Does *Iris sikkimensis* Dykes occur in India?

Iris sikkimensis Dykes has been considered as a mysterious species and believed to have originated under cultivation, since its publication by Dykes[1] in 1913. Initially this species was described by Dykes[2] as *I. kumaonensis* var. *caulescens* from the rootstock said to be supplied by Messrs. Barr. & Sons from an unknown locality of Sikkim Himalaya. After four years he raised it as a species (*I. sikkimensis*) in his monograph with a note: 'It is with some hesitation that I publish the account of this *Iris*, because although after cultivating it for at least four years side by side with *I. kumaonensis* and *I. hookeriana*, I have no doubt that it is distinct from both of these, I am yet not satisfied that it may not be merely a hybrid between the two. This however can hardly be possible, if as I was led to understand, the plant was wild in Sikkim, for *I. hookeriana* does not seem to extend as Far East as that'. The account of this species in all the subsequent revisionary works on *Iris* is not satisfactory.

A thorough search in all the Indian herbaria, including BSIS, Sikkim State Forest Department Herbarium, ARUN and ASSAM, could not help find any specimen of *I. sikkimensis* and no live specimens were seen anywhere in Sikkim Himalaya or in gardens in the Himalayan region during the present work. This species is neither treated nor do any of the descriptions of *Iris* match with it in any Indian flora, including *Plants of Darjeeling and Sikkim Himalayas*[3], *Spring Flora of Sikkim Himalaya*[4], *Flora of Sikkim*[5], *Flora of Eastern Himalaya*[6–8], floras of northeastern Indian states (Assam, Meghalaya, Manipur and Tripura) and those of Nepal[9,10], Bhutan[11] and China[12,13]. The type or other specimen of *I. sikkimensis* is untraceable in Kew, where all Dykes collections are deposited. The only evidence of its existence is its description and illustration, which is somewhat intermediate between *I. hookeriana* and *I. kumaonensis* (Table 1). Its origin through hybridization between *I. hookeriana* and *I. kumaonensis* is quite apparent, since it resembles the former by its well-developed scape with 2–3-flowered spathe and the latter by narrower leaves and long perianth tube. A critical analysis of the given characters reveals that *I. sikkimensis* is closer to *I. hookeriana* than *I. kemaonensis*. However, it differs from *I. hookeriana* by slightly narrower leaves, 2–3-flowered spathe (two-flowered in *I. hookeriana*) and 3.5–5 cm long perianth tube (1.5–3 cm in *I. hookeriana*), faintly mottled at base of standards (uniform in *I. hookeriana*) and refusal to set any seed (normal fruiting in *I. hookeriana*). But *I. hookeriana* has no representation in the central and eastern Himalayas. Thus, a question of natural hybridization between *I. hookeriana* and *I. kumaonensis* in the Sikkim Himalayas does not arise. The absence of seed set in *I. sikkimensis* strengthens the possibility of hybridization of *I. hookeriana* ($2n = 24$) with *I. kumaonensis* ($2n = 22$) under cultivation.

Noltie (pers. commun.) had not seen the species during his extensive collection tour in Sikkim Himalayas and presumes it to be described by Dykes from the material obtained by him from a British nursery and 'said to have come from the wild in Sikkim'. Service[14] confirms that the species has never been collected again after it was published. In the absence of any material that could be identified as *I. sikkimensis* in the herbaria and in the field, its occurrence in India is quite doubtful. There is also no evidence of its survival under cultivation, since several

Table 1. Comparison of characters in *Iris sikkimensis* with *I. hookeriana* and *I. kumaonensis*

I. hookeriana Foster	*I. sikkimensis* Dykes	*I. kumaonensis* Wall. *ex* Royle
Distributed in western and Trans Himalayas	Said to be distributed in unknown locality of Sikkim Himalayas	Distributed from western to eastern Himalayas
Leaves pale green, 15–25 × 0.7–1 cm in flowers, elongating up to 40–50 × 1.5 cm	Leaves pale green, 10–20 cm long in flowers, elongating up to 30–45 × 1.2–2 cm	Leaves light green, 8–15 cm long in flowers, elongating up to 60 × 1 cm
Scape 10–20 cm, bearing a terminal head of two flowers	Scape 10–15 cm long, bearing a terminal head of 2–3 flowers	Scape acauliscent or rarely 2–6 cm long, with one-flowered spathe
Spathe valves pale green, 5–7.5 cm long, subscarious.	Spathe valves pale green, 5–7.5 cm long, scarious in upper third and along the edge	Spathe valves green, unequal, 5–8 cm long, margin narrowly scarious
Pedicel 0.5–2 cm long	Pedicel 1.2–2 cm long	Pedicel 0.2–1 cm long
Perianth tube with purple stