

## A Biosystematic Study of *Elymus charkeviczii*, *E. mutabilis* and *E. subfibrosus* from the Peninsula Kamchatka

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**Abstract:** A comparative study of inter-specific hybrid viability was conducted among accessions of *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis*, together with accessions of *E. kamczadalarum*. Our results from the hybridizations and genetic analysis of morphological characters together with the protein analyses support the previous conclusion that *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis* on Kamchatka are just morphological variants of the same taxon, which probably this is endemic to Kamchatka. *Elymus kamczadalarum* is a distinct species although introgression may occur between this species and *E. charkeviczii* s.l. at places where the two species are growing together.

**Keywords:** biosystematics; SDS-electrophoresis; hybridization; recombination; gene pool; *Elymus*

About 10 to 14 species of *Elymus* grow in the peninsula Kamchatka (PROBATOVA 1985; YAKUBOV & CHERNYAGINA 2004). *Elymus kamczadalarum* (Nevski) Tzvel. is one of the most widely spread species, whereas *E. charkeviczii* Provat. is only recorded from very few places, including the surroundings of Esso village. The latter species differs from *E. kamczadalarum* by its scabrous rachillas, bigger anthers and shorter lemmas. *Elymus subfibrosus* (Tzvel.) Tzvel. has also been recorded from Kamchatka. This is an insufficiently known taxon from the northern regions of Siberia and continental part of the Russian Far East (TZVELEV 1976). The morphological descriptions of this species differ considerably between the floras of Siberia (PESHKOVA 1990) and Russian Far East (PROBATOVA 1984). Either the two descriptions are relating to different taxa or it is a taxon with very large morphological variation. *Elymus mutabilis* collected in the Russian Far East also differs from the Siberian materials. In Eastern Siberia only a few marginal populations of *E. mutabilis* have been recorded (PESHKOVA 1990, 2001; HULTÉN & FRIES 1986). Further to the East

there is a distribution gap (except a single specimen from the Magadan area) before it reappears in Kamchatka (PROBATOVA 1985).

Our previous results from an analysis of endosperm storage proteins patterns, suggested that the accessions from Kamchatka corresponding to *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis* belong to a single taxon, which is morphologically variable (AGAFONOV & SALOMON 2002). The aim of the present paper was to conduct an extended biosystematic evaluation of these "taxa", below referred to as the *ChSM* complex.

### MATERIAL AND METHODS

Seeds of *Elymus* species were collected in the peninsula Kamchatka by the authors or kindly provided by colleagues at the Institute of Northern meadows research, Academy of Sciences of the Jacutia-Sakha Republic (Table 1; Figure 1). The materials were identified using PROBATOVA (1985) for the Far-Eastern accessions and PESHKOVA (1990) for the Siberian accessions. The plants were

Table 1. Origin of accessions *E. kamczadalarum*, *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis*. **Bold** – accessions included in hybridization

Code	Species sensu TZVELEV (1976), PROBATOVA (1985)	Origin, collector
Kamchatka		
<b>KSO-9605</b> ; KSO-9610–12	<i>E. kamzchadalarum</i>	Kamchatka, 20 km SSE of Elyzovo, near Sosnovka (A. Agafonov & B. Salomon) [1]
KSO-9613	<i>E. kamzchadalarum</i> *	[1]
KSO-96, population	all morphotypes**	[1]
KAD-9614	<i>E. charkeviczii</i>	Kamchatka, 60 km NW of Elyzovo, Poperechnaya river (A. Agafonov & B. Salomon) [2]
<b>KES-9631</b> ; <b>KES-9632</b>	<i>E. charkeviczii</i>	Central Kamchatka, near Esso (A. Agafonov & B. Salomon) [3]
<b>KES-9612</b> ; <b>KES-9634</b>	<i>E. subfibrosus</i> (?)	[3]
<b>KES-9625</b> ; <b>KES-9633</b> ; <b>KES-9639</b> ; <b>KES-9640</b>	<i>E. mutabilis</i> (?)	[3]
KES-96, population	all morphotypes**	[3]
Yakutia		
1 JAK-8737	<i>E. subfibrosus</i>	near Khandiga (V. Doronkin)
2 JAE-8951	<i>E. subfibrosus</i>	near Elgjay (E. Filippov)
3 JAV-8953	<i>E. subfibrosus</i>	near Verkhnevilyujsk (E. Filippov)
4 JAC-8331	<i>E. subfibrosus</i>	Njurba breeding station, cultivar (G. Denisov)
5 JAC-0101; JAC-0102	<i>E. subfibrosus</i> ***	30 km NE of Jakutsk (N. Ermakov)
6 JAN-8916	<i>E. subfibrosus</i>	Southern Yakutia, near Nerjungri (O. Potemkin)

