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Isoetes in Alaska and the Aleutians

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ABSTRACT.—Three species of *Isoetes* are recognized as present in the study area: *I. echinospora* Durieu (diploid, $2n=22$), *I. maritima* Underw. (tetraploid, $2n=44$) and *I. occidentalis* L.F. Hend. (hexaploid, $2n=66$). Three interspecific hybrids are expected and two are known to be present: *I.* ×*pseudotruncata* D.M. Britton & D.F. Brunton (triploid, $2n = 33$) and *I.* ×*truncata* (Eaton) Clute (pentaploid, $2n = 55$). The missing hybrid taxon is *I. echinospora* (diploid) × *I. occidentalis* (hexaploid).

The genus *Isoetes* was largely unknown in Alaska and adjacent areas until recent years. The floristic treatment by Hultén (1968) was a major advance at the time. He recognized three taxa in Alaska, *I. muricata* Durieu var. *braunii* (Durieu) C.F. Reed, *I. muricata* ssp. *maritima* (Underw.) Hultén, and *I. truncata* (Eaton) Clute. In the same publication, *I. maritima* ssp. *maritima* received synonyms of *I. maritima* Underw., *I. macounii* A.A. Eaton and *I. beringensis* Kom., whereas, *I. truncata,* had the query, "*I. asiatica* with respect to Alaskan specimens," attached to it.

Britton and Brunton (1993) considered Alaskan taxa in an assessment of the pentaploid hybrid *I.* ×*truncata* (A.A. Eaton) Clute in western North America. Further biosystematic studies on Alaska *Isoetes* are a natural extension of that hybrid investigation. Almost no cytological determinations of Alaskan taxa had been undertaken before Britton and Brunton (1993). Similarly, no Scanning Electron Microscope (SEM) studies of spores had been conducted. Britton and Brunton (1993) cited four Alaskan collections of *I. occidentalis* L.F. Hend., including a large collection by S. S. Talbot from Auke Lake that was determined cytologically as $2n=66$.

Our objectives in the present study were to determine the number of species and interspecific hybrids of *Isoetes* in Alaska and to document their cytology, spore morphology, and distribution. Another longer term objective was to clarify the relationship of *I. maritima* with *I. macounii* and *I. beringensis*.

The Asian north Pacific species *I. asiatica* (Makino) Makino is $2n=22$ and has many similarities to *I. echinospora* (Takamiya et al., 1994, 1996; Wantanabe et al., 1996). However, *I. asiatica* should not be confused with either *I.* ×*truncata* (pentaploid) or *I. occidentalis* (hexaploid) (Britton and Brunton, 1993), as was done by Boivin (1961).

Materials and Methods

Living plants were collected by Stephen S. Talbot or Stephen and Sandra Talbot from 1992–1997 and were grown in distilled water in a growth chamber at the University of Guelph. The cytological methods and SEM methods employed in this investigation were those of Britton and Brunton (1989, 1992).

Alaskan *Isoetes* specimens are widely scattered in herbaria; they were examined in the following collections: AKFWS, ALA, BYU, CAS, ISC, OAC, S, SASK, CAN, DAO, and US.

The seven large living collections from Adak Island were particularly interesting. Adak Island is approximately half-way along the Aleutian chain and can be considered as representative of the whole archipelago. Further, these were the first collections received by the first author with data obtained by GPS (Global Positioning Satellite). It is possible to map to within ca. 1 m from data such as Raine Lake 51°46.3560′N 176°48.0009′W (*S. & S. Talbot 61* [OAC]).

Aquatic *Isoetes* are best studied from autumn collections. At that time of year, the spores are mature and will yield the best possible SEM photographs. This is sometimes logistically difficult to achieve, however, in a northern location where the summer and fall seasons are short.

Chromosome counts were determined for each of the five Alaskan taxa investigated during this study from the following specimens (cytological vouchers in OAC):

Isoetes echinospora ($2n=22$): Kenai NWR, Lake 5, 60°37.7′N, 150°48.6′W, *S. S. Talbot 5A5-1a*; Kenai NWR, Lake 19, 60°43.7′N, 150°35.7′W, *S. S. Talbot 19A3-4A*; Kenai NWR, Lake 23, 60°43.8′N 150°35.7′W, *S. S. Talbot 23A3-4A*.

