

# Taxonomical features of stigmas of tribe *Lithospermeae* DC. (Boraginaceae) species in Asiatic Russia

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**Abstract.** With the help of scanning electron microscopy, morphology of stigmas and shapes of papillae were studied for 11 species (from Asiatic Russia) of genera *Lithospermum* L., *Buglossoides* Moench, *Cerinth* L., *Echium* L., and *Onosma* L., belonging to tribe *Lithospermeae*, and two species of the genus *Botriospermum* Bunge of unknown taxonomical affiliation in the family Boraginaceae. It was revealed that the shapes of the stigma and papillae can be useful for clarifying relationships among the species and genera, thus complementing the data for compiling a fuller and more correct taxonomy of the family Boraginaceae.

## 1 Introduction

The first systematic data on stigma morphology of flowering plants appeared in the works of Y. Heslop-Harrison, K. R. Shivanna [1] and Y. Heslop-Harrison [2], where they determined that stigmas of family Boraginaceae species are of the type “dry, papillate, where papillae are unicellular,” abbreviated as DPU. This means that stigmas are of the dry type without cells on the surface excreting a liquid, and that papillae have unicellular structure.

Taxonomical value of stigma features was first indicated by German botanists J. Grau and A. Schwab [3] during classification of the genus *Myosotis* L. s. l. (tribe *Myosotideae* Reichenb., Boraginaceae). Subsequently, the idea of studying stigmas of Boraginaceae species has been supported by M. Bigazzi and F. Selvi [4]. They have described morphological diversity of stigmas and papillae in 62 species of 15 genera of tribe *Boragineae*, developed terminology for describing features of papillae, and showed that every papilla consists of two parts: a neck that is connected to the surface of the stigma and an inflated cup of various shapes.

More detailed history of stigma research for Boraginaceae species is described in a study by the present author [5].

To date, only for two genera of tribe *Lithospermeae* DC. (Boraginaceae)—South-African *Lobostemon* Lehm. and ancient Mediterranean *Onosma* L. [6, 7]—stigmas and papillae have been studied.

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Because at present, systematic information on stigma morphology of Eurasian species of tribe *Lithospermeae* is absent, we decided to study them, by means of a scanning electron microscope.

## 2 Materials and methods

Materials for the study were herbarial collections of the Central Siberian Botanical Garden SB RAS (NSK, NS). Stigma morphology and papillae shape were investigated under a Carl Zeiss EVO MA 10 scanning electron microscope. The methods of this study have been described by the present author elsewhere [5]. For the description of stigmas and papilla shape, the terminology proposed by Bigazzi and Selvi [4] was used.

## 3 Results and discussion

### 3.1 Tribe *Lithospermeae* DC.

This tribe includes ~25 genera and 460 species, which mainly grow in the Mediterranean and Iran-Turan provinces of the Ancient Mediterranean. The modern taxonomy of tribe *Lithospermeae* [8], based on molecular-genetic research into Boraginaceae species, is much different from M.G. Popov’s taxonomy outlined in the “Flora of USSR” [9]. The tribe is now considered in a broader scope and includes genera previously placed in independent tribes *Alkaniaceae* DC., *Cerinthaceae* DC., *Echieae* DC., and *Moltkiopsidinae* Riedl. This classification, in our opinion, more precisely matches the placement of genera in the family Boraginaceae and is consistent with the notion of greater significance of fruit features (gynobases and ems) compared to the features of the flower.

Eleven species of six genera grow in Asiatic Russia. The genus *Onosma* L. includes four species, *Lithospermum* L. includes two, and the other genera (*Buglossoides* Moench, *Echium* L., *Cerintho* L., and *Botriospermum* Bunge) are single-species taxa.

#### 3.1.1 Genus 1: *Lithospermum* L.

This genus includes ~50 species, mainly growing in North and South America and the Mediterranean. On the Asiatic-Russia territory, two species occur: *L. officinale* L. and *L. erythrorhizon* Siebold et Zucc.

Below are the results of our study on these species’ stigmas.

*L. officinale* has a stigma that is pronouncedly low-pyramidal, 170–180  $\mu\text{m}$  long, and up to 87  $\mu\text{m}$  wide; in the middle, it is slightly divided into two lobes. Papillae are well spaced on the stigma surface and are long, vermiform, 5–9  $\mu\text{m}$  long, and 2.0–3.5  $\mu\text{m}$  wide; on the convex cap of a papilla, round tubercles are clearly visible. Vermiform papillae are revealed for the first time; therefore, here we provide a description of this shape: papillae are long and vermiform; their length is twice (or more) greater than width, and a neck is absent; they are connected to the stigma surface via the base along the entire length.

*L. erythrorhizon* has a stigma that is flatter, 168–170  $\mu\text{m}$  long, and up to 40  $\mu\text{m}$  high; the lobes are rounded and more pronounced. Papillae are moderately densely located on the stigma surface and are represented by two shapes. Most papillae are lageniform: the neck is short, and the cap is rounded, 4.0–4.8  $\mu\text{m}$  long, and 3.5–4.7  $\mu\text{m}$  wide; the surface is convex, with clearly visible round tubercles. Between lageniform papillae, a few vermiform papillae are located, 5.0–6.0  $\mu\text{m}$  long and 1.2–2.0  $\mu\text{m}$  wide.

### 3.1.2 Genus 2: *Buglossoides* Moench

This genus includes 10–15 species, among which, *B. arvensis* (L.) I.M. Johnst is widespread. In the “Flora of USSR,” it was assigned to the genus *Lithospermum*, from whose species it is distinguished by erems with a tuberos surface without spines; these erems are not smooth and shiny.

*B. arvensis* has a bilobate stigma, which is 227  $\mu\text{m}$  long and 116  $\mu\text{m}$  high; the lobes are rounded-spherical. Papillae are moderately densely located on the stigma surface and are represented by two shapes. Most papillae are lageniform, 9.0–10.0  $\mu\text{m}$  long, and ~5.0  $\mu\text{m}$  wide. The neck is short, via which a papilla is connected to the stigma surface. Furthermore, a few vermiform papillae can be seen. The presence of the vermiform papillae on the stigma surface in *B. arvensis* indicates a relationship between this species and the genus *Lithospermum*.

### 3.1.3 Genus 3: *Cerinth* L.

In the “Flora of USSR,” this genus was included in monotypic tribe *Cerintheae*. On the Asiatic-Russia territory, only one species grows: *C. minor* L., which sporadically occurs in Altai and West Siberia.

To characterize morphological diversity of the features of stigmas and papillae, stigmas of the widespread species *C. minor* from two regions—Altai Krai and the Caucasus—were studied. It was found that stigma shapes and papilla shapes are similar between the plants from the different regions, but papilla surfaces differ. The Caucasian plants have almost smooth papilla surfaces, whereas the Altai plants have tuberculate papilla surfaces.

*C. minor* has a flat stigma with a small depression in the middle, which divides it into faint lobes; the stigma is 137–305  $\mu\text{m}$  long and 48–85  $\mu\text{m}$  high, similarly to the stigma of the genus *Lithospermum*, but in terms of papilla shapes, it is close to species of the genus *Onosma*. Papillae are densely located on the stigma surface, are lageniform, big, 12–14  $\mu\text{m}$  long, and 9–10  $\mu\text{m}$  wide; the neck is very short and thick, and the cap is lobate and convex, with different numbers of lobes; thus, the shapes and sizes of papillae are also different.

### 3.1.4 Genus 4: *Echium* L.

This ancient Mediterranean genus includes more than 40 species. On the Asiatic-Russia territory, only one annual-biennial species grows—*Echium vulgare* L.—which occurs in dry open spaces, forest steppes, fallows, and weedy locations. In the “Flora of USSR,” this genus was included in tribe *Echieae* because its species have a zygomorphic corolla and a bifid column. Nonetheless, in terms of gynobase shape and erem morphology, it is close to tribe *Lithospermeae*.