\*plant of introgressive origin

\*\*morphotypes corresponding to *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis*

\*\*\*morphotypes deviating from *E. subfibrosus*

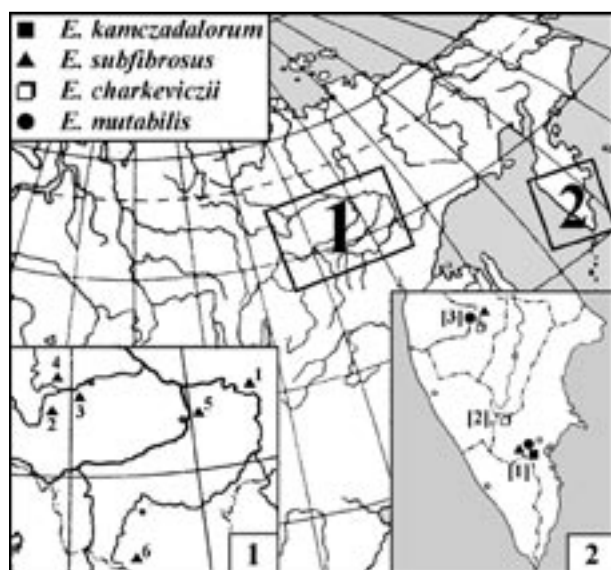


Figure 1. The origin of *Elymus* accessions used in the study

cultivated both in a glass house and outdoors in a field plot.

Preparation of endosperm protein extracts and SDS-electrophoresis followed KOSTINA *et al.* 1998. Cluster analysis was performed using Statistica 6.0 (Statsoft, Inc.) and dendrograms were obtained by complete linkage from the Manhattan distance coefficients. Plant crossing procedures were carried out following LU and BOTHMER (1990). Crossability was estimated as the ratio of filled seeds to the total number of flowers in a spike (AGAFONOV 1994) and estimation of levels of reproductive compatibility followed AGAFONOV and SALOMON (2002).

## RESULTS AND DISCUSSION

### Morphological variation

Plants of *E. kamczadalarum* when growing together with *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis*

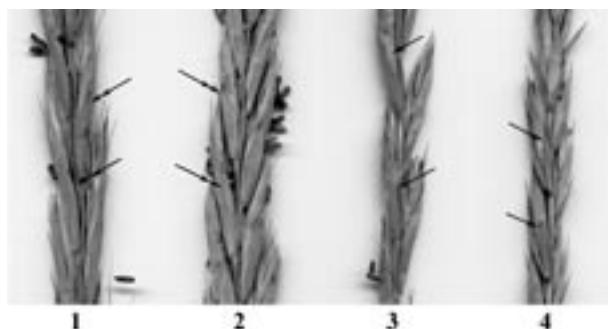


Figure 2. Fragments of spikes: 1. *E. charkeviczii* KES-9632; 2. *E. "mutabilis"* KES-9633; 3. *E. "subfibrosus"* KES-9612, lower part; 4. *E. "subfibrosus"* KES-9612, upper part. Arrows show glumes  $k = 0.6-0.7$ , double-arrows show glumes  $k = 0.7-0.8$

were easily distinguished on their narrower leaves and longer glumes (lengths ratio of lemma and adjacent lemmas  $L_{gl}/L_{lem} = k = 0.9-1.0$ ). Still, we collected a few fertile individuals of *E. kamczadolorum* with shorter glumes ( $k = 0.8-0.9$ ). Cultivation confirmed that this character was stable and electrophoretic analysis of seeds showed presence of protein components typical of the *ChSM* complex (see Figure 5).

Accessions belonging to the *ChSM* complex possessed shorter lemmas ( $k = 0.6-0.8$ ) and wider leaves (up to 18 mm). Variation on characters  $L_{gl}/L_{lem}$  and range in lemma pubescence made PROBATOVA (1984) to describe a new species *E. charkeviczii* (glabrous lemmas and scabrous rachillas) and to recognize specimens with partially pilose lemmas

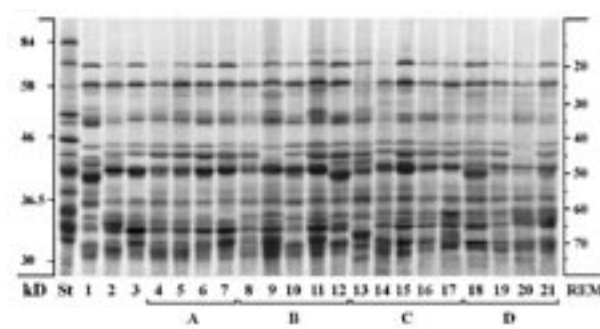


Figure 3. SDS-PAGE of endosperm proteins in the heterogeneous morphologically population KES-96 (*ChSM* complex) in comparison with studied biotypes. Single seed per plant. Electrophoretic variant + Me. 1. KES-9633 (*E. "mutabilis"*); 2. KAD-9614 (*E. charkeviczii*); 3. KES-9634 (*E. "subfibrosus"*); A, B, C, D – different morphotypes of lemma and rachilla. St – *Elymus sibiricus*, ALT-8401

and evenly pubescent lemmas as *E. subfibrosus* and *E. mutabilis*, respectively.

A heterogeneous population of the *ChSM* complex was studied in detail and high variation in lemma and rachilla hairiness was observed. In all, four variants could be identified among 158 spikes collected from separate plants. A, lemma and rachilla long-pubescent ("*mutabilis*",  $n = 17$ ); B, Pilose lemma, pubescent rachilla ("*subfibrosus*",  $n = 53$ ); C, lemma pilose near apex and along sides and rachilla pubescent ("*subfibrosus*",  $n = 76$ ); D, Lemma glabrous and rachilla scabrous ("*charkeviczii*",  $n = 12$ ). Additional variation was found in leaves (green vs. glaucous), relative length of

Table 2. Morphological characters of the biotypes of Kamchatic taxa *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis* included in crossing

Accession code	Leaves, adaxial surface	Spikes	Lemma back	Rachillas	Additional characteristics
KES-9612 <b>sub</b>	DLP	MD	PA	LP	Spike length up to 24 sm
KES-9625 <b>mut</b>	SP	RD	LP	LP	Spike length up to 18 sm
KES-9631 <b>char</b>	SC	RD	GL	P	Short pubescent abaxial surface of leaves
KES-9632 <b>char</b>	SC*	RD	GL	P	*Sparsely pubescent upper leaves to apex
KES-9634 <b>sub</b>	SP	MD	PA	LP	Spike length up to 24 sm
KES-9639 <b>mut</b>	DLP**	RD	LP	LP	**4 lower leaves are scabrous
KES-9640 <b>mut</b>	SC*	RD	LP	LP	*Sparsely pubescent upper leaves to apex

Leaves upper surface: DLP – densely long pubescent; SP – sparsely pubescent; SC – scabrous

