Crepis desertorum (Asteraceae, Cichorieae), a new species from northern Xinjiang (China) based on morphological and molecular data

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1. Introduction

Crepis L. is a large taxonomically controversial genus in tribe Cichorieae of Asteraceae containing over 200 species that are mainly distributed throughout the Northern Hemisphere and Asia (Enke and Gemeinholzer, 2008; Shi et al., 2011). The genus presumably originated in the Altai/Tien Shan region in Central Asia (Babcock, 1947). The present center of diversity of Crepis is the circum-Mediterranean area (Enke and Gemeinholzer, 2008; Babcock, 1947). The present center of diversity of Crepis is the circum-Mediterranean area (Enke and Gemeinholzer, 2008). Babcock, 1947 divided Crepis into 27 sections worldwide and included Lagoseris as a section of the genus Crepis (e.g., Lack, 2007; Enke, 2010). However, recent molecular studies based on nuclear ITS and plastid matK sequences indicated that sect. Lagoseris may be a distinct genus (Enke and Gemeinholzer, 2008; Enke, 2010; Kilian et al., 2009). ITS and matK phylogenetic trees of the Lagoseris clade include Crepis species from sections Lagoseris, Pterotheca, Microcephalum, Intybelia, as well as the genera Rhagadiolus and Lapsana (Enke and Gemeinholzer, 2008; Enke, 2010).

During fieldwork (2012–2013) in a sand desert area of the Junggar Basin of northern Xinjiang, northwestern China, we discovered an ephemeral species of Asteraceae that could not be identified. Its annual habit and the receptacle with only a few setiform paleae indicated that the plant clearly was a member of Crepis sect. Pterotheca (Babcock, 1947a, 1947b) or Lagoseris subg. Pterotheca (Czerepanov, 1964). Detailed examination of these plants and a survey of the literature, including Flora of the U.S.S.R (Czerepanov, 1964), Flora of China (Shi et al., 2011), Flora Xinjiangensis (An, 1999) and Babcock’s monographs of Crepis (Babcock, 1947a, 1947b), showed that this unknown plant was similar to Crepis sancta subsp. bifida (Vis.) Thell. ex Babc. but distinguished from it by glandular hairs on the stem, bract shape, style color and shape of the dimorphic achenes. Morphological comparisons and nuclear ITS and plastid matK sequences indicated that this plant represented an undescribed species of Crepis, which we named Crepis desertorum.

2. Material and methods

2.1. Morphological description

The morphological description is based on live material. Details of bracts, flowers and achenes were examined under a Nikon...
SMZ1000 stereoscopic microscope (Nikon Corp., Tokyo, Japan) and measured with a digital caliper. Distribution, habitats and phenology of the new species are based on field observations. Live material was collected from Mosuowan, Shihezi city of Xinjiang, China (44°25.42′N, 86°01.21′E).

2.2. SEM observations on pollen and achene morphology

Mature pollen grains and achenes were mounted directly on aluminum stubs with double-coated conductive glue, coated with gold-palladium and examined under a Zeiss SUPRA 55VP (Carl Zeiss, Oberkochen, Germany) scanning electron microscope (SEM). Terminology for the description of pollen follows Skvarla and Turner (1966) and Ma et al. (2009). Vouchers for the pollen and achene material studied are provided in Table 1. All voucher specimens have been deposited in the Herbarium of Xinjiang Agricultural University (XJA) (see Table 1).

2.3. Karyotype analysis

Mature achenes were collected from a natural population (Mosuowan, Shihezi city of Xinjiang, China) in June 2013. They were incubated on wet filter paper in Petri dishes at 20/10 °C (12/12 h) and a 12 h daily light period for 4−5 days, during which time germination occurred. Root-tips were removed from roots 1−1.5 cm in length and pretreated with 0.002 M 8-hydroxyquinoline at room temperature (c. 20 °C) for 3 h and then fixed in ethanol:glacial acetic acid (3:1v/v) for 24 h. Subsequently, fixed root-tips were hydrolyzed in 1 M HCl for 20 min at 60 °C, stained with carbol fuchsin and then squashed in a drop of 45% acetic acid and mounted for cytological observations. Photomicrographs were taken under a Zeiss Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany) using a 100 × oil lens. Karyotypic analysis of chromosomes at mitotic-metaphase was determined from five well-spread metaphases. The symbols used to describe the karyotypes followed Levan et al. (1964) and Li and Chen (1985). The asymmetry classification of the karyotype follows Stebbins (1971) and Li and Chen (1985).

