THE GENUS PIKEA (DUMONTIACEAE, RHODOPHYTA) IN ENGLAND AND THE NORTH PACIFIC: COMPARATIVE MORPHOLOGICAL, LIFE HISTORY, AND MOLECULAR STUDIES

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ABSTRACT

A Pikea species attributed to Pikea californica Harvey has been established in England since at least 1967. Previously, this species was believed to occur only in Japan and Pacific North America. Comparative morphological studies on field-collected material and cultured isolates from England, California, and Japan and analysis of organellar DNA restriction fragment length polymorphisms, detected using labeled organellar DNA as a non-radioactive probe, showed that English Pikea is conspecific with P. californica from California. Both populations consist of dioecious gametophytes with heteromorphic life histories involving crustose tetrasporophytes; 96% of organellar DNA bands were shared between interoceanic samples. A second dioecious species of Pikea, P. pinnata Setchell in Collins, Holden et Setchell, grows sympatrically with P. californica near San Francisco but can be distinguished by softer texture, more regular branching pattern, and elongate cystocarpic axes. Pikea pinnata and P. californica samples shared 49-50% of organellar DNA bands, consistent with their being distinct species. Herbarium specimens of P. robusta Abbott resemble P. pinnata in some morphological features but axes are much wider; P. robusta may represent a further, strictly sub-tidal species but fertile material is unknown. Pikea thalli from Japan, previously attributed to P. californica and described here as Pikea yoshizakii sp. nov., are monocious and show a strikingly different type of life history. After fertilization, gonimoblast filaments grow outward through the cortex and form tetrasporangial nemathecia; released tetraspores develop directly into erect thalli. Tetrasporoblastic life histories are characteristic of certain members of the Phyllophoraceae but were previously unknown in the Dumontiaceae. Japanese P. yoshizakii shared 55 and 56% of organellar DNA bands with P. californica and P. pinnata, respectively; phylogenetic analysis indicated equally distant relationships to both species. Pikea yoshizakii or a closely similar species with the same life history occurs in southern California and Mexico.

Key index words: Dumontiaceae; introduced algae; Pikea: Pikea californica; Pikea pinnata; Pikea yoshizakii sp. nov.; organellar DNA RFLPs; Rhodophyta; taxonomy

An abundant population of a Pikea species attributed to Pikea californica Harvey (1853:246) was discovered in 1983 in the Isles of Scilly, Cornwall, where it has been established since at least 1967 (Maggs and Guiry 1987). This seaweed is regarded as an alien in the British Isles, and further release is prohibited under Schedule 9 of the Wildlife and Countryside Act 1981; it is therefore important to clarify its identity and, if possible, determine its provenance. The identification as P. californica by Maggs and Guiry was based on the morphology of cystocarp-bearing branches, which had swollen spindle-shaped tips containing numerous small cystocarps, as in Californian material of P. californica (Abbott 1968). In contrast, the cystocarps of P. robusta Abbott (1968:180), the only other species of Pikea recognized at the time, were reported to occur in small rounded wart-like nemathecia (Abbott 1968). Prior to the discovery by Maggs and Guiry (1987), the genus Pikea was believed to be confined to the Pacific Ocean, in Japan and from Alaska to Baja California, Mexico (Lindstrom and Scagel 1987). In various taxonomic treatments, the number of Pikea species recognized varies according to the authors' interpretation of the observed morphological variability. Abbott (1968) considered that P. pinnata Setchell in Collins, Holden et Setchell (1899:648) could "be placed easily within the limits of Pikea californica" but that P. robusta was a distinct species. In contrast, Lindstrom and Scagel (1987) regarded P. pinnata and P. robusta as conspecific and suggested that the typical morphologies exhibited by P. pinnata-robusta (branching regularly pinnate; main axes to 4 mm in width) and P. californica (branching irregular; main axes more cylindrical) probably represented young and older thalli of a single species, the name P. californica having priority. They noted, however, that critical field observations or experiments should be carried out to verify their conspecificity.

Although Maggs and Guiry (1987) suggested that the English population of P. californica could have been introduced from California by wartime flying boats, there was only circumstantial evidence to support this hypothesis. It could instead have originated in Japan, from which there is a well-established pattern of seaweed introductions into Europe (Farnham 1980). Equally, its North Atlantic distribution could be relictual. There are thus four possible interpretations of the presence of P. californica in the British...
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Isles: it has been introduced 1) from California or 2) from Japan; 3) either a Japanese or a Californian original population has given rise to both the other Pacific population and the Atlantic population; or 4) it is a relict of a previous wider distribution. An understanding of the provenance of the British population could provide evidence for the transport vectors involved in its introduction. Life history data were expected to be important because contrasting life histories of *P. californica* have been reported from Japan and California. Scott and Dixon (1971) demonstrated a heteromorphic life history involving a crustose tetrasporophyte for seven Californian isolates, whereas in Japan Chihara (1972) found a direct-type development of carpospores into erect gametophytes. Maggs and Guiry were unable to examine the life history of the English population because mature cystocarps were not present in collections made in 1983 and 1984.

In higher plants, restriction site analysis of chloroplast DNA is now the most popular technique in systematics for phylogenetic reconstruction below the family level, partly due to its conservation rate of evolution (Soltis et al. 1992). In the algae also, differences in organellar DNA restriction patterns have been used to investigate the systematics of red algae (Goff and Coleman 1988, Parsons et al. 1990, Maggs et al. 1992). Restriction fragment length polymorphisms (RFLPs) have rarely been compared quantitatively, however, due to the general belief that there is insufficient interspecific similarity between banding patterns; chloroplast genomes of even relatively closely related taxa are reported to be highly divergent (Manhart and McCourt 1992). Nevertheless, phylogenetic analysis can be valid if more than 25% of fragments are shared (Dowling et al. 1990). The results obtained in the *Gracilaria verrucosa* (Hudson) Papenfuss complex using RFLP analysis (Rice and Bird 1990) correspond well with relationships inferred from 18S sequence data (Bird et al. 1992).

In the present study, we address the question of the identity and origin of the English *Pikea* population by comparing English, Californian, and Japanese field-collected material and cultured isolates, in terms of morphology, life history, and organellar DNA RFLPs.