Isoetes maritima ($2n=44$): Adak Island, Lake Leone above dam [6 plants], 51°50.7′N, 176°38.3′W, *S. & S. Talbot 51B*; Adak Island, elev. 185 m, Raine Lake area, 51°46.356′N, 176°48.009′W, *S. & S. Talbot 60*; same locality, *S. & S. Talbot 61*; Adak Island, Palisades Lake, 51°54.762′N, 176°36.061′W, *S. & S. Talbot 53*; Adak Island, "Shotgun" Lake, 51°56.320′N, 176°35.782′W, *S. & S. Talbot 54*; Izembek NWR, Cold Bay, Rescue Lake, 55°15.652′N, 162°49.764′W, *S. & S. Talbot 408*; Izembek NWR, Cold Bay, 55°10.851′N, 162°42.698′W, *S. & S. Talbot 410*.

Isoetes occidentalis ($2n=66$): Juneau, Auke Lake, *S. S. Talbot 920818*; Izembek NWR, Cold Bay, Frosty Mountain, 55°08′N, 162°50.0′W, 25 July 1993, *S. & S. Talbot* s.n.; Izembek NWR, Blinn Lake, 55°14.974′N, 162°45.787′W, 23 July 1993, *S. Talbot* s.n.; Adak Island, Lake Marie area, 51°49.964′N, 176°40.773′W, *S. & S. Talbot 50B*.

Isoetes ×truncata ($2n=55$): Adak Island, E side of Lake Leone, 51°50.701′N, 176°38.342′W, *S. & S. Talbot 52*; Adak Island, Lake Leone above the dam [3 plants], 51°50.7′N, 176°38.3′W, *S. & S. Talbot 51A*.

Isoetes ×pseudotruncata ($2n=33$): Kenai NWR, Lake 6 (Mosquito Lake), *S. S. Talbot 6A3-1*.

FIG. 1. *Isoetes maritima* (tetraploid) (left), *I. occidentalis* (hexaploid) (right) and their interspecific hybrid *I.* ×*truncata* (pentaploid) (centre) from Adak Island, Aleutian Islands. Vertical scale bar = 5 cm.

OBSERVATIONS

Cytological studies of the living plants allowed them to be classified as diploid ($2n$=22), triploid ($2n$=33), tetraploid ($2n$=44), pentaploid ($2n$=55) or hexaploid ($2n$=66). Examples of mitotic plates for these can be found in Britton and Brunton (1993) for *I. maritima* (tetraploid), *I. occidentalis* (hexaploid), and their interspecific hybrid, *I.* ×*truncata* (pentaploid). Similarly, mitotic plates for *I. maritima* (tetraploid), *I. echinospora* (diploid), and their interspecific hybrid, *I.* ×*pseudotruncata* (triploid), are shown in Britton and Brunton (1996).

The seven collections from Adak Island were classified as *I. maritima* (tetraploid), *I. occidentalis* (hexaploid), and their interspecific hybrid, *I.* ×*truncata* (pentaploid) (Fig. 1). This hybrid has pronounced hybrid vigor and the spores have extreme polymorphism. Views from SEM of the spores of these three taxa in Alaska (emphasizing Adak Island populations) are shown in Fig. 2. These are shown in full array, with proximal, lateral, and distal views of megaspores together with microspores. They are arranged for easy comparison between the three taxa, with the interspecific hybrid placed between the two parents.

Important features to note for *I. occidentalis* are the cristate megaspores with short, sharp, irregular spines (Figs. 2a, c) and a narrow girdle (Fig. 2b), and papillate spiny microspores (Fig. 2d). The large spores are quite spherical, unlike those of *I. maritima,* which often have an enlarged distal portion, presenting a subdued, almost "acorn-like" shape.

For *I. maritima,* the megaspore spines are usually shorter and more blunt than those of *I. echinospora* (Fig. 3). The triradial face is somewhat flattened

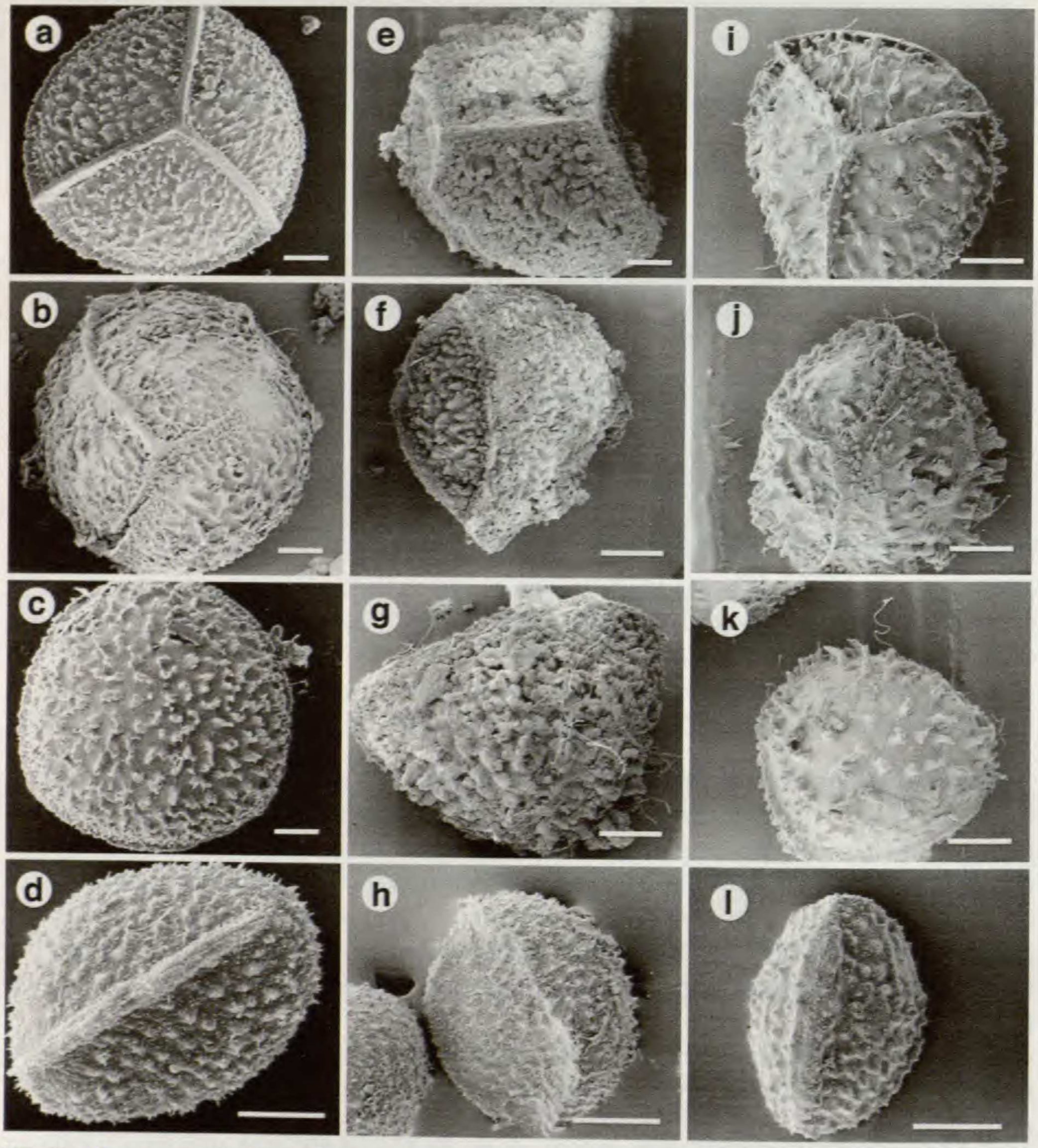

FIG. 2. Spore morphology of *Isoetes occidentalis* (left column), *I. maritima* (right column) and their interspecific hybrid *I. ×truncata* (center column). Voucher specimens are at OAC. a–d) *I. occidentalis* (a, b, d: Auke Lake, *S. S. Talbot 920818*; c: Lake de Marie, Adak Island, *S. & S. Talbot 50B*). e–h) *I. ×truncata* (Lake Leone, Adak Island, *S. & S. Talbot 52*). i–l) *I. maritima* (Palisades, Adak Island, *S. & S. Talbot 53B*). a, e, i) Proximal view of megaspore. b, f, j) Lateral view of megaspore. c, g, k) Distal view of megaspore. d, h, l): Microspore. Scale bars = 100 μm for megaspores, 10 μm for microspores.

