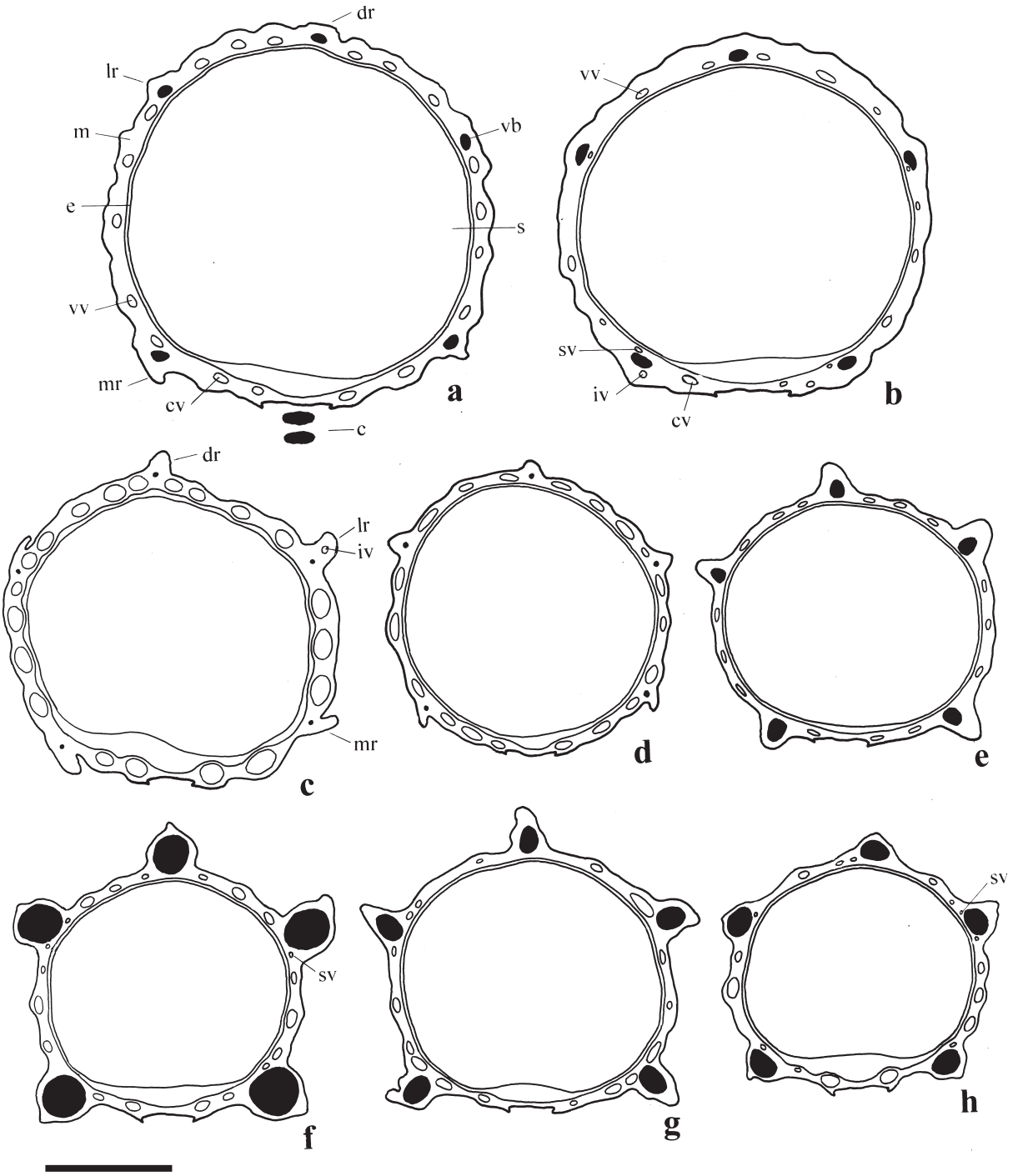

Figure 3: Line drawings of all eight species of *Bupleurum* of northeastern China showing terminology and differences in shape of mericarps, prominence of ribs, vascular bundle size, arrangement and size of vittae (oil ducts), and shape of the ventral endosperm. (a) *B. longiradiatum*, (b) *B. komarovianum*, (c) *B. euphorbioides*, (d) *B. sibiricum*, (e) *B. bicaule*, (f) *B. scorzonerifolium*, (g) B. *chinense*, (h) *B. angustissimum*. c = carpophore; cv = commissural vitta i.e. oil duct in the area where the two mericarps making up the fruit join; dr = dorsal rib; e = endocarp; iv = intrajugal oil duct; lr = lateral rib; m = mesocarp; mr = marginal rib; s = seed; sv = scattered vitta (relatively small, position not fixed but close to the endocarp); vb = vascular bundle; vv = vallecular vitta. Scale bar = 0.7mm

and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are absent, vallecular vittae 2–4 in each furrow and commissural vittae four or five. They are small and arranged irregularly. There are no small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are large and elliptic. The ventral endosperm is slightly concave.