**MATERIALS AND METHODS**

In England, *Pikea californica* thalli were sampled randomly in the shallow sublittoral zone at Gap Point, St Mary's, Isles of Scilly, Cornwall (Table 1), and mailed to Belfast overnight in damp cloth. The individual thalli, weighing 1.0–13.4 g, were frozen separately in liquid nitrogen and kept at −70°C. *Pikea californica* was collected intertidally in California (Table 1) at Duxbury Reef, near Bolinas, Marin County, and at Lime Point, north end of the Golden Gate Bridge, Marin County (all other Californian locations mentioned here are shown in Abbott and Holllenberg 1976: 10–13). The Lime Point collection also included one thallus of *P. pinnata*. Californian material was either dried immediately in silica gel (Chase and Hills 1991) or kept on ice and transported alive back to Belfast. In Japan, *Pikea* sp. was collected by M. Yoshizaki in the shallow sublittoral at Inubo-saki, Choshi Peninsula, on 26 August 1993, and a sample was dried in silica gel. Representative material of all collections was fixed in 4% seawater formalin, and herbarium specimens have been deposited in the Natural History Museum, London (BM). Morphological studies were carried out on live or formalin-fixed material. Sections made by hand were either stained with 1% aqueous anilin blue, rinsed, post-fixed in dilute HCl, and mounted in 60% Karo corn syrup, or placed directly in Wittman's hematoxylin (Wittman 1965) to stain nuclei. Herbarium specimens from US, UC, NCU, and BM (herbarium abbreviations as in Holmgren et al. 1990) were examined. Small fragments were removed, rehydrated in formalin-seawater, and treated like formalin-fixed material.

**Cultured isolates.** Cultures of *P. californica* from California and the Isles of Scilly were initiated from clean cystocarpic tips left in sterile seawater to release carpospores; these were pipetted onto glass slides in fresh petri dishes. *Pikea pinnata* was grown from segments of mature axes that had been surface-sterilized by soaking for 2 min in 1% bleach in seawater. Japanese *Pikea* cultures were isolated from clean fertile tips that were grown for 14 d in enriched seawater until spores were released and from gametophytic tips repeatedly excised until unialgal. All isolates were grown in modified von Stosch's medium (Guiry and Cunningham 1984) at 15°C, 14:10 h LD, unless otherwise stated, at ca. 20 amol photons m⁻² s⁻¹.

**DNA extraction.** Frozen or silica gel-dried algal samples, 2.5–5 g fresh weight, were ground to a fine powder in 1 mL of extraction buffer (100 mM Tris pH 8, 50 mM NaCl, 50 mM EDTA, 0.6% SDS) using a liquid nitrogen-chilled mortar and pestle. The powder was mixed with 14 mL extraction buffer, 15 μL RNase A (10 mg mL⁻¹), and 75 μL proteinase K (20 mg mL⁻¹) and incubated in a waterbath at 57°C for 1.5 h. Samples were phenol–chloroform–extracted (Sambrook et al. 1989), and the DNA was precipitated from the final aqueous phase with 0.1 volume 7 M sodium acetate, pH 7.0, and 2.5 volumes prechilled 100% ethanol. The floating DNA precipitate was removed using a sterile glass loop, washed with prechilled 70% ethanol, air-dried for 50 min, and resuspended overnight at 4°C in 2–4 mL sterile water.

**Organellar DNA separation.** Organellar DNA was separated from nuclear DNA on Hoescht–cesium chloride gradients. Cesium chloride was dissolved in the DNA solution to give a refractive index of 1.40 and Hoescht 33258 added to a final concentration of 160 μg mL⁻¹. The gradients were formed in 3.8-M cesium chloride tubes centrifuged (Beckman TL-100) in a vertical rotor at 100,000 rpm for 3 h at 20°C. Two well-separated bands were present on the gradients (Fig. 1). The heavy lower band apparently consisted principally of nuclear DNA that formed a continuous smear after restriction digestion and electrophoresis on agarose gels. The less dense upper band was composed largely of organellar DNA, with variable amounts of nuclear DNA contamination, and produced distinct patterns of restriction fragments after digestion and electrophoresis (Fig. 55). The upper band on the gradient was removed by side-puncture while illuminated with ultraviolet light. The DNA was precipitated by the addition of 5 volumes sterile water and 2.5 volumes prechilled 100% ethanol.

After incubation overnight at −20°C, the organellar DNA pellet was collected by centrifugation at 8000 rpm for 20 min at 4°C, washed with prechilled 70% ethanol, air-dried for 1 h, and re-suspended overnight at 4°C in 100 μL sterile water. The volume of DNA solution for each digest was determined empirically by digesting and electrophoresing 10–50 μL aliquots. Each 3–5-g sample produced enough organellar DNA for about 10 restriction digests, but in some cases the organellar DNA restriction fragment patterns were partially obscured by contaminating nuclear DNA. To allow more digests and to produce better resolved RFLPs, we used a hybridization technique developed for our study of the genus *Ceramium* (Maggs and Ward 1993). The least contaminated
organellar DNA preparations were labeled and used to probe Southern blots of small quantities of digested organellar DNA. Restriction endonuclease digestion, Southern transfer, and hybridization. Organellar DNA aliquots (2–10 μL) were digested with the 6-base cutting endonucleases EcoRI, PstI, PvuII, BglII, HindIII, and KpnI using standard techniques (Sambrook et al. 1989), except that 1 μL 100 mM spermidine was added to each 20 μL reaction mixture to aid digestion. Each digest was replicated, and no differences were observed between runs. Restriction fragments were separated on 0.5% agarose gels (containing 300 μg/mL of digested organellar DNA. Probes were prepared by nonradioactive labeling using the Digoxigenin DNA labeling and detection kit (Boehringer Mannheim), as detailed in the manufacturer’s instructions. Organellar DNA probes were prepared by nonspecific labeling using the Digoxigenin DNA labeling and detection kit (Boehringer Mannheim), as detailed in the manufacturer’s instructions. Each 20 μL aliquot of organellar DNA solution provided 50 μL of probe, sufficient to label approximately 1000 cm² of filter. The filters were hybridized and detected according to the manufacturer’s protocol except that they were prehybridized for 4 h at 55°C in 0.5x SSC, 0.1% SDS. Sizes of the fragments were determined using Digoxigenin-labeled and unlabelled size markers run on each gel.