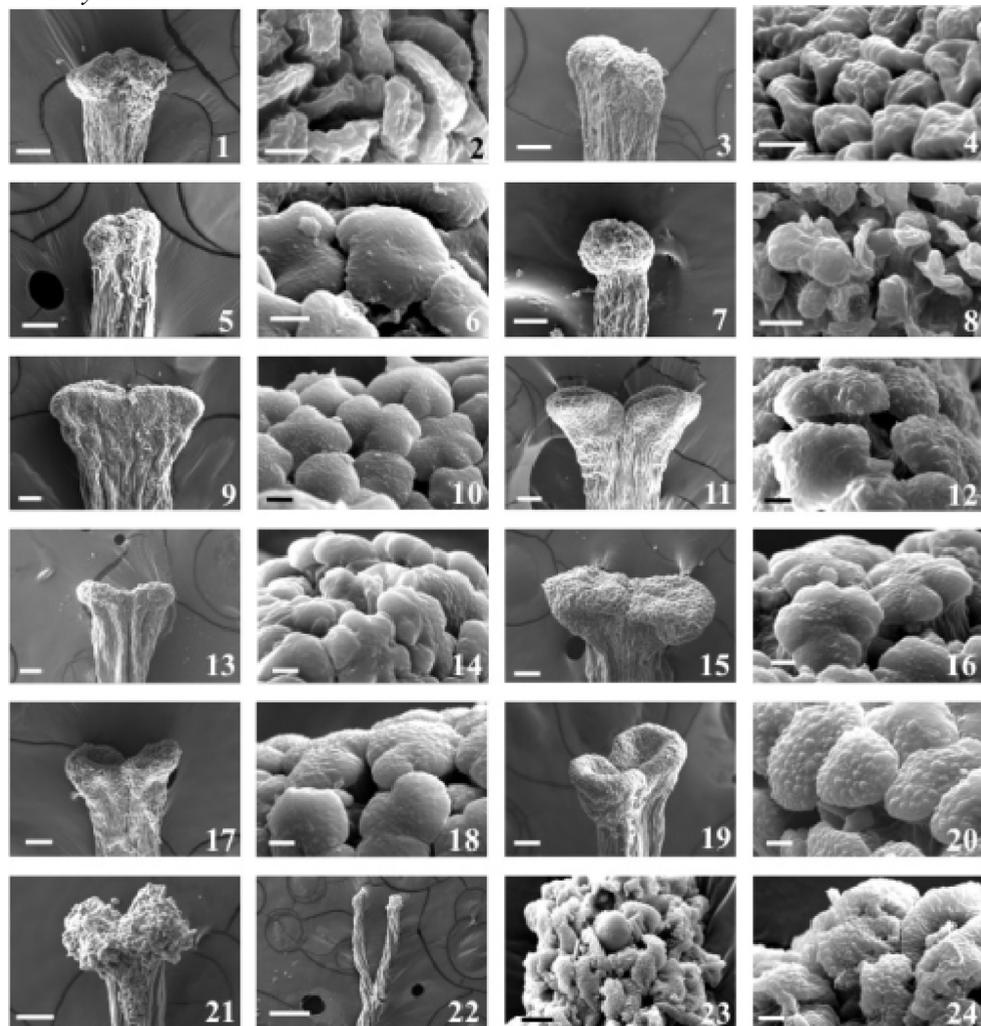
The stigma is undivided, hemispherical, 80–81  $\mu\text{m}$  long, and 58–60  $\mu\text{m}$  wide. Papillae are vermiform, 15–20(25)  $\mu\text{m}$  long and 5.0–6.5  $\mu\text{m}$  wide, located mainly along the stigma. The cap of the papillae is swollen and tuberos, and tubercles have different sizes and are spherically swollen. The vermiform shape of the papillae of *Echium vulgare* shows its relationship with the species of tribe *Lithospermeae*, especially *L. officinale*.

### 3.1.5 Genus 5: *Onosma* L.

This genus includes 150 species, and the greatest diversity is noted for the Ancient Mediterranean territory.