B. angustissimum mericarps (Figures 1h, 2h and 3h) are narrowly oblong-elliptic in dorsal view, about 3mm long and 0.8mm wide, and 5-angular in transverse section. Ribs are prominent. Intrajugal oil ducts are absent, vallecular vittae 1–3 in each furrow and commissural vittae two. They are small and arranged irregularly. There are some small vittae scattered in the mesocarp close to the endocarp. Vascular bundles are large and transversely elliptic. The ventral endosperm is slightly concave.

A summary of various structural characters of the mericarps of the eight species of *Bupleurum* is given in Table 1. The results show that the fruit anatomy is sufficiently variable to distinguish between the species and possibly also their respective sections and series. It was therefore possible to create the following key, based exclusively on fruit anatomical characters. Differences between the species and notes on the systematic implications are given below.

Key

1. Outline of mericarps in transverse section round, ribs not prominent
   2. Intrajugal oil ducts absent .....*B. longiradiatum*
   2. Intrajugal oil ducts present .....*B. komarovianum*
1. Outline of mericarps in transverse section 5-angular, ribs prominent
   3. Vascular bundles minute, much less than the pericarp thickness
      4. Intrajugal oil ducts present .....*B. euphorbioides*
      4. Intrajugal oil ducts absent .....*B. sibiricum*
   3. Vascular bundles very large, more than three times the pericarp thickness
      5. Ventral endosperm convex .....*B. bicaule*
      5. Ventral endosperm not convex
         6. Vascular bundles more than four times the pericarp thickness .....*B. scorzonerifolium*
         6. Vascular bundles up to three times the pericarp thickness
            7. Vascular bundles elliptic (tangential diameter <radial diameter) .....*B. chinense*
            7. Vascular bundles transversely elliptic (tangential diameter >radial diameter) .....*B. angustissimum*

Discussion

The eight species of the genus *Bupleurum* in northeastern China can be separated into two groups based on the mericarp structure being round in outline or 5-angular with prominent ribs (Table 1 and Key). In the first group, *B. komarovianum* has a similar fruit structure, leaf venation and hollow stem to *B. longiradiatum* and therefore they should be placed in the same group — sect. *Longifolia* (Wolff 1910, Su *et al.* 1998). The second group includes *B. euphorbioides*, *B. sibiricum*, *B. bicaule, B. scozonerifolium*, *B. chinense* and *B. angustissimum*, all belonging to sect. *Eubupleura* (Wolff 1910, Su *et al.* 1998). The fruit anatomy closely supports the traditional placement of the species by Wolff (1910), Anon. (1977) and Fu (1995). The only exception is *B. komarovianum* which should not be placed in sect. *Eubupleura* as was done by Anon. (1977) and Fu (1995). The six species in the sect. *Eubupleura* can be further divided into two groups. *Bupleurum euphorbioides* and *B. sibiricum* with large vittae and very small vascular bundles form group 1 (*B. euphorbioides* group) and both species belong to the ser. *Ranunculoidea* (Wolff 1910, Su *et al*. 1998). *Bupleurum bicaule, B. scozonerifolium*, *B. chinense* and *B. angustissimum* with small vittae and large vascular bundles form group 2 (*B. bicaule* group) and these species all belong to the ser. *Falcata* (Wolff 1910, Su *et al.* 1998). There are no major differences in fruit anatomy of the *B. bicaule* group except that in some species small vittae are present in the mesocarp close to the endocarp (Figures 3b, 3f and 3h). Previous studies of gross morphology also found uniformity within the *B. bicaule* group (Anon. 1977, Fu 1995). We therefore suggest that these four species are indeed closely related.

An interesting aspect of the study is that the vittae are shown to vary greatly between the species studied. Intrajugal oil ducts, if present, are associated with varying numbers of vascular bundles (Figures 3b and 3c). Vallecular and commissural vittae are arranged regularly in some species (Figures 3a and 3c–f) but irregularly in others (Figures 3b and 3g–h). There are some small vittae scattered in the mesocarp in some species (Figures 3b, 3f and 3h), a character also present in some genera of Saniculoideae (Drude 1898). The exclusion of the genus *Bupleurum* from the rest of the Apioideae with a stable arrangement of vallecular and commissural vittae and its placement in a basal position close to the tribe Heteromorpheae and various other anomalous genera (e.g. *Annesorhiza*, *Polemanniopsis* and *Steganotaenia*) suggested by DNA evidence (see Plunkett 2001) therefore seems reasonable.