Morphology of spikes: MD – medium dense; RD – rather dense

Lemma back: LP – long pubescent; PA – pilose to apex; GL – glabrate

Rachillas: LP – long pubescent; P – pilose

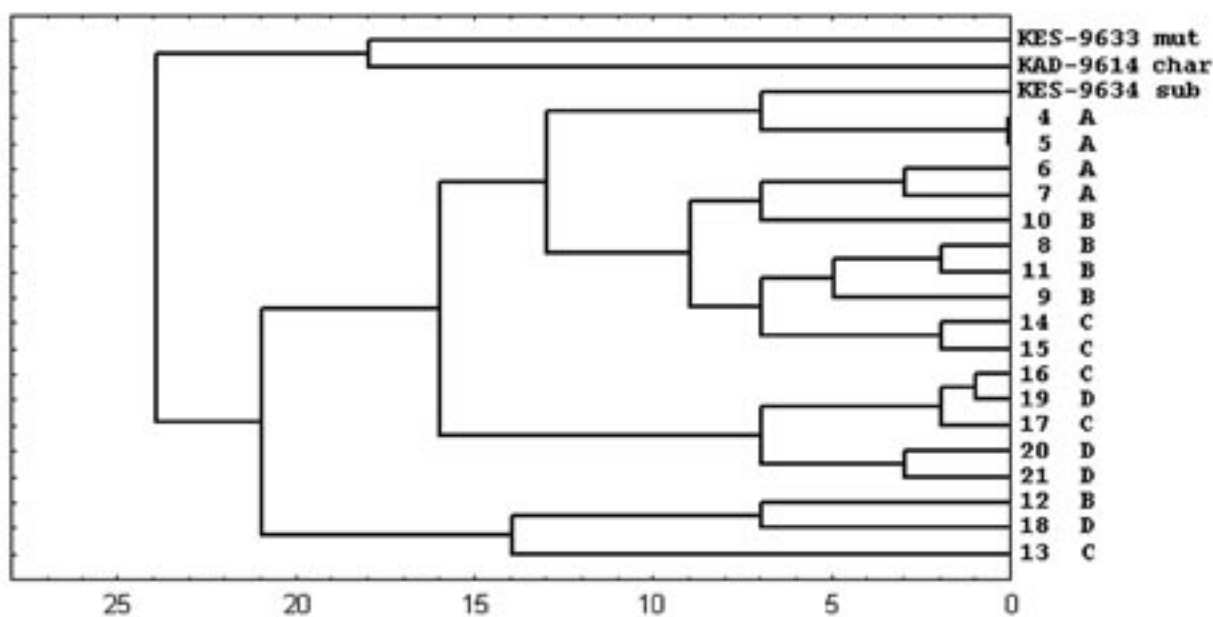


Figure 4. Complete linkage dendrogram of the Manhattan distance coefficients obtained from electrophoretic data (Figure 3). **A, B, C, D** – different morphotypes of lemma and rachilla (see text). The scale indicates levels of dissimilarity

glumes, and width of the membranous margins of lemma. The analysis also showed that the character “scabrous rachilla” varied among plants identified as *E. “charkeviczii”*.

The population KSO-96 was considerably smaller and less variable but still contained the three “taxa” included in the *ChSM* complex.

### Electrophoretic analysis

SDS-electrophoresis of storage endosperm proteins is an effective method for evaluating *Elymus* species (AGAFONOV & BAUM 1998; KOSTINA *et al.* 1998; AGAFONOV *et al.* 2002; KOSTINA *et al.* 2002). The high level of polymorphism makes it possible to

study intraspecific differentiation and interspecific relationships.

Our previous study (AGAFONOV & SALOMON 2002) on selected *E. kamczadalarum* and *ChSM* complex accessions from Kamchatka showed that the two groups have: *i*, unique banding patterns; *ii*, low levels of variability within the groups, and, *iii*, bidirectional genetic introgression seems to occur. The present extended analyses have confirmed these findings (cf. Figure 5). In Figure 3 an example of the variation in population KES-96 is shown, including typical accessions of “*mutabilis*”, “*charkeviczii*” and “*subfibrosus*”. In order to reveal any correlation between the four morphological variants and polypeptide composition, a dendro-

Table 3. Seed fertility of hybrids and sexual compatibility Cs of biotypes in the Kamchatic ChSM complex in generations

No	Cross combination	Highest value of seed set (%)				Level of Cs
		F <sub>1</sub>	N <sub>F1</sub>	F <sub>2</sub>	N <sub>F2</sub>	
1	KES-9612 <b>sub</b> × KES-9625 <b>mut</b>	85.9	3	80.0	2	α1
2	KES-9634 <b>sub</b> × KES-9625 <b>mut</b>	82.8	3	87.3	2	α1
3	KES-9634 <b>sub</b> × KES-9631 <b>char</b>	92.4	3	84.5	2	α1
4	KES-9639 <b>mut</b> × KES-9632 <b>char</b>	88.6	1	94.5	6	α1
5	KSO-9605 <b>kam</b> × KES-9625 <b>mut</b>	0.0	1	–	–	β?

N<sub>F1</sub> and N<sub>F2</sub> – number of plants analyzed in F<sub>1</sub> and F<sub>2</sub>.