(Fig. 3i) and the lateral view (Fig. 3j) exposes a distinct girdle that is unmarked or ornamented with very short, narrow spines. The microspores are similar to those of *I. occidentalis*, with papillae and subdued spines.

The spores of *I. ×truncata* (Figs. 2e–h) are so misshapen that it is difficult to obtain an aesthetically pleasing photo. All are distorted and present a lab-

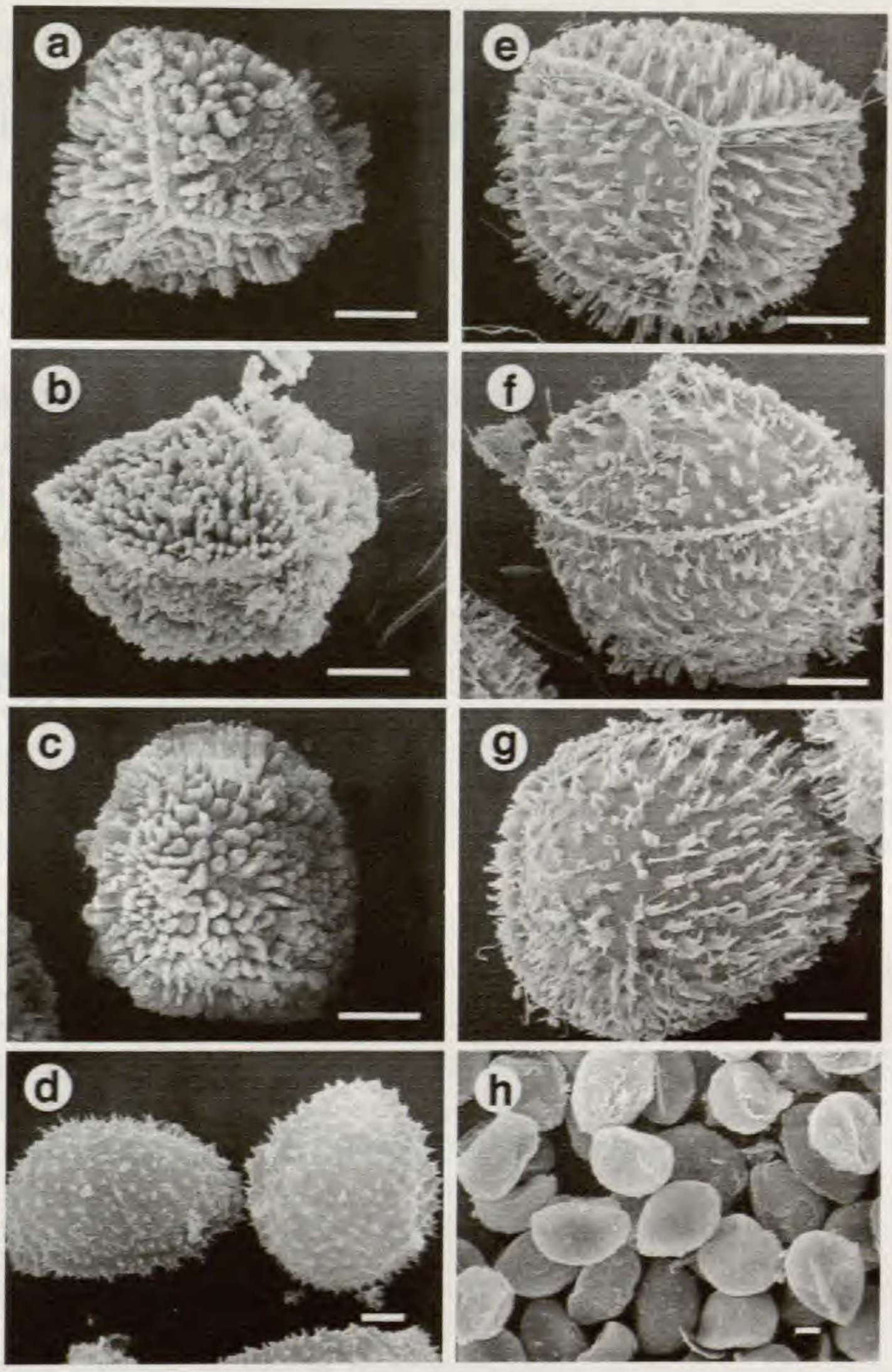

FIG. 3. Spore morphology of the interspecific hybrid *Isoetes ×pseudotruncata* (left column) and *I. echinospora* (right column) from Alaska (the other parent, *I. maritima,* is illustrated in Fig. 2i–l). Voucher specimens are at OAC. a–d) *I. ×pseudotruncata* (Mosquito Lake, Kenai NWR, *S. S. Talbot 6A3-1*). e–h) *I. echinospora* (Lake 23, Kenai NWR, *S. S. Talbot 19A3-4A*). a, e) Proximal view of megaspore. b, f) Lateral view of megaspore. c, g) Distal view of megaspore. d, h) Microspores. Scale bars = 100 μm for megaspores, 10 μm for microspores.

yrinth of rough spines, which give the megaspores a "brain coral" appearance at high magnification. This is the polymorphic spore pattern found in all *Isoetes* hybrids (variable in size, shape, and ornamentation; often with "brain-coral" pattern; many megaspores broken and aborted). The microspores (Fig. 2f) have reduced papillae, but are still far from smooth in appearance.

One interesting cytological result concerned a large collection of 48 plants from the Kenai Peninsula, Lake #6 (Mosquito Lake) (*Talbot 6A3-1*). Thirty-nine of the plants had $2n=33$. Their megaspores varied in size, were usually flattened and had "brain-coral" ornamentation. The remaining nine plants were

very small and immature. One or two suggested *I. maritima* in appearance and some had flexuous leaves as in *I. echinospora* in Alaska. The unsolved problem, however, is why there were so many hybrid plants if hybrids are indeed unable to reproduce sexually. Representative spores of the 39 plants, which are referable to *I. ×pseudotruncata,* are shown in Figs. 3e–h. The spores of *I. echinospora,* a parent species of *I. ×pseudotruncata* (Britton and Brunton, 1996), are shown in Figs. 3e–h. For comparative purposes one should visualize *I. maritima* in Figs. 2i–l to the left of *I. ×pseudotruncata.*