We studied stigmas of four species (Fig.): *O. simplicissimum* L., *O. gmelinii* Ledeb., *O. transrhymense* Klok. ex Popov, and *O. setosa* Ledeb. The first three species grow in

Siberia; in the East, their areal is limited by the Yenisei River; to the east of it, *Onosma* species are absent. *O. setosa* is a West-European species closely related to *O. transrhymense*.



**Fig.** Shapes of stigmas and papillae in species of tribe *Lithospermeae* DC. from Asiatic Russia. Panels 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22: the shape of the stigma; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 23, and 24: the shape of papillae; 1 and 2: *Lithospermum officinale*; 3 and 4: *L. erythrorhizon*; 5 and 6: *Cerintho minor*; 7 and 8: *Botriospermum tenellum*; 9 and 10: *Onosma simplicissimum*; 11 and 12: *O. gmelinii* (Khakassia); 13 and 14: *O. gmelinii* (Altai Republic); 15 and 16: *O. transrhymense* (Novosibirsk Oblast); 17 and 18: *O. setosa*; 19 and 20: *O. transrhymense* (Krasnoyarsk Krai); 21: *Buglossoides arvensis*; and 22–24: *Echium vulgare*. Scale bars: 60  $\mu\text{m}$  (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22) and 4  $\mu\text{m}$  (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 23, and 24).

*O. simplicissimum* has a slightly bilobate stigma, which is sinuate throughout almost the entire, length, 250–400  $\mu\text{m}$  long, and 90–108  $\mu\text{m}$  high. Papillae are moderately densely located on the stigma surface, are lageniform, 11–13  $\mu\text{m}$  long, and 9–11  $\mu\text{m}$  wide; the neck is short, the cap is rounded and slightly lobed, and the lobes have different sizes; their surface is almost smooth, with subtle small tubercles.

For comparative morphological analysis, we investigated the stigmas of *O. simplicissimum* from different geographical areas: Altai Krai and the Republic of

Khakassia. It was revealed that stigma and papilla shapes are stable throughout the areal, but the Khakassian plants have more prolonged papillae and big lobes.

*O. gmelinii* has a pronouncedly bilobate stigma, which is sinuate almost throughout the entire length, is 260–408  $\mu\text{m}$  long and 71–100  $\mu\text{m}$  high. Papillae are dense on the stigma surface, are lageniform, 12–20  $\mu\text{m}$  long, and (11)14–20  $\mu\text{m}$  wide; the neck is more or less long; the cap is rounded and big-lobed, with lobes of different sizes.

Stigmas of *O. gmelinii* from two locations—the Altai Republic (Kosh-Agach) and the Republic of Khakassia—were studied too. It was revealed that stigma and papilla shapes are stable, i.e., do vary among geographic locations, but the papillae surface varies: specimens from Kosh-Agach have a slightly tuberous surface, whereas Khakassian specimens have a coarse tuberous surface.

*O. transrhymense* has a flat bilobate stigma, which is deeply sinuate almost throughout the entire length, is 420–469  $\mu\text{m}$  long and 105–106  $\mu\text{m}$  high. Papillae are dense on the stigma surface, lageniform, 14–20  $\mu\text{m}$  long, and 9–18  $\mu\text{m}$  wide; the neck is more or less long; the cap is rounded and big-lobed, sometimes with almost solid edges; the surface is coarse and tuberous.

*O. transrhymense* is a polymorphous species and was studied on specimens from two locations, Novosibirsk Oblast and Krasnoyarsk Krai. The specimens from Novosibirsk Oblast have papillae that are more or less spaced on the stigma surface, the neck is longer, and the surface is almost smooth, with subtle tubercles. The specimens from Krasnoyarsk Krai have a shorter neck of the papilla and a coarse tuberous surface.