Acknowledgements — We thank the curators and staff of HANU (Harbin Normal University, China), IFP (Forestry and Pedology Institute of the Chinese Scientific Institution, Shenyang, China) and NEFI (Botanical Institute of North-East Forestry University, Harbin, China) for their help or allowing us to collect samples for the study.

References

Anonymous (1977) Flora Plantarum Herbacearum Chinae Boreali-Orientalis, Vol 6. Forestry and Soil Institute of Liao Ning Province, Scientific Publishing House, Shen Yang, pp 188–200

Drude O (1898) Umbelliferae. In: Engler A, Prantl K (eds), Die Natürlichen Pflanzenfamilien, Vol 3. W Engelmann, Leipzig, pp 63–150

Exell AW (1960) Systematics Association Committee for descriptive terminology, I. Preliminary list of works relevant to descriptive biological terminology. Taxon **9**: 245–257

Feder N, O'Brien TP (1968) Plant microtechnique: some principles and new methods. American Journal of Botany **55**: 123–142

Fu P (1995) Clavis Plantarum Chinae Boreali-Orientalis, Vol 2. Scientific Publishing House, Beijing, pp 463–465

Pimenov MG, Leonov MV (1993) The Genera of the Umbelliferae. Royal Botanic Gardens, Kew, United Kingdom

Table 1: Summary of structural characters of mericarps in the eight species of *Bupleurum* indigenous to northeastern China. Diagnostic characters for groups (sections and series) are shown in bold

	1 Sect. *Longifolia*		2 Sect. *Eubupleura*					
			Ser. *Ranunculoidea*		Ser. *Falcata*			
Mericarp shape and structure	*B. longiradiatum*	*B. komarovianum*	*B. euphorbioides*	*B. sibiricum*	*B. bicaule*	*B. scozonerifolium*	*B. chinense*	*B. angustimum*
Shape in dorsal view*	narrowly oblong-elliptic	elliptic	narrowly oblong	narrowly oblong	oblong	narrowly oblong	elliptic-oblong	narrowly oblong-elliptic
Shape in transverse section	**round**	**round**	5-angular	5-angular	5-angular	5-angular	5-angular	5-angular
Ribs	**not prominent**	**not prominent**	prominent	prominent	prominent	prominent	prominent	prominent
Vascular bundle size**	small	small	**very small**	**very small**	**large**	**very large**	**large**	**large**
Vascular bundle shape***	transversely elliptic	transversely elliptic	round	round	elliptic	round	elliptic	transversely elliptic
Intrajugal oil ducts	absent	present	present	absent	absent	absent	absent	absent
Vallecular and commissural vittae arrangement	regular	irregular	regular	regular	regular	irregular	irregular	irregular
Vallecular and commissural vittae size****	small	small	**large**	**large**	small	small	small	small
Scattered vittae in the mericarp*****	absent	present	absent	absent	absent	present	absent	present
Ventral endosperm shape	flat	flat	slightly concave	convex	convex	flat	slightly concave	slightly concave

* Terminology according to Exell (1960)

** Very large: more than four times the pericarp thickness; large: less than three times the pericarp thickness; small: less than the pericarp thickness; very small: much less than of pericarp thickness

*** Elliptic: tangential diameter <radial diameter (see Figures 3e and 3g); transversely elliptic: tangential diameter >radial diameter (see Figures 3a, 3b and 3h)

**** Vittae large: the largest ones nearly as wide as the pericarp thickness; vittae small: some of them may be nearly as wide as the pericarp but most of them much smaller

***** Scattered vittae: vittae are much smaller than vallecular vittae and scattered in the mesocarp close to the endocarp

Plunkett GM (2001) Relationship of the order Apiales to subclass Asteridae: a re-evaluation of morphological characters based on insights from molecular data. Edinburgh Journal of Botany **58**: 183–200

Shan RH, Li Y (1974) On the Chinese species of *Bupleurum* L. Acta Phytotaxonomica Sinica **12**: 261–294

Shan RH, Sheh ML (1979) Flora Reiplicae Popularis Sinicae, Vol 55. Scientific Publishing House, Beijing, pp 215–295

Su Pu, Yuan CQ, She ML, Liu YZ, Xiang BR, An DK (1998) Numerical taxonomy of medicinal *Bupleurum* species in China. Acta Botanica Boreali — Occidentalia Sinica **18**: 277–283

Wolff H (1910) Umbelliferae — Apioideae — *Bupleurum*, *Trinia* et reliquae Ammineae heteroclitae. Das Planzenreich IV, **228**: 1–214

Edited by IB Friis