Key features in Fig. 3 include the long, thin spines of *I. echinospora,* which extend to the equator (no girdle). *Isoetes echinospora* microspores (Fig. 3h) are very smooth in contrast to those of *I. ×pseudotruncata* (Fig. 3d). The thickened megaspore spines again show the striking "brain-coral" pattern under high magnification (Figs. 3a–c).

We have constructed a key to distinguish the five taxa of *Isoetes* in Alaska, based almost exclusively on characters observed and/or measured on the cytologically confirmed Alaskan material noted in Materials and Methods above.

1. Plants larger than associated plants with uniformly-shaped spores, found in mixed populations with one or both putative parents; megaspores polymorphic; interspecific hybrids
   2. Intact megaspores ca. 560 μm (± 45 μm); ornamentation congested and with ± short spines . . . . . ***I. ×truncata*** (2*n*=55)
   2. Intact megaspores ca. 400 μm (± 40 μm); ornamentation densely congested and with ± tall spines . . . . . ***I. ×pseudotruncata*** (2*n*=33)
1. Plants and their megaspores ± uniform in size and shape, found in pure or mixed populations; sexual plants, not of hybrid origin
   3. Leaves succulent, thick; megaspores 605 μm (± 45 μm); microspores 35–45 μm . . . . . ***I. occidentalis*** (2*n*=66)
   3. Leaves wiry, thin; megaspores < 550 μm; microspores 30–40 μm
      4. Megaspores 490 μm (± 30 μm), with short, blunt spines; prominent equatorial girdle present, smooth or marked with very short, thin spines; microspores papillose, 30–40 μm . . . . . ***I. maritima*** (2*n*=44)
      4. Megaspores 400–500 μm with long, sharp-ended spines; equatorial girdle not present; microspores smooth, 20–30 μm . . . . . ***I. echinospora*** (2*n*=22)