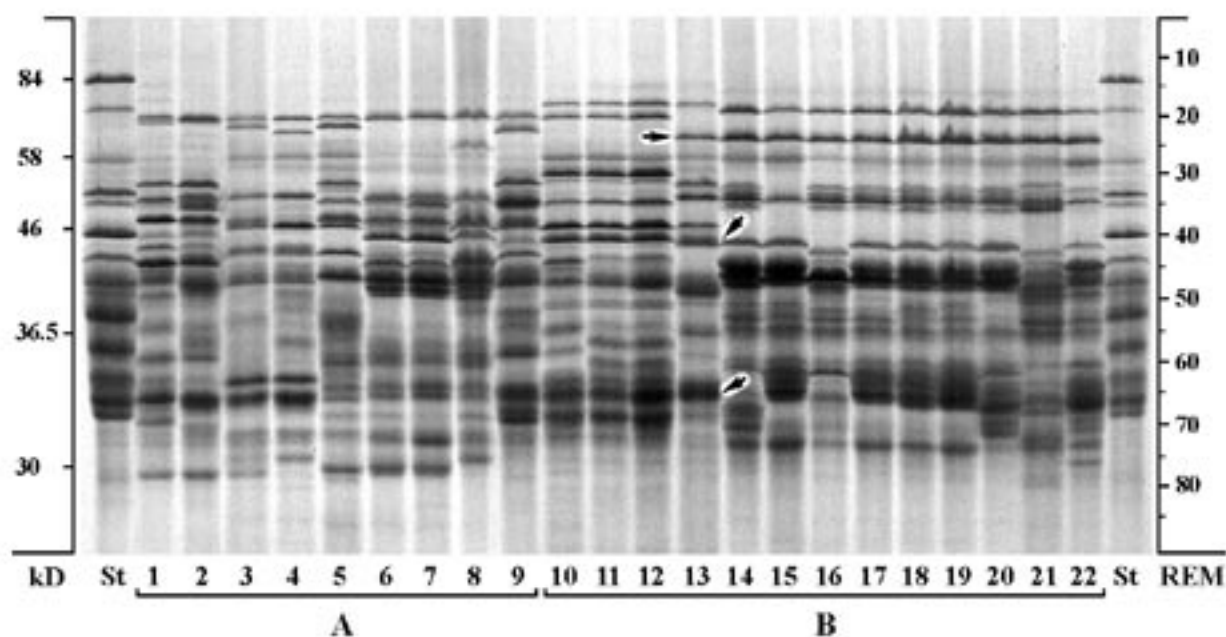


Figure 5. SDS-PAGE polypeptide spectra of endosperm proteins of continental accessions *E. subfibrosus* and deviating morphotypes (A), Kamchatic taxa *E. kamczadalarum* and ChSM complex (B). Single seed per accession. Electrophoretic variant + Me

A: 1. JAC-0102; 2. JAC-0101; 3. JAE-8951; 4. JAV-8953; 5. JAK-8737; 6–8. JAC-8331; 9. JAN-8916

B: *E. kamczadalarum*: 10. KSO-9610; 11. KSO-9611; 12. KSO-9612; 13. KSO-9613, *E. charkeviczii*: 14. KSO-9608; 15. KAD-9614. *E. "subfibrosus"*: 16. KSO-9619; 17. KSO-9620; 18. KSO-9621; 19. KSO-9622; 20. KSO-9623. *E. "mutabilis"*: 21. KES-9633; 22. KES-9639. St – *Elymus sibiricus*, ALT-8401. Arrows point to polypeptides of the accession KSO-9613 indicating a possibility of introgression between *E. kamczadalarum* and ChSM complex

gram was made (Figure 4), but no such correlation was found.

The comparative analyses also included continental accessions of *E. subfibrosus*. They showed higher level of variation than materials from Kamchatka. Proximate accessions had most overlapping banding patterns, despite of differences in spike

morphology. However, very few bands were in common with the ChSM complex.

#### Hybrid analysis

A series of crosses was made between the morphological variants (Table 2). High seed fertility

Table 4. Segregation of hybrid *E. mutabilis* KES-9639 × *E. charkeviczii* KES-9632 in F<sub>2</sub> on characters "glabrate – pubescent lemma back" and "pilose – pubescent rachilla"

Phenotype	No. of individuals observed	No. of individuals expected	$\chi^2, P$
<b>Pubescence of lemma back</b>			
Glabrate	15	9.625	$\chi^2 = 4.04$ (0.2 > P > 0.05)
All intermediate	127	134.75	
Long pubescent	12	9.625	
<b>Pubescence of rachilla</b>			
Pilose	10	9.625	$\chi^2 = 4.22$ (0.2 > P > 0.05)
All intermediate	128	134.75	
Long pubescent	16	9.625	

of hybrids confirmed the assumption that population KES-96 form a single reproductive gene pool (Table 3) and is best treated as one morphologically variable species. The single hybrid *E. kamzchadalarum* KSO-9605 × *E. "mutabilis"* KES-9625 had closed anthers and no seed set during two field vegetations. However, genetic introgression between *E. kamzchadalarum* and the *ChSM* complex is probably still possible as indicated by the electrophoresis data.

### Genetic analysis of diagnostic characters

The seed fertility of hybrids within the *ChSM* complex corresponds to a reproductive compatibility at the 1 level (cf. AGAFONOV 1994). This made it possible to carry out a genetic analysis of the distinctive characters. For this purpose an  $F_2$  population from a hybrid between *E. mutabilis* KES-9639 and *E. charkeviczii* KES-9632 was produced, i.e., using the most contrasting parental variants. Totally 154 plants were scored for the following characters: (i) upper leaf surface scabrous vs. sparsely to densely pubescent; (ii) lemma glabrous, pilose or pubescent; (iii) rachilla pubescent vs. pilose.

Only nine individuals had scabrous leaves and the rest were distributed among phenotype classes from sparsely pubescent upon apex up to densely long pubescent. As clear boundaries between classes of pubescence were impossible to define, a hypothesis of digenic inheritance was tested. The recessive phenotype **a1a1a2a2**, corresponding to scabrous leaves, would be expected in the ratio 1:15. The observed ratio 9:145 has a probability  $P > 0.95$  suggesting that leaf pubescence is controlled by two independently inherited genes.

It was even more difficult to distinguish phenotype classes for the degree of lemma hairiness. Not less than six phenotypes were finally suggested but without distinct borders: *i*, glabrous; *ii*, sparsely short pilose near apex; *iii*, evenly short pilose; *iv*, long pilose; *v*, sparsely long pubescent; *vi*, densely long pubescent. The high number of phenotype classes and probability of subjective errors make a genetic analysis dubious. However, the easiest classes to distinguish were the two extremes (glabrous and densely long pubescent). An attempt to evaluate probability of digenic inheritance of the character with equal effect on phenotype was made. The hypothesis of  $F_2$  segregation in a ratio 1:14:1 was checked. All intermediate classes should correspond to frequency 14 in a classic ratio of di-

genic additive segregation 1:4:6:4:1. The observed ratio was 15:127:12.

The character "rachillas hairiness" was also difficult to score into distinct phenotype classes. To some extent this character varied even among florets from a single spike. This phenomenon is particularly visible for florets of different order in a spikelet. The lower florets have shorter rachillas and longer hairs. It was only possible to record a general ratio for the extreme individuals having short pilose or long pubescent rachillas. All others were classified as intermediates. The ratio was then 10:128:16.

The results of the  $\chi^2$ -tests are shown in the Table 4. The probabilities for the floral characters were only in the range of  $0.2 > P > 0.05$ , which do not confirm a digenic additive inheritance of the investigated characters.

In conclusion, our results from the hybridizations and genetic analysis of morphological characters together with the protein analyses support the conclusion that *E. charkeviczii*, *E. subfibrosus* and *E. mutabilis* on Kamchatka are just morphological variants of the same taxon, which probably is endemic to Kamchatka. This taxon we tentatively call *E. charkeviczii* awaiting further taxonomic and nomenclatural studies. Molecular methods like DNA markers and gene sequencing should be performed in order to further reveal the relationships of the *E. charkeviczii* complex and its relationship to the continental *E. mutabilis* and *E. subfibrosus* and possible connections to the Northern American taxa *E. trachycaulus* and *E. alaskanus*.

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