RESULTS

Pikea californica from the Isles of Scilly. In 1983, Pikea californica was distributed throughout the five inhabited islands and numerous rocks and islets comprising the archipelago of the Isles of Scilly (Magg and Guiry 1987: fig. 1). It was most abundant in the Western Rocks, which are fully exposed to the southwesterly swell, where it was often the dominant species from around extreme low water to 3 m below Chart Datum. It also occurred in smaller quantities at some less exposed sites and to a depth of 1.4 m. In October 1988, P. californica was common near Gap Point, a relatively sheltered site on the east coast of St. Mary’s; in February 1993, P. californica still occurred in abundance at this site. From these observations, the population appeared to be winter-fertile. Immature reproductive structures were present at the beginning of October and cystocarps matured between November and February (Table 1). The thalli collected in February 1993 were closely similar, both vegetatively and reproductively, to those from Scilly in 1983 and 1984, described by Maggs and Guiry (1987).

Carpospores, 10–13 μm in diameter, were released in large numbers from fertile cystocarps axes by virtual dissolution of the pod-like tips. They germinated by division into two cells that gave rise by pseudodichotomous branching to compact discoid thalli up to 50 μm in diameter after 8 d (Fig. 2). After 16 d the discs were several cells thick in the centers (Fig. 3), and by 60 d they were 1.1 mm across. Crustose thalli (Fig. 4) formed numerous secondary pit connections in the basal layer and between cells of the erect filaments by fusion of nucleate conjunctor cells, resulting in multinucleate vegetative cells (Fig. 5). Occasional cell fusions also took place in the basal layer (Fig. 6) and between cells of erect filaments. Poorly attached spores developed into filaments (Fig. 2) and later into loosely anchored callus-like growths (Fig. 8). The filaments were sparsely to profusely branched, with little-pigmented conjunctor cells (Fig. 7) that formed secondary pit connections to other filaments when these were close enough to be contacted; occasional cell fusions also took place (Fig. 7).

Two replicate cultures were transferred at 30 d from 15°C 14:10 h to 15°C 8:16 h and then moved at 60 d to 10°C 8:16 h. Ten days later, callus-like thalli in these cultures had formed tetrasporangia

![Fig. 1. Cesium chloride gradients of two Pikea DNA samples, P. californica from Duxbury Reef, California (left), and P. pinnata from Lime Point, California (right), showing good separation of upper organellar DNA band and lower nuclear DNA band.](image-url)
Figs. 2–12. Life history of *Pikea californica* from Isles of Scilly, England. Fig. 2. Germinating carpospores 8 d after release, forming compact discs (right) or poorly attached filamentous growths (left). Fig. 3. Young tetrasporophyte crusts 16 d after release of carpospores. Fig. 4. Crustose tetrasporophytes. Fig. 5. Basal cell layer of tetrasporophyte, stained with Wittman’s hematoxylin, showing nucleate conjunctor cell fused to neighboring cell (arrow) and resulting multinucleate cells (arrowhead). Fig. 6. Basal cell layer of tetrasporophyte, with conjunctor cell (arrow) and direct cell fusions (arrowhead). Fig. 7. Loosely attached tetrasporophyte filament with conjunctor cell (arrowhead) and direct cell fusion (arrow). Fig. 8. Callus-like spherical tetrasporophytes after transfer to short days, surrounded by germinating tetraspores. Fig. 9. Squash of callus-like tetrasporophyte with terminal mature tetrasporangia (t) and developing tetrasporocyte (tc). Fig. 10. Gametophytes, grown from tetraspores released in culture, consisting of extensive basal discs with young erect axes. Fig. 11. Sparsely branched gametophytes grown at 10° C, 8:16 h. Fig. 12. Gametophyte tips grown at 15° C, 16:8 h, showing wider axes and more regular branching than those in Figure 11.
and released spores (Fig. 8), but crustose thalli remained nonreproductive. Neither callus-like nor crustose thalli in four replicate cultures kept at 14-h days became fertile. Tetrasporangia were formed terminally on erect filaments (Fig. 9), surrounded by elongate, little-pigmented apical cells of other erect filaments. Mature tetrasporangia, 15–21 μm long and 8–13 μm wide, were irregularly cruciately divided (Fig. 9) and released tetraspores 6–8 μm in diameter.

Tetraspores grown at 10° C 8:16 h developed into discs, each of which formed a centrally positioned erect axis after 55 d. Thalli grew faster after transfer to 15° C 14:10 h and formed rings of pinnately branched, uniaxial, erect axes (Fig. 10). The internal fishbone pattern characteristic of *Pikea* axes (Maggs and Guiry 1987:figs. 6, 7) was visible through the cortex. Branching pattern varied somewhat depending on growth conditions but was essentially irregularly pinnate. Tips maintained at 10° C 8:16 h grew into sparsely branched thallii with main axes up to 0.5 mm in width (Fig. 11), whereas those transferred to 15° C 16:8 h formed main axes to 0.9 mm wide and branched more regularly and frequently (Fig. 12). Reproduction was not observed in erect thalli under any conditions, although they were subjected to various transfers between long-day conditions at 15° C and 8- or 4-h days at 10° and 15° C.

*Pikea californica* from California. Thalli sampled from a small population of *Pikea californica* in a wave-exposed gulley near extreme low water of spring tides at Duxbury Reef (Table 1) generally had reproductive structures of fertile thallii that were confined to the branches borne by a few older major axes, whereas the bulk of each thallus was not obviously reproductive. Thalli (Fig. 13) were 4–13 cm high and to 15 cm broad and consisted of groups of erect axes of different ages attached by solid discoid holdfasts up to 12 mm in diameter. They were bushy and three-dimensional rather than complanate, branched to at least eight orders, with a tough cartilaginous texture. The main axes were 2.0–2.9 mm wide at the base, often denuded of branches below, then branched sparsely and irregularly dichotomously, becoming very densely branched distally in an irregularly pinnate pattern, with a tendency to second branching near tips, the main axis curving away from the ultimate branches (Fig. 14). The typical fishbone pattern was clearly visible toward tips where major axes were up to 0.9 mm wide. Although branching was much denser than in the lectotype of Harvey's *P. californica* (Maggs and Guiry 1987:fig. 5) and the tips were narrower, the irregularity of the branching pattern corresponded well to Harvey's material, and the Duxbury Reef samples were identified accordingly as *P. californica*.

The site of our second collection, Lime Point near the north end of the Golden Gate Bridge, approximates the type locality of *P. californica*. Harvey indicated only that his collection was from Golden Gate (the broad channel into San Francisco Bay), without specifying a particular locality. At Lime Point, a small patch of *P. californica* was growing on a boulder at ca. 60 cm above extreme low water level, among dense foliose algae. Thalli collected (Table 1) were 4–11 cm high and 2–16 cm broad, with main axes ca. 2 mm wide and young axes to 1.2 mm in diameter (Fig. 15). Main axes were denuded only near the holdfast. One juvenile thallus, which had only a single erect axis, had a similar branching pattern to the mature thallus, although its overall shape was narrower. The Lime Point collection was more similar to the lectotype of *P. californica* than were the Duxbury Reef thalli because young axes were wider, with more secund branching. Harvey's specimens were obviously collected in the drift, and allowing for the loss of some branches by wave damage one of the erect axes of our Lime Point thallii (Fig. 15) quite closely resembles the single erect axis of the holotype.