The distribution in Alaska of 13 localities from 17 records of *I. occidentalis* and 21 localities from 34 records of *I. maritima,* as well as two localities of their interspecific hybrid, *I. ×truncata,* are shown in Fig. 4. In Fig. 5, the distribution of *I. maritima* is presented again, as are seven localities from 14 records of *I. echinospora* and one locality from three records of their interspecific hybrid, *I. ×pseudotruncata.* Harms (1966) noted the difficulty in determination of the collections from Harding and George Lakes east of Fairbanks. Cytological data and SEM photos have clarified the differences among the taxa present at these locations.

Discussion

Isoetes in Alaska is considered at this time to include three species and two interspecific hybrids. In all, there are five levels of ploidy (diploid to hexaploid), with one taxon at each level. The taxon that is missing in this scenario is the tetraploid interspecific hybrid, *I. echinospora* (diploid) × *I. occidentalis*

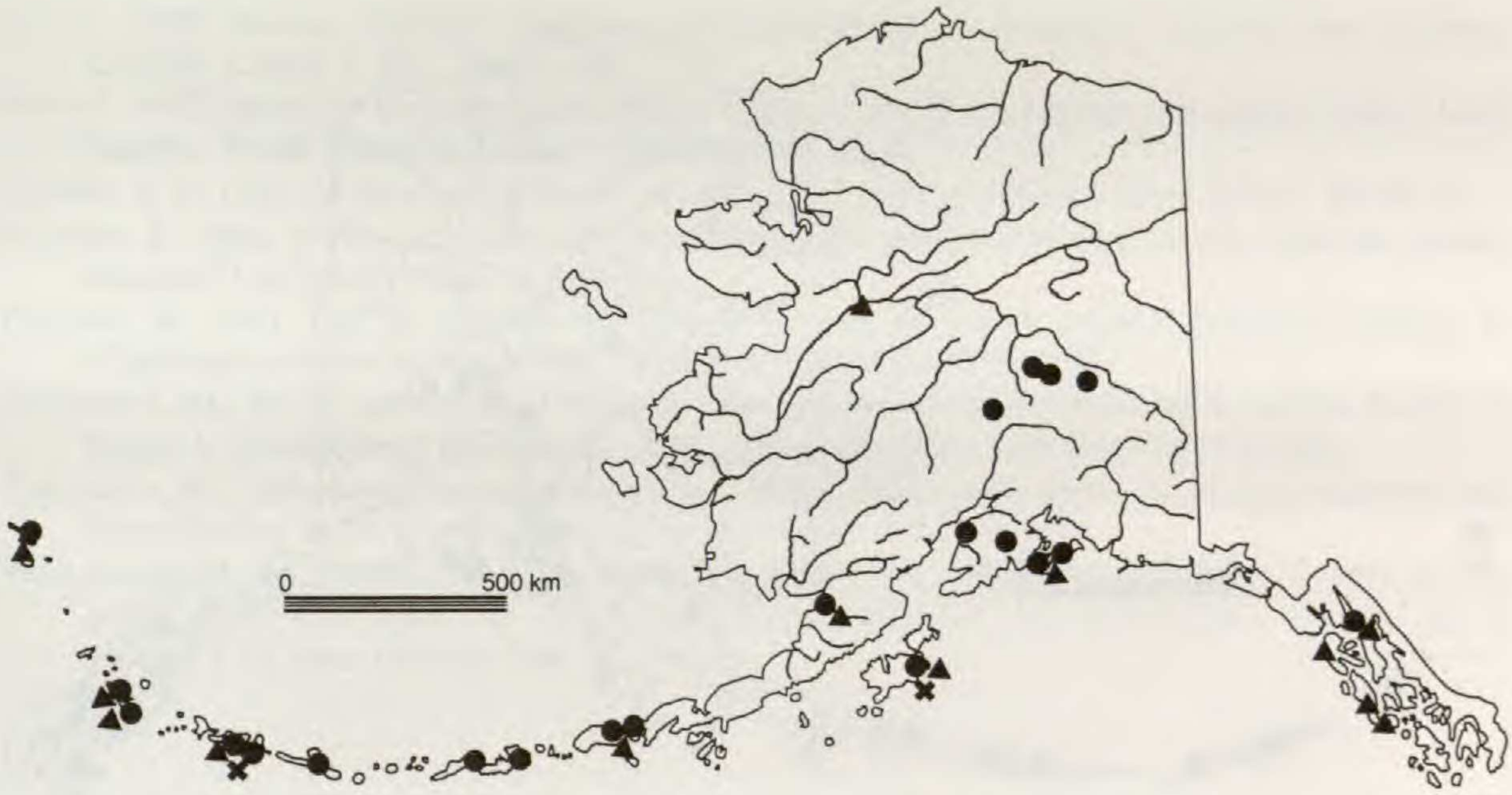

FIG. 4. Locations of representative populations documenting the distributions of *Isoetes maritima* (circles), *I. occidentalis* (triangles), and their interspecific hybrid, *I.* ×*truncata* (crosses), in Alaska.

(hexaploid). The most likely locations to initiate a search for this hybrid would be the seven localities shown for *I. echinospora* in Fig. 5 or the 13 localities shown for *I. occidentalis* in Fig. 4. At this time, however, we know of no localities in Alaska where both species have been found together.