2.4. Molecular analysis

We sampled 17 populations representing 14 species of Crepis s.l., including the presumed new species, for molecular analyses. Askelia flexuosa was chosen for the outgroup based on Enke and Gemeinholzer (2008), Zhang et al. (2011), and our data. Sequences were aligned in MAFFT v7.311 (Katoh et al., 2002; Katoh and Standley, 2013) and manually adjusted with MEGA 7.0.14 (Kumar et al., 2016). Phylogenetic analyses were conducted based on Bayesian inference (BI) in MrBayes v3.2.6 (Ronquist et al., 2012), Maximum Likelihood in RAxML 8.2.10 (Stamatakis, 2014) on CIPRES (Miller et al., 2010) and Maximum Parsimony (MP) in PAUP version 4.0b10 (Swofford, 2002).

For Bayesian inference (BI) analysis, jModeltest v2.1.7 (Darriba et al., 2012) was used to select the best-fit model of nucleotide substitution based on the Akaike information criterion (AIC). The SYM + I + G model was selected for the nrITS dataset and the GTR + I model for the cpDNA dataset. Four simultaneous Monte Carlo Markov chains (MCMCs) were run for five million generations for both nrITS, matK and combined sequences, and one tree was sampled every 1000 generations. The first 1250 trees (25% of total trees) were discarded as burn-in. The remaining trees were summarized in a 50% majority-rule consensus tree, and the Bayesian posterior probabilities (BP) were calculated.

For the maximum likelihood bootstrap (ML) analyses, we constructed the phylogeny using GTRCAT as the nucleotide substitution model and performed 1000 replicates for the bootstrap analyses. For the maximum parsimony bootstrap (MP) analyses, 100 replicates of random stepwise addition with tree bisection-reconnection (TBR) branch swapping were performed using heuristic searches, with all most-parsimonious trees saved at each replicate (MulTree on). Support for each branch was assessed using bootstrap analyses with 100 bootstrap replicates, each with 10 stepwise additions.

| Table 1 |
|---|
| Voucher material for the Crepis desertorum J. Qiu & D.Y. Tan pollen, achene morphology and karyotype analysis. |
| Location | Coordinate | Altitude | Date | Voucher |
|---|---|---|---|---|
| **Pollen material** | | | | |
| China, Xinjiang, Shihezi City Mosuowan District, 147 Regiment 22 Company | 44°25.42′N, 86°01.21′E | 300 m | 6 May, 2013 | J. Qiu 2013-0171 (XJA) |
| China, Xinjiang, Jimsar County, Wuciaowan District, China national highway 216 514 km | 44°23.71′N, 88°47.21′E | 530 m | 27 Apr, 2013 | J. Qiu 2013-0129 (XJA) |
| **Achene material** | | | | |
| China, Xinjiang, Shihezi City Mosuowan District, 147 Regiment 22 Company | 44°25.42′N, 86°01.21′E | 300 m | 15 May, 2013 | J. Qiu 2013-0195 (XJA) |
| China, Xinjiang, Jimsar County, Wuciaowan District, China national highway 216 514 km | 44°23.71′N, 88°47.21′E | 530 m | 12 May, 2013 | D.Y. Tan 274 (XJA) |
Fig. 1. Illustrations of *Crepis desertorum* J. Qiu & D.Y. Tan sp. nov. Drawn by C.D. Cui. (A) individual; (B) capitulum; (C) outer bract; (C′) inner bract; (D) floret; (E) ligule teeth; (F) receptacle; (G) infructescence; (H) peripheral achene; (H′) central achene.
Fig. 2. Photographs of *Crepis desertorum* J. Qiu & D.Y. Tan. (A) individual; (B) capitulum; (C) involucre; (D) bracts (ob = outer bracts, ib = inner bracts); (E) ligulate florets; (F) style branches; (G) details of ligule teeth; (H) receptacle; (I) infructescence; (J) peripheral achene; (K) central achene. Scale bar: 1 mm.
3. Results

3.1. Taxonomic treatment

*Crepis desertorum* J. Qiu & D.Y. Tan, sp. nov (Figs. 1 and 2).

**Type:** CHINA. Xinjiang, Mosuowan District, Shihezi city, sand dune, 44°25.42′N, 86°01.21′E, alt. 300 m, 30 Apr. 2013, J. Qiu & D. Y. Tan 2013-0146 (holotype: XJA, isotypes: KUN and PE).

3.2. Diagnosis

*C. desertorum* is similar to *C. sancta* subsp. *bifida*, but it is easily distinguished from the latter species by habit, stem with glandular hairs, style color, receptacle and dimorphic achenes.

3.3. Description

Annual. Roots slender, hard. Stem erect or ascending, 7–25 cm high, cylindrical with glandular hairs, finely ribbed. Basal leaves numerous and forming a rosette, leaf blade linear-lanceolate, lanceolate or oblanceolate, 4–10 cm long (including petiole) and 0.7–1.4 cm wide, glabrous, base attenuate to petioles, apex obtuse to acute, margin irregularly sinuate-dentate or pinnatifid, spiny hairy marginally; cauline leaves arising from the branches, linear, ca. 0.3–0.4 mm long. Capitula many in corymb. Involucre narrowly cylindrical to narrowly campanulate, 7–11 mm long and 2–4 mm wide. Involucral bracts with glandular hairs; outer bracts 4–5, triangular, longest 1/3–1/4 as long as inner ones, margin white scarious, apex acute; inner bracts 8–12, lanceolate, 10–12 mm long, ca. 2 mm wide, margin white scarious, apex acute. Receptacle covered with erect setaceous hairs, 3–7 mm long. Florets ligulate, 12–30, corolla 6–9 mm long, tube white, ca. 3 mm long, ligule yellow in lower part and yellowish-white in upper part, apex 5-dentate with teeth ca. 0.01 mm long; anther ca. 2 (–2.5) mm long; style branches yellowish, 0.5 mm long. Infructescences numerous. Achenes dimorphic; peripheral achenes 2–15, whitish-yellowish, terete, linear, 4.2–6.1 mm long and 0.2–0.5 mm wide, with 10 ribs covered with papilliform protuberances; central achenes 7–30, reddish-brown, terete, linear, 4.2–5.6 mm long and 0.2–0.4 mm wide, with 10 smooth ribs. Pappus many fine white bristles, 3–6 mm long. Fl. and fr. Apr.–Jun.

**Distribution and habitat** — *C. desertorum* is widely distributed in the Junggar Basin. It grows in stationary sand dunes at altitudes of 300–530 m (Fig. 5). Associated species include *Haloxylon ammodendron* (C.A.Mey.) Bunge, *Calligonum leucocladum* (Schrenk) Bge., *Eremurus anisopterus* (Kar. et Kir.) Regel, *E. inderiensis* (M. Bieb.) Regel, *Soranthus meyeri* Ledeb., *Astragalus flexus* Fisch. and some annual desert ephemerals.

**Etymology** — The specific epithet is derived from the desert habitat, from which the type of this species was collected.

**Chinese name** — Huang Mo Huan Yang Shen (荒漠还阳参).

**Morphological evidence** — By comparing the morphological characteristics of *C. desertorum* with those of its related species (Table 3), we found that *C. desertorum* is similar to the species *C. sancta* (with three subspecies) and more specifically to *C. sancta* subsp. *bifida*.
However, it differs from *C. sancta* in having glandular hairs on the stem (Fig. 1A) (vs. yellow setiform hairs, subglabrous or glandular on the stem of *C. sancta*), a flat receptacle with some erect setaceous hairs in the center of the receptacle (Figs. 1F and 2H) (vs. flat receptacle with scales in *C. sancta*), style usually yellow (vs. green in *C. sancta* subsp. *bifida*) and dimorphic achenes (vs. trimorphic achenes in *C. sancta* and *C. sancta* subsp. *bifida*). The related species *C. frigida* is a perennial with monomorphic achenes (Table 3), whereas *C. desertorum* is an annual herb usually with dimorphic achene (Figs. 1H, H’ and 2J, K).

**Pollen evidence** — *C. desertorum* pollen is echinoloplate and belongs to the *Chichorium intybus* type. It is isopolar, radially symmetrical, tricolporate and spheroidal. Grain size ranges from 28.21 to 35.48 µm (Fig. 3A). The endexine (a distinct exine layer between ektexine and intine) is thick, and the columellae are very thin and short.

**Achene morphology** — Peripheral and central achenes of *C. desertorum* differ in pericarp micro-morphology. Rough longitudinal ribs (Fig. 3B) on the surface of peripheral achenes are composed of papilliform cells covered with white granules (Fig. 3C). Epidermal cells between the longitudinal ribs are scaly and glabrous. However, thin longitudinal ribs (Fig. 3E) on the surface of central achenes are composed of glabrous scaly cells (Fig. 3F), and the epidermal cells between the longitudinal ribs also are scaly and glabrous.

**Karyotype evidence** — The chromosome number of *C. desertorum* is 2n = 2x = 10 (Fig. 4). The karyotype includes 5 pairs of chromosomes (Table 2): pair 1 subtelocentric (st), pairs 2–3 submetacentric (sm), and pairs 4–5 metacentric (m). The first pair has a satellite. The karyotype formula is 2n = 10 (4m + 4sm + 2nd) (2SAT). Karyotype asymmetry is of the 3A type.

**Phylogenetic evidence** — The Bayesian tree with MP, ML and BP support values for each clade is shown in Fig. 6. The sampled species of *Crepis* s.l. form a monophyletic group with strong support of BP = 1.00, when the genera *Lapsana* and *Rhagadiolus*, which are deeply nested within the clade of *Crepis* s.l., are included. The clade of *Lapsana* and *Rhagadiolus* is sister to the *Lagoseris* clade, which includes the new species *C. desertorum* and some species of the sect. *Lagoseris* (*C. frigida*, *C. sancta*, *C. purpurea*, *C. sahendi*, *C. multicaulis*, *C. elongata*). Within the clade, *C. desertorum* is sister to *C. frigida* but with moderate support of BP = 0.78.

**Paratypes** — CHINA. Xinjiang: Mosuowan District, Shihezi City, alt. 300 m, 12 June, 2012, D. Y. Tan 2012-0102 (XJA); same locality, 6 May, 2013, J. Qiu 2013-0171 (XJA); Wujiaqu City, alt. 490 m, 12 May, 2013, J. Qiu 2013-0129 (XJA); Wutonggou, Fukang City, alt. 446 m, 27 Apr., 2013, J. Qiu 2013-0142 (XJA); same locality, 14 May, 2013, J. Qiu 2013-0182 (XJA); Wucaiwan District, Jimsar County, alt. 530 m, 27 Apr., 2013, J. Qiu 2013-0093, 2013-0129 (XJA).

### 4. Discussion

The genus *Crepis* comprises about 18 species (five endemic) in China, nine of which are distributed in Xinjiang (Shi et al., 2011). The *Lagoseris* species previously reported as occurring in Xinjiang is *L. sancta* (L.) K. Maly (Hou, 1982). *L. sancta* also is recorded in *Flora Xinjiangensis* (An, 1999). However, neither Hou (1982) nor An (1999) described the distribution or habitat of

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**Table 2**

| Chromosome pair | Relative length (chromosome length/total length of chromosome set) × 100, long arm length (L), short arm length (S), total length of chromosome set (TLC), centromeric index (CI = 100 × [S/TLC]) and arm ratio (AR = L/S) | Chromosome types |
|-----------------|-------------------------------------------------------------------------------------------------|-----------------|
| 1*              | 3.86 1.21 5.06 22.86 3.20 st                                                                 |                 |
| 2               | 3.44 1.61 5.04 31.96 2.14 sm                                                                   |                 |
| 3               | 3.39 1.21 4.60 25.92 2.81 sm                                                                   |                 |
| 4               | 2.36 1.57 3.94 39.94 1.50 m                                                                    |                 |
| 5               | 2.26 1.37 3.63 38.01 1.65 m                                                                    |                 |