Reproductive structures were similar in both our collections. Spermatangial sori (Fig. 16) extended over the surface of axes of the last three orders of branching, in older parts of the thallus. They were visible to the naked eye in fresh and liquid-preserved material because they were paler than the vegetative cortex and could be seen as glossy patches on herbarium material. Spermatangia, cut off obliquely in pairs by spermatangial mother cells, were 6.5–9 μm long and 3–4 μm wide. Elongated pigmented sterile cortical cells were intermixed with fertile spermatangial mother cells.

Carpogonial branches were of variable length, and the terminal five cells were strongly recurved with twisted trichogynes (Fig. 21). Lateral cells were frequent on the lower cells of the carpogonial branch; these cells were intermediate in staining qualities between the upper and the lower carpogonial branch cells. The five terminal carpogonial branch cells were larger than the other cells, wider than high, and contained enlarged darkly staining nuclei (Fig. 21). Auxiliary cell filaments were similar in shape to carpogonial branch filaments. They were variable in length and in the sizes and shapes of cells and usually bore short lateral filaments (Fig. 22). The terminal five to seven cells were strongly differentiated, being larger, more darkly staining, and with enlarged granular nuclei. The auxiliary cell itself, usually two to three cells from the end of the filament, was distinguishable from the other enlarged cells by the homogeneous cytoplasm and clear nucleus with a smaller nucleolus (Fig. 22). Auxiliary cells were ovoid, 12–14 μm long by 15–19 μm wide. Postfertilization development was not studied in detail. Connecting filaments, occasionally branched, were seen in connection with auxiliary cells (Fig. 17), and gonimoblast initials arose in the region of the fusion between auxiliary cell and connecting filament (Fig. 23). Mature cystocarpic tips (Fig. 18) were broad and irregularly swollen, with cystocarps extending...
from the penultimate order of branching into the small last-order branches, and contained numerous small cystocarps up to 200 μm in diameter.

Carpogores from the Duxbury Reef thalli were released by female tips with mature cystocarps by degeneration of the pod-like fertile axes. Germination and development (Fig. 19) were as for the English isolate. One replicate culture including sev-
Fig. 21. Immature 18-celled carpogonial branch with short lateral branches (arrowheads) and enlarged differentiated terminal five cells (c = carpogonium, h = hypogynous cell).

Fig. 22. Auxiliary cell branch, bearing short lateral branches (arrowheads), with terminal five cells strongly differentiated from the other cells by their larger nuclei and more densely stained cytoplasm. Auxiliary cell (a) is distinct, with a clear rather than granular nucleus.

Fig. 23. Auxiliary cell branch after auxiliary cell (a) has been contacted by connecting filament, seen as incoming filament (arrowhead) and ongrowing filament with gonimoblast initial (gi).

Typical specimens of this species have been collected from central California (Marin County) southward to San Luis Obispo. No herbarium material has been seen from south of Point Conception; all southern Californian specimens are attributable to other species.

Pikea pinnata. At Lime Point, in addition to the patch of Pikea californica on a boulder, a separate group of Pikea thalli was observed on the vertical face of a very large rock, a slightly more sheltered habitat than the boulder. The single, male thallus collected from this group corresponded to the type material of Pikea pinnata (Fig. 24), the type locality for which is Fort Point, at the south end of the Golden Gate Bridge, so it was provisionally identified as this species. Our specimen (Fig. 25) was 16.5 cm high and very bushy; the texture, particularly of young axes, was much softer and more flexible than in P. californica from the same site. The thallus consisted of a single main axis up to 1.8 mm wide, terete basally and compressed above, bearing a regularly pinnate distichous array of second-order branches, each of which was regularly or irregularly pinnately branched to five further orders of branching (Fig. 26). Main axes near the apices bore dense regular opposite-distichous fringes of elongate last-order branches. A fishbone pattern similar to that seen in Pikea californica was visible only in young axes. In transverse section (TS) (Fig. 27), the central and lateral axial filaments were 80–110 μm in diameter, surrounded by dense intertwined rhizoidal filaments. Mature axes in TS consisted of a solid mass of rhizoidal filaments 8–50 μm in diameter.

Developing spermatangial sori were continuous over the last two orders of branching in parts of the thallus. A few mature spermatangial mother cells bore pairs of ovoid spermatangia measuring ca. 9 × 4 μm that were interspersed with elongate sterile cortical cells ca. 14 μm long and covered with a layer of mucilage to 13 μm thick (Fig. 28). Female thalli were observed only in dried herbarium material. Carpogonial branches and developing gonimoblasts were present in both of two thalli collected at Fort Point on 18 November 1926 by K. M. Drew (Drew #1065 in BM, as Pikea sp.). Mature cystocarps were evident only in collections made in February: Setchell's type collection, from Fort Point, 18 February 1898; and Bolinas, Marin County, collected by Yamada in February 1929, in UC. Female reproductive structures were formed in elongate, pod-like penultimate branches (Fig. 29). Carpogonial branches were strongly recurved and closely similar to those of P. californica. Some evidence of postfertilization development was noted but not examined in detail. Developing and mature cystocarps extended along the fertile branches, sometimes appearing to be borne in two rows (Fig. 29).