The tetraploid *I. maritima* has a broad band of distribution that extends from Washington state along the coast of British Columbia and westward to at least the end of the Aleutians archipelago. The species is also apparently disjunct inland in British Columbia and Alberta (Britton and Brunton, 1993) and in Alaska at the lakes east of Fairbanks (Fig. 4). It is much more frequent in Alaska than *I. echinospora* (Fig. 5), which has only 7 localities mapped from 12 records. The latter species is best known from records in the Alaskan panhandle and on the Kenai peninsula. It is not known from the Aleutian Island archipelago.

Impressive biosystematic studies have been undertaken of related *Isoetes* taxa in Japan by Takamiya and his associates. Takamiya et al. (1994) considered the somatic chromosome numbers present in Japanese *Isoetes* and delineated both euploids and aneuploids. Watanabe et al. (1995) studied spore morphology from SEM and the measurements of spores. Takamiya et al. (1996) examined meiosis for all the cytotypes in both microspore and megaspore formation. It will be interesting to see the taxonomic conclusions resulting from these investigations, particularly as they relate to North American taxa. For example, is the Asian *I. asiatica* conspecific with North American *I. echinospora*?

It is surprising that none of the five taxa now known from Alaska have been reported west of the international date line, especially when *I. maritima* and *I. occidentalis* are found on the outermost Aleutian Islands (Fig. 4). The dip-