Note: * Chromosomes with satellites. st (subtelocentric), sm (submetacentric) and m (metacentric).
Table 3
Morphological comparison of *Crepis desertorum*, *C. frigida*, *C. sancta*, and *C. sancta* subsp. *bifida*.

| Characters                  | *Crepis desertorum* | *Crepis frigida*<sup>a,b</sup> | *Crepis sancta*<sup>c</sup> | *Crepis sancta* subsp. *bifida*<sup>c</sup> |
|-----------------------------|---------------------|-------------------------------|-----------------------------|-----------------------------------------------|
| **Life form**               | annual              | perennial                     | annual                      | annual                                        |
| **Stem indumentum**        | glandular hairs     | mostly scapelike,             | yellow setiform hairs,      | —                                             |
|                             |                     | tomentose and pubescent       | subglabrous or glandular    |                                               |
| **Outer bracts**           | triangular, longest | linear to ovate, 2.0–3.0 mm   | deltoid to subovate, acute  |                                               |
| as long as inner ones, c. 0.3  | 0.4 mm long, margin| long, c. 1.5 mm wide,         | 1/8–1/3 as long as the inner|                                               |
| white scarious, apex acute |                     | subglabrous or thinly         | bracts                       |
|                             |                     | glandular, margin white       | scarios                      |
| **Receptacle**             | flat, covered with  | flat, with scales              | flat, with scales            | —                                             |
|                            | erect setaceous     |                               | —                            |                                               |
| **Style color**            | yellow              | yellow dimorphic               | greenish or yellow           |                                               |
| **Achene type**            | dimorphic           | dimorphic or sometimes trimorphic | white, corticeous,          |                                               |
|                             | whiskish-yellowish, terete, | light brown,            | strongly obcompressed,      |                                               |
| **Peripheral achenes, shape** | line, 4.2–6.1 mm   | cylindrical, c. 5.0 mm         | linear-lanceolate,          |                                               |
|                             | long and 0.2–0.5 mm | long, straight or curved,      | dorsally smooth,            |                                               |
|                             | wide, with 10 ribs  | with 15 smooth ribs,           | mostly winged,              |                                               |
|                             | covered with dense  | slightly attenuate;           | stramineous (sometimes     |                                               |
|                             | papilliform protuberances | epidermal cells obscure in   | absent)                     |                                               |
|                             |                     | outline with acute end wall,   | —                            |                                               |
|                             |                     | outer cell wall                | —                            |                                               |
|                             |                     | ornamentation smooth           | —                            |                                               |
| **Intermediate achenes, shape** | —                    | terete, slender, coarsely or | terete, slender, coarsely or |
|                             |                     | finely spiculate               | finely spiculate             |
|                             |                     | brown or greenish,            | brown or greenish,           |
|                             |                     | narrowly fusiform, 3.0–4.0 mm  | narrowly fusiform,           |
|                             |                     | long, c. 0.4 mm wide,         | 0.4 mm wide,                |
|                             |                     | sometimes scabridulous        | sometimes scabridulous      |                                               |
| **Central achenes, shape** | reddish-brown, line, | —                            | —                            |                                               |
|                             | 5.6 mm long and 0.2 | terete, slender, coarsely or | terete, slender, coarsely or |
|                             | —0.4 mm wide, with 10 | finely spiculate               | finely spiculate             |
|                             | smooth ribs; the epidermal | brown or greenish,           | brown or greenish,           |
|                             | cells obscure in    | narrowly fusiform, 3.0–4.0 mm  | narrowly fusiform,           |
|                             | outline with acute  | long, c. 0.4 mm wide,         | 0.4 mm wide,                |
|                             | end wall            | sometimes scabridulous        | sometimes scabridulous      |

Note: —, Not described.

<sup>a</sup> http://cichorieae.e-taxonomy.net/portal/node/8.
<sup>b</sup> Kalmuk et al., 2018.
<sup>c</sup> Babcock, 1947.

Fig. 6. Bayesian consensus tree of *Crepis desertorum* (bold) and related species of *Crepis*. Numbers above branches indicate Bayesian posterior probability (BP), numbers below branches are maximum likelihood bootstrap (ML) and maximum parsimony bootstrap (MP).