A vegetative isolate from Lime Point grown in culture formed long unbranched main axes bearing regularly pinnate short lateral branches (Fig. 30) and differed from P. californica grown under the same conditions in the lack of main axis dichotomies. Herbarium specimens identifiable with certainty as P. pinnata were collected only in the San Francisco area (from the lower intertidal zone at Fort Point, Lime Point, and Duxbury Reef), and this species may have a restricted geographical distribution.
Fig. 24–30. Field-collected and cultured *Pheoa pinnata* from California. Fig. 24. Isotype (BM) from Fort Point, with regular distichous branching along main axis. Figs. 25–28. Field-collected specimen from Lime Point. Fig. 25. Complete thallus with regularly distichous branches along main axis and bushy higher order branching. Fig. 26. Single major branch to show higher order branching. Fig. 27. TS of young axis showing large axial filament and four major lateral filaments in plane of compression. Fig. 28. TS through immature spermatangial sorus. Fig. 29. Branches of fertile female with developing cystocarps, Fort Point, 18 November 1926, K. M. Drew #1065, BM. Fig. 30. Cultures from axial segments of Lime Point thallus grown at 15°C, 16:8 h.
ever, a clear line of demarcation could not be drawn between *P. pinnata* and *Pikea robusta*. Fresh material of *P. robusta* has not been available for study. Herbarium material attributed to this species (Fig. 31) is regularly pinnately branched and highly morphologically variable, with main axes varying from 1.5 to 4 mm in width. Female reproductive material is unknown: the structures previously identified as cystocarps are vegetative wart-like growths (Fig. 32) with a loose filamentous construction (Fig. 33). Examination of herbarium collections indicates that this species may be exclusively subtidal and occurs to a depth of at least 20 m (UC 1454971, dredged from the south end of Monterey Bay, 25 July 1966 by D. P. Abbott). *Pikea robusta* appears to have an extensive geographical distribution, at least from Oregon (UC 552419, Squaw Island, 28 June 1926) to Pacific Mexico (US 85140, Punta Cabras, 10-m depth, 31 August 1980, W. J. North).

*Pikea from Japan.* One complete *Pikea* thallus and several fragments collected at Choshi initially appeared to be cystocarpic. When spores were released in culture, they originated from external tetrasporangial nemathecia growing over gametophytic tissue. The tetraspores grew directly into erect thalli. This species of *Pikea* differs strikingly, both morphologically and in its life history, from all other members of the genus, and it is here proposed as a new species, *Pikea yoshizakii* sp. nov., named after Makoto Yoshizaki, who has studied this species over a long period and first elucidated its life history.

**Pikea yoshizakii** Maggs et Ward sp. nov.

*Thalli erecti, 5-25 cm alti, ex axibus irregulatim ramosi et compressi, 0.8 mm lati, 0.6 mm crassi. Anatomia vegetativa typica generis. Filamentum centrale axialeque 110 μm diam. Filamenta lateralia in plano compressa 16-40 μm diam. Thalli monoecii. Sori spermatangiorum super corticem ramorum vetiorum lateraliurnque continue formati. Spermatangia ovoidea, 6-9 × 2.5-5 μm. Rami carpogoniales 10-13-cellulares. Rami cellularum auxiliarium 5-8-cellulares. Filamenta gonimoblasti primum interne crescentia, tum per corticem emergentia et textura exi- teriorem nemathecia formantia, e strato cellularum basaliurn prostrato constatem atque filamenta erecta quae tetrasporangia terminalia et irregulariter cruciatae divisae portant, 13-20 × 10-12 μm maturitate. Tetrasporae thallo gametophyticos efficiens.

Holotypus: In herbario SAP 060884 (Fig. 34), Yazaki, Ibaraki Prefecture, in parte orientali et centrali Honshu, Japan; 27 March 1971, leg. M. Katado et T. Konno.

*Thalli erect, 5–25 cm high, composed of irregularly branched compressed axes 0.8 mm wide and 0.6 mm thick; vegetative anatomy typical of the genus, central axial filament 110 μm in diameter, lateral filaments in plane of compression 16–40 μm in diameter; thalli monoecious; spermatangial sori formed continuously over cortex of older lateral branches, spermatangia ovoid, 6–9 × 2.5–5 μm; carpogonial branches 10–13-celled. Auxiliary cell branches 5–8-celled; gonimoblast filaments initially growing internally, then emerging through cortex and forming external nemathecial growth, consisting of prostrate basal cell layer and erect filaments bearing terminal irregularly cruciately divided tetrasporangia, 13–20 × 10–12 μm when mature; tetraspores giving rise to gametophytic thalli.*
Holotype: SAP 060884 (Fig. 34), Yazaki, Ibaraki Prefecture, east-central Honshu, Japan; 27 March 1971, leg. M. Katado and T. Konno.

Description. Thalli consisted of groups of cartilaginous erect axes, 5–25 cm high, attached by a solid discoid holdfast. Main axes were slightly compressed to 0.8 mm wide and 0.6 mm thick, branched irregularly dichotomously or pinnately to four to five orders of branching (Fig. 35), with spine-like or adaxially curved, nearly terete, ultimate branches. A fishbone pattern of axial filaments could not be seen through the cortex, but in younger tissue the axial filament was usually visible. Older parts of thalli bore conspicuously enlarged deep red branches with obtuse tips (Fig. 36), which resembled cystocarpic branches in other *Pikea* species.
Main axes in transverse section (Fig. 37) consisted of dense rhizoidal medullary filaments 2–4 \( \mu \text{m} \) in diameter surrounding a central thick-walled axial filament to 110 \( \mu \text{m} \) in diameter and two pairs of major lateral axial filaments varying from 16 to 40 \( \mu \text{m} \) in diameter, formed in the plane of compression. Subcortical cells were 16–20 \( \mu \text{m} \) wide \( \times \) 10–12 \( \mu \text{m} \) long and intergraded into cortical cells decreasing from 10 to 7 \( \mu \text{m} \) in diameter. Outermost cortical cells were 3 \( \mu \text{m} \) wide \( \times \) 4 \( \mu \text{m} \) long.

The thalli were monoecious. Spermatangia (Fig. 38) were formed in sori covered by a thickened layer of mucilage. They were cut off obliquely in pairs from spermatangial mother cells in the outer cortical layer among elongated sterile cortical cells and when mature were ovoid, 6–9 \( \mu \text{m} \) \( \times \) 2.5–5 \( \mu \text{m} \), vacuolate proximally, with an apical nucleus (Fig. 43). Transverse sections through fertile axes showed numerous secondary simple or branched filaments of short cells with dense contents (Fig. 39) at the boundary between outer medulla and subcortex. These resembled reproductive branches but seemed to remain sterile. The carpogonial branches (Fig. 44) were 9–10-celled, with the terminal five cells enlarged and the lower cells ovoid, 4–7 \( \mu \text{m} \) high \( \times \) 7–13 \( \mu \text{m} \) wide, and slightly differentiated from ordinary subcortical cells by their reduced pigmentation. The five terminal carpogonial branch cells were distinguishable from the other cells of the reproductive filament by their dense nonpigmented contents, larger size when mature, and lack of lateral cells. The terminal five cells of the carpogonial branches were strongly recurved so that the carpogonium was adjacent to the fourth cell from the carpogonium. The basal three differentiated cells of the carpogonial branch were ovoid, wider than long, and the hypogynous cell was triangular or wedge-shaped (Fig. 44). Carpogonia were conical, 8–11 \( \mu \text{m} \) long \( \times \) 7–13 \( \mu \text{m} \), with trichogynes ca. 7 \( \mu \text{m} \) in diameter that were spirally twisted basally and usually strongly curved one or more times as they passed between the cortical cells before emerging through the cuticle. Auxiliary cell branches (Figs. 39, 45) were six to eight cells long, and there was frequently a short lateral branch on the suprabasal cell. The five terminal cells of the reproductive filament, of which the auxiliary cell was the third or fourth (Fig. 45), were differentiated from the others, resembling carpogonial branch cells. Auxiliary cells were ovoid, 5–8 \( \mu \text{m} \) long \( \times \) 14–15 \( \mu \text{m} \) wide.