*O. setosa* (from the Republic of Kalmykia) has a bilobate stigma, which is deeply sinuate almost throughout the entire length, is 308–320  $\mu\text{m}$  long and 80–85  $\mu\text{m}$  high. Papillae are densely located on the stigma surface, are lageniform, 14–20  $\mu\text{m}$  long, and 9–18  $\mu\text{m}$  wide; the neck is more or less long; the cap is rounded and big-lobed, and the surface is coarse and tuberous.

Comparative morphological analysis suggested that stigma and papilla shapes of *O. setosa* are similar to those of a closely related species, *O. transrhymense*.

### 3.1.6 Genus 6: *Botriospermum* Bunge

This East-Asian genus includes approximately four annual species; on our territory, only one species—*B. tenellum* (Hornem.) Fisch. et C.A. Mey—grows. The placement of this genus in the family Boraginaceae is still ambiguous. In the “Flora of USSR,” it was assigned to independent tribe *Botriospermeae*, but the flat type of gynobases brings it together with the genera of tribe *Lithospermeae*; therefore, additional research, in particular, on stigma features, should help to reveal its relationships.

*B. tenellum* has an undivided stigma that is almost spherical, 130–135  $\mu\text{m}$  long, and 95–100  $\mu\text{m}$  high. Papillae are spaced on the stigma surface, are vermiform, 5.0–8.5  $\mu\text{m}$  long, and 1.0–2.5  $\mu\text{m}$  wide; the neck is high, the cap is fusiform-tortuous, and the surface is smooth.

*B. secundum* has an undivided stigma that is almost spherical, 136  $\mu\text{m}$  long, and 93–95  $\mu\text{m}$  high. Papillae are spaced on the stigma surface, are vermiform, 7–9  $\mu\text{m}$  long, and 2.2–4.0  $\mu\text{m}$  wide; the neck is high, the cap is fusiform-tortuous, and the surface is smooth.

Therefore, for the species of tribe *Lithospermeae*, three types of stigma are characteristic: undivided or slightly bilobate, bilobate, or bifid. For genera *Onosma* and *Cerithe*, lageniform papillae are characteristic. *Lithospermum officinale*, *Echium vulgare*, *Botriospermum tenellum*, and *B. secundum* have vermiform papillae. On the stigmas of *Lithospermum erythrorhizon* and *Buglossoides arvensis*, papillae of two types occur: lageniform and vermiform. The lageniform papilla shape of species of tribe *Lithospermeae* is different from that of species of tribes *Myosotideae* and *Boragineae*: the latter have a

short neck and a rounded-lobate cap. In terms of stigma and papilla shapes, the genus *Onosma* is an outlier in tribe *Lithospermeae*: its species have a bilobate, deeply sinuate stigma and lageniform papillae, which are big-lobed and morphologically similar to the papillae of ancient Mediterranean species *Onosma pachypoda* Boiss. and *O. gaubae* Bornm. This finding gives us a reason to suppose Ancient Mediterranean genesis of the Asiatic-Russia species. Thus, in the family Boraginaceae, a new vermiform shape of papillae was revealed.

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## Figure caption

### **O.D. Nikiforova “Taxonomical features of stigmas of tribe Lithospermeae DC. (Boraginaceae) species in Asiatic Russia”**

Figure. Shapes of stigmas and papillae in species of tribe *Lithospermeae* DC. from Asiatic Russia.

Panels 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22: the shape of the stigma; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 23, and 24: the shape of papillae; 1 and 2: *Lithospermum officinale*; 3 and 4: *L. erythrorhizon*; 5 and 6: *Cerintho minor*; 7 and 8: *Botriospermum tenellum*; 9 and 10: *Onosma simplicissimum*; 11 and 12: *O. gmelinii* (Khakassia); 13 and 14: *O. gmelinii* (Altai Republic); 15 and 16: *O. transrhymense* (Novosibirsk Oblast); 17 and 18: *O. setosa*; 19 and 20: *O. transrhymense* (Krasnoyarsk Krai); 21: *Buglossoides arvensis*; and 22–24: *Echium vulgare*.

Scale bars: 60  $\mu\text{m}$  (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 22) and 4  $\mu\text{m}$  (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 23, and 24).