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**Table 3**

Morphological comparison of *Crepis desertorum*, *C. frigida*, *C. sancta*, and *C. sancta* subsp. *bifida*.
northern Xinjiang, China; the Indo-Himalayas, whereas C. desertorum belongs to the Cichorium intybus subgroup, while that of C. sancta belongs to the Taraxacum officinale subgroup (Enke, 2010). However, C. desertorum has a chromosome number of \(x = 5\), which differs from that of C. frigida \((x = 4)\) (Enke and Gemeinholzer, 2008).

Molecular studies based on nuclear ITS and plastid matK sequences indicated that C. desertorum is more closely related to C. frigida than to C. sancta subsp. bifida (Fig. 6) but with low support \((PP = 0.78)\). Although the micromorphology of the central achenes of C. desertorum is similar to that of C. frigida (Kalmuk et al., 2018), the two species differ greatly in life form, stem indumentum and outer bracts (Table 3). Babcock, 1947a, 1947b even treated L. sancta as a synonym of C. desertorum subsp. bifida. C. desertorum is known only from the Junggar Basin in northern Xinjiang, China; C. sancta is distributed in Europe, the Caucasus region, Balkans-Asia Minor, Palestine, Afghanistan and the Indo-Himalayas, whereas C. sancta subsp. bifida is distributed in Afghanistan, Turkistan and N.W. Himalayas (Babcock, 1947; Czerepanov, 1964). Thus, the geographical distribution of C. desertorum is disjunct from that of C. sancta and C. sancta subsp. bifida. C. desertorum has some unique morphological characters, and it differs from C. sancta subsp. bifida in having glandular hairs on the stem, yellowish style branches, peripheral whitish-yellowish linear achenes with 10 ribs covered with papilliform protuberances and white pappus (Table 3).

Africa is a major distribution area of the genus Crepis with more than 40 species, and the achenes of some African species are dimorphic, such as C. aspera, C. amplexifolia, C. pulchra, and others (http://cichorieae.e-taxonomy.net/portal/node/8). There are significant differences in the achene shape and number of the ribs, surface structure and the pappus between C. desertorum and these African species with dimorphic achenes (Enke, 2010; Kalmuk et al., 2018; http://cichorieae.e-taxonomy.net/portal/node/8).

Declaration of Competing Interest

The author declares no conflict of interest.

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Appendix S1

Taxon name, voucher information (for the first published sequences in part I and the downloaded sequences from GenBank that was placed in part II) and GenBank accession numbers for ITS and matK sequences used in this study.

| Taxa | Voucher | ITS | matK |
|------|---------|-----|------|
| Crepis desertorum J. Qiu & D.Y. Tan, 1 | LW-079 (XJA), Wucaiwan, Xinjiang, China | MN750316 | MN764299 |
| Crepis desertorum J. Qiu & D.Y. Tan, 2 |  | MN750317 | MN764300 |
| Crepis desertorum J. Qiu & D.Y. Tan, 3 |  | MN750318 | MN764301 |
| Crepis elongata Babcock | SunH2522 (KUN), Demuwa, Tibet, China | MN750319 | MN764302 |

Part II:

| Taxa | ITS | matK |
|------|-----|------|
| Askellia flexuosa (Ledebour) W. A. Weber | EU363596 | EU363544 |
| Crepis frigida (Boiss. & Balansa) Babcock | EU363612 | EU363555 |
| Crepis rubra L. (— C. incarnata Vis.) | EU363608 | EU363551 |
| Crepis multiculus Ledeb. | EU363642 | EU363573 |
| Crepis palasitina (Boiss.) Bormrn. | EU363639 | — |
| Crepis praemorsa (L.) Walther | EU363654 | EU363578 |
| Crepis pteochooides Boiss. | — | EU363570 |
| Crepis pulchra L. | AJ633639 | AJ633144 |
| Crepis purpurea (Wild.) M. Bieb. | EU363653 | — |
| Crepis sahendi Boiss. & Buhse | — | EU363651 |
| Crepis sancta (L.) Bornm. | AJ633637 | AJ633150 |
| Lapsana communis L. | AJ633285 | AJ633137 |
| Lapsana communis L., 2 | AJ633286 | AJ633138 |
| Rhagadiolus stellatus (L.) Gaertn. | DQ451823 | AJ633224 |

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