A taxonomic study of the genus Padina from Japan, Southeast Asia, and Hawaii based on morphology and gene sequence data (rbcL and cox3) resulted in the recognition of four new species, that is, Padina macrophylla and Padina ishigakiensis from Ryukyu Islands, Japan; Padina maroensis from Hawaii; and Padina usoehtunii from Myanmar and Thailand. All species are bistratose and morphologically different from one another as well as from any known taxa by a combination of characters relating to degree of calcification; the structure, position, and arrangement of hairlines (HLs) and reproductive sori; and the presence or absence of rhizoid-like groups of hairs and an indusium. Molecular phylogenetic analyses demonstrated a close relationship between P. ishigakiensis, P. macrophylla, P. maroensis, and Padina australis Hauck. The position of P. usoehtunii, however, was not fully resolved, being either sister to a clade comprising the other three new species and P. australis in the rbcL tree or more closely related to a clade comprising several other recently described species in the cox3 tree. The finding of the four new species demonstrates high species diversity particularly in southern Japan. The following characters were first recognized here to be useful for species delimitation: the presence or absence of small rhizoid-like groups of hairs on the thallus surface, structure and arrangement of HLs on both surfaces either alternate or irregular, and arrangement of the alternating HLs between both surfaces in equal or unequal distance. The evolutionary trajectory of these and six other morphological characters used in species delineation was traced on the phylogenetic tree.