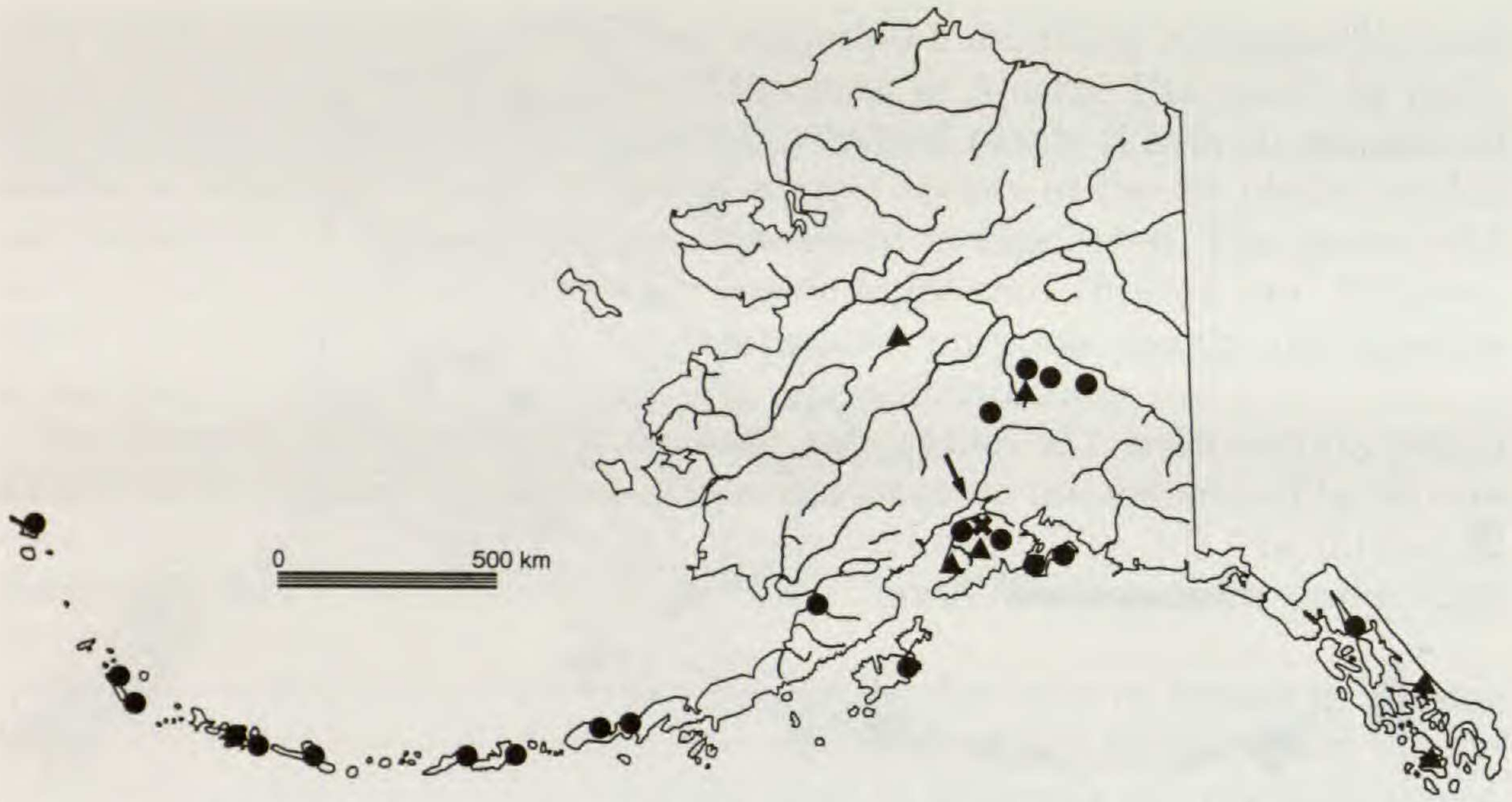

FIG. 5. Locations of representative populations documenting the distributions of *Isoetes maritima* (circles), *I. echinospora* (triangles), and their interspecific hybrid, *I.* ×*pseudotruncata* (cross), in Alaska.

loid *I. asiatica* and *I. beringensis* (tetraploid?), however, are considered to constitute the only *Isoetes* species of Asian Beringia. Pietsch (1991) states that *I. asiatica* was studied in 24 lakes and *I. beringensis* in 8 lakes in the southeast of Sakhalin Island. He also reports previously studying *I. asiatica* in eight lakes and *I. beringensis* in four lakes on the Kamchatka peninsula.

Hultén (1968) considered *I. beringensis* to be a synonym of *I. maritima*. We have examined the type collection of *I. beringensis* (LEN) and also consider that it represents *I. maritima*. Although the material was quite variable and plants on the holotype sheet had been attacked by fungi, some well-formed megaspores and microspores were available for examination with a light microscope and documentation with SEM (Britton and Brunton, 1993).

The occurrence of *I. occidentalis* in Asia, on the Kamchatka peninsula, and/or on islands along the western edge of the Bering Sea, is likely. The presence of *I.* ×*truncata* would also be a possibility at such sites. Similarly, the triploid hybrid *I. asiatica* × *maritima* (*I.* ×*pseudotruncata*, if *I. asiatica* is established to be conspecific with *I. echinospora*) may well be found on the Kamchatka peninsula, Sakhalin Island, and/or the Kurile Island chain of the Asian Beringia. In addition, investigation of possible subspecific variation between Asian and North American populations along the Aleutian Island archipelago may assist in clarifying the apparent contradiction between Old World and New World interpretation of Beringian *Isoetes*.

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