Early postfertilization development was not studied in detail due to the lack of appropriate material. However, auxiliary cells were seen in contact with connecting filaments (Fig. 47), and the processes immediately following fertilization appeared similar to those in *Pikea californica*. In later stages, irregularly branched gonimoblast filaments of pigmented cells 6.5–10 \( \mu \text{m} \) in diameter and 1–6 diameters long grew through the medulla, around the axial filaments, to form a dense interwoven mass. They then emerged through the cortex (Figs. 40, 48) and grew over the spermatangial sori (Fig. 41), forming an external nemathecial growth. This consisted of a prostrate basal cell layer (Fig. 41), with secondary pit connections and cell fusions (not shown), bearing erect filaments with terminal irregularly cruciately divided tetrasporangia, which measured 13–20 \( \times \) 10–12 \( \mu \text{m} \) when mature (Fig. 42).

**Life history.** Field-collected fertile tetrasporoblastic tips (Fig. 36) released tetraspores after 2 wk in very low light at 15°C, 14:10 h; cultures were then moved to an irradiance of ca. 20 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \). Tetraspores, 8–10 \( \mu \text{m} \) in diameter, germinated to give discs that coalesced after 18 d. At 55 d, the coalesced discs gave rise to erect axes that grew to 600 \( \mu \text{m} \) in height after a further 10 d (Fig. 49). After being maintained for 12 mo under these culture conditions in small petri dishes, erect axes had reached only 5 mm in height, but following transfer to deep culture dishes at 15°C, 16:8 h, ca. 40 \( \mu \text{mol} \text{m}^{-2} \text{s}^{-1} \), they grew rapidly. Two months later, erect axes (Fig. 50) were up to 15 mm high, slightly compressed and up to 500 \( \mu \text{m} \) wide. Their branching pattern was very irregular, occasionally pinnate in places, often unbranched for distances of 4–5 mm, and lateral branches were constricted at the bases. Reproductive structures were not observed.

An excised field-collected gametophytic tip continued growth, without branching, and after 8 mo at 15°C, 14:10 h formed numerous emergent trichogynes (Fig. 51). Spherical released spermatia, 5–5.5 \( \mu \text{m} \) in diameter, were first observed 2.5 mo later but had probably been released earlier as postfertilization development was then apparent. Spermatoria were observed adhering to trichogynes (Figs. 46, 51), forming cytoplasmic continuity with the trichogynae. During the next 6 mo, trichogynes and spermatoria were formed continuously near the growing apex. The older part of the same axis formed gonimoblast tissue, which grew internally and out through the cortex, giving rise to a tetrasporangial nematheicum 3 mm long; this released tetraspores 7 mo after trichogynes were first observed. Sixty days after tetraspore release, discs up to 700 \( \mu \text{m} \) in diameter were forming erect axes.

In the field, tetrasporoblasts have been collected in March, May (Chihara 1972), August (this study), and July (NCU, Togawa, Choshi, 9 July 1971, leg. M. H. Hommersand); they are probably present throughout the year.

**Distribution.** In Japan, *Pikea* has been reported to occur at extreme low water on the central Honshu Coast from Inubo-saki to Kinkwasan (Okamura 1921). It is also common in eastern Honshu at depths of 3–4 m (S. Ninomiya, pers. commun.). All Japanese *Pikea* examined appeared to be *P. yoshizaki*.

Several herbarium collections previously attributed to *Pikea californica* from southern California and the Pacific Coast of Baja California Norte, Mex-
Pikea in England and North Pacific

Field-collected Pikea yoshizakii from Choshi, Japan, maintained alive in crude culture. Fig. 43. TS through spermatangial sorus with spermatangial mother cells (m) bearing pairs of developing and mature spermatangia (s). Fig. 44. Carpogonial branch with reflected terminal cells (c = carpogonium, h = hypogynous cell). Fig. 45. Auxiliary cell branch with lateral branch (arrowhead) and five terminal differentiated cells, of which auxiliary cell (a) is the middle. Fig. 46. Spermatium fused to trichogyne of mature carpogonial branch. Fertilization may have taken place as the trichogyne cytoplasm has been cut off from the carpogonium. Fig. 47. Auxiliary cell (a) in contact with connecting filaments. Fig. 48. Gonimoblast filament emerging through cortex and giving rise to basal layer of external nemathecial tissue.

Details of these collections are as follows: UC 132920, La Jolla, drift, August 1907, S. Stokes, with tetrasporoblasts; UC 95023, Santa Barbara, 1874, Mrs. S. P. Cooper; BM, Phycocaea boreali-americana, Collins, Holden, and Setchell, #897 "Pikea californica" Harv. Pacific Beach, Mrs E. Snyder"; NCU, reef, south side Puerto Santo Tomas (31°33'N, 116°41'W), 8 November 1969, M. H. Hommersand, with tetrasporoblasts (Figs. 52-54); NCU, S. Middle Coronado Is., Los Coronados Is. (32°23.7'N, 117°15.5'W), 2-10-m depth, 11 July 1969, M. H. Hommersand et al.; NCU, Santa Marta, Punta Descanso (32°16'N, 117°1'W), lower intertidal, crevices and undersides of rocks, M. H. Hommersand, 13 June 1968, with tetrasporoblasts; NCU, Santa Marta, Punta Descanso, intertidal and drift, M. H. Hommersand, 30 July 1969, spermatangial.

North American thalli (Fig. 54) were 4–9 cm high, with more-or-less terete main axes up to 1.5 mm in diameter, denuded basally, branched sparsely and irregularly distally. Toward the tips branching was abundant, mostly irregularly dichotomous, rarely pinnate, forming fan-like arrays of compressed to flattened minor branches 0.4–1 mm wide. Last-order branchlets were spine-like or adaxially curved. On older parts of thalli in some collections, tetrasporoblastic tissue was present on branchlets of the last two orders of branching. The conspicuous dark red thickened axes were 1–1.5 mm long. In section (Fig. 52), these were seen to consist of tetrasporoblastic nemathecia up to 150 μm thick growing over spermatangial sori. Mature tetrasporangia (Fig. 53) measured 20–28 × 12–14 μm. Spermatangial sori only were present on the material from Santa Marta; spermatangia were 6–8 × 2.5–3 μm.

Organellar DNA RFLPs. Organellar DNA restriction fragments larger than 2 kb were usually well resolved on gels (Fig. 55), but smaller fragments could be seen clearly only on blots probed with labeled organellar DNA (Fig. 56). Gels of upper-band
DNA from *Pika pineata*, both undigested and digested with various restriction enzymes, showed bands at 4.0 and 2.2 kb (Fig. 55, arrows) which appeared to be plasmids. Plasmids were not evident in the other samples, and the *P. californica* organellar DNA probe did not hybridize to the putative plasmid bands of *P. pinnata*.

Comparison of restriction fragments between samples showed that some were shared between all samples, some were found only in two or three of the samples, and others were specific to one species or another. The restriction patterns of English and Californian samples of *P. californica* were similar, but a few bands were present only in one or the other.

The distance matrix based on shared organellar DNA bands (Table 2) shows that the English *Pika* sample shared 96% of bands with *P. californica* from California. *Pika pinnata* and *P. californica* samples shared 49.0–50.5% of bands. Japanese *P. yoshizakii* shared 55.6–55.8% of bands with *P. californica* and 54.5% with *P. pinnata*. Parsimony analysis produced a single shortest tree of 201 steps (Fig. 57); the next shortest tree was 266 steps.

**DISCUSSION**

Initially, we expected that life history data might be important in determining whether the *Pika* population in the Isles of Scilly originated in Japan or California because contrasting life histories had been reported from these areas (Scott and Dixon 1971, Chihara 1972). Life history data have actually proved to be more significant than we had hoped because the life history of Japanese *Pika* differs strikingly from that of *Pika californica*.

The English *Pika* and Californian *P. californica* populations have identical life histories, involving
Figs. 55, 56. Restriction digests of *Pika* organellar DNA. Fig. 55. Ethidium bromide-stained gel of organellar DNA from *P. californica* California (cc) and *P. pinnata* (p) digested with *Pst*I, with size marker lane (M) and sizes in kb indicated beside it. Larger restriction fragments are well resolved. Arrows indicate band at 4.0 kb and position of 2.2 kb band (not visible here) which appear to be plasmids. Fig. 56. Southern blot of restriction digests of *Pika* organellar DNA probed with nonradioactively labeled organellar DNA of *P. californica* from Duxbury Reef, California. Restriction enzymes are as indicated; the four lanes in each group are *P. californica* England (ce), *P. californica* California (cc), *P. pinnata* (p), and *P. yoshizakii* (y). Size marker lane (M) has sizes in kb indicated beside it. Some restriction fragments are shared among all four samples (e.g. arrowheads), some were found only in two to three of the samples (e.g. triangles), and others are specific to one species or another (e.g. dot). English and Californian samples of *P. californica* had very similar RFLPs, but a few bands are present only in one (e.g. small arrows).
crustose tetrasporophytes and dioecious erect gametophytes. This life history is similar to that described for seven Californian isolates from Morro Bay, San Luis Obispo County by Scott and Dixon (1971) except that they reported the formation of monosporangia on loose filaments of the crustose phase. The structures they described and illustrated as monosporangia (Scott and Dixon 1971:fig. 4) strongly resemble conjunctor cells (Fig. 7), and the occurrence of monosporangia in a member of the Gigartinales is anomalous (Guiry 1990). In both Scott and Dixon's study and the present study, tetrasporangia were observed in culture on spherical but not crustose tetrasporophyte thalli. This phenomenon is commonly seen in crustose algae in culture, and we have observed it in several unrelated species. The crustose phase is unique in the British Isles because it forms both secondary pit connections and cell fusions. Except for the secondary pit connections, the crusts resemble *Rhodophysema elegans* (P. Crouan et H. Crouan) J. Agardh ex P. Dixon. They were not observed on pebbles in the Isles of Scilly during field surveys, but, like the gametophytes, they may be confined to bedrock, which we were unable to sample. The development of tetrasporangia following transfer to a lower temperature and short daylength indicates that tetrasporogenesis is probably a short-day response and that the crusts are winter-fertile, like many crustose red algal species and tetrasporophyte phases in temperate waters. Although Scott and Dixon (1971) found that tetrasporangia were formed under long-day conditions in their isolates, their cultures had previously been maintained in short days, and sporogenesis may have been initiated under these conditions.

Gametophytes have never become fertile in any cultures of *P. californica*, so crossability tests between the English and Californian isolates were not possible. Nevertheless, our life history data are consistent with these two populations being conspecific. The only obvious difference noted was phenological. The English population does not begin to reproduce until late September, whereas in California we found mature males and females with developing and mature cystocarps in July. This was surprising in view of published reports of spermatangia in late August to mid-November (Lindstrom and Scagel 1987), female structures only in early October (Lindstrom and Scagel 1987), and cystocarps in November to January (Scott and Dixon 1971). However, in general, we observed reproductive structures of fertile thalli to be confined to the branches borne by a few older major axes, whereas the bulk of each thallus was not obviously reproductive. Herbarium material from central California showed the same pattern. Three of 13 thalli collected during June to August (in UNC) had mature spermatangia or cystocarps. Although fertile branches may be remnants from an earlier reproductive period, the difference between the reproductive phenologies of English and Californian *Pikea* could also be related to the annual daylength regimes. The longest daylength for San Francisco, at ca. 38° N, is 14.5 h (calculated using equations in Dring 1984) in contrast to the 16-h maximum daylength in the Isles of Scilly at 50° N. However, possible photoperiodic effects on reproduction could not be investigated in the laboratory as gametophytes did not reproduce in culture.

Comparison of organellar DNA RFLPs from English and Californian *P. californica* showed that banding patterns were very similar, differing by only a few fragments. The 96% similarity between samples clearly indicates conspecificity when compared with
maximum RFLP similarity levels of 62–72% (with various restriction enzymes) between separate Graciilaria and Gracilariopsis species (Rice and Bird 1990, Bird et al. 1992) and of about 60% between closely related Ceramium species (unpubl. data). It was beyond the scope of this study to determine the nature and origin of the 4% of bands present in one sample only. It is possible, for example, that mitochondrial DNA was present in higher concentrations in one sample than another, so that mitochondrial bands were seen in that sample. The fragments are unlikely to indicate a plastid DNA length polymorphism because extra bands would be seen in one sample with all restriction enzymes, whereas we found that the patterns of both samples were identical with some enzymes (e.g. BglII).

Morphological, life history, and molecular data have all confirmed that the Pikea population in the Isles of Scilly is conspecific with Pikea californica from Duxbury Reef, California, near the type locality at Golden Gate. Its habitat on strongly wave-exposed bedrock from just above extreme low water into the subtidal zone is also similar to that favored in California. Female reproductive morphology of P. californica was not studied in detail but the features observed were consistent with previous studies of Pikea (Abbott 1968, Lindstrom and Scagel 1987) and of other Dumontiaceae (Lindstrom 1984).

At the start of the present investigation, there was considerable confusion as to the number of species of Pikea in North America, some workers including P. pinnata Setchell and/or P. robusta Abbott in synonymy with P. californica. We were able to clarify the status of P. pinnata because Pikea material from near Golden Gate, San Francisco, the type locality of both P. pinnata and P. californica, appeared morphologically to consist of two separate forms, corresponding to the type specimens of these two species. The differences, especially texture and color, were quite obvious in fresh material but would have been much harder to identify in herbarium material. Contrasting branching patterns were maintained in cultures grown under the same conditions, P. pinnata having longer branches with more regularly distichous branching and fewer dichotomies. Analysis of organellar DNA RFLPs clearly indicated that the material provisionally identified as P. pinnata represents a species distinct from P. californica. A further difference was that plasmid-like DNA bands were seen only in this species. Plasmids are common in several genera of red algae (Goff and Coleman 1988, 1990, Villemur 1990). Labeled organellar DNA probes did not hybridize to the plasmid bands; previous studies (Goff and Coleman 1990, Villemur 1990) have likewise shown no homology between plasmid and nuclear or plastid DNA. The life history of P. pinnata is unknown, but it is dioecious, tetrasporophytes are unknown, and it can be presumed to have a heteromorphic life history. In the related genus Farlowia, both species studied have crustose tetrasporophytes (DeCew and West 1981).

Fresh material of Pikea robusta was not available, and reproductive material has never been found. It is not possible at present to demarcate clearly between it and P. pinnata on the basis of herbarium specimens. Some specimens are readily separable from P. pinnata by wider main axes and less dense higher order branching, but thalli with narrow main axes are extremely difficult to identify from herbarium material. The key feature of P. robusta reported by Abbott (1968), the occurrence of cystocarps in distinct, wart-like branches, has not been confirmed and may be a misinterpretation of the galls in this species. Morphological studies of fresh material of P. robusta are required, preferably including winter collections since P. robusta may be winter-fertile, but its subtidal habitat at extremely wave-exposed sites would make this difficult. Nevertheless, on the basis of its vegetative habit and possibly exclusively subtidal habitat, it seems likely that P. robusta is a distinct species of Pikea.

Pikea yoshizakii forms tetrasporoblasts, in a life history strikingly different from that of P. californica and P. pinnata, and it undoubtedly represents a new species. Chihara (1972) reported a direct-type life history, having mistaken tetrasporoblasts with tetrasporangia for cystocarps, which is not surprising because they closely resemble cystocarpic axes. He interpreted his observations as direct development of carpospores into female gametophytes, a life history that has been observed in many red algae (see Maggs 1988). This type of tetrasporoblastic life history was previously known only in the Phyllophoraceae. Its occurrence in the unrelated family Dumontiaceae correlates well with evidence from the Phyllophoraceae showing multiple origins of the tetrasporoblastic life history (Fredericq and Ramirez 1996). Although the genus Ahnfeltiopsis was recently segregated from Gymnogongrus because it lacks tetrasporoblasts (Silva and DeCew 1992), Fredericq and Ramirez (1996) showed that tetrasporoblastic entities are more closely related to taxa with cystocarps than to other tetrasporoblastic species. They argued that the occurrence of a tetrasporoblastic life history can no longer be regarded as evidence for the recognition of different genera, a finding with which we concur, because P. yoshizakii is clearly congeneric with P. californica and P. pinnata. Tetrasporoblasts correspond merely to a shortcut in the life cycle, bypassing the free-living tetrasporangial stage (Fredericq and Ramirez 1996). Phylogenetic analysis did not group the two Pikea species with heteromorphic life histories.

North American specimens attributed here to P. yoshizakii appear to have an identical life history to the Japanese isolate, and all major features of reproductive morphology are similar in size. Also, many species are common to California and north-
east Japan (Hommersand 1972), so this distribution is not unexpected. American specimens show some vegetative morphological differences in comparison with Japanese material, however. These may be habitat-related but could be significant, and American material may therefore represent a separate, but closely related, species.

Sporadic observations over a 10-year period have shown that *P. californica* is well established in the Isles of Scilly, but searches of suitable habitats off the Lizard peninsula of south Cornwall in 1994 found no evidence that this species has spread since its establishment prior to 1967. The question of where the Cornish *P. californica* may have originated has now been answered to some extent. The discovery that "*P. californica*" in Japan represents a different and very distinct species means that Japan can be eliminated as a possible source of the Cornish population, as proposed by Maggs and Guiry (1987). Maggs and Guiry suggested that flying boats were the most likely vector. The Catalina I was manufactured in San Diego and, from 1941 onward, was ferried across the United States by civilian crews to Bermuda, where British crews picked them up for the final leg of the flight to England to join Coastal Command. Photos from this period (Scaborough 1983:23) show the use of canvas sea-anchors on the water; these would have been stored in the hull while flying and would make excellent transporters for live seaweeds, keeping them damp but aerated. We have now shown, however, that *P. californica* does not occur around San Diego, having a southern limit at Point Conception. There is no history of oyster cultivation in Scilly (the most usual vector of marine introductions), so we are unable at present to put forward any explanation of the presence of *P. californica* in the Isles of Scilly. Now that the situation has been clarified by elucidating the taxonomy of *Pikaia* in California to some extent, and finally identifying the Japanese species, the central question of the provenance of the Cornish population can be readdressed, perhaps by sequencing an appropriate genomic region such as the ribosomal internal transcribed spacer (Bakker et al. 1995) or by employing RAPD techniques (Patway et al. 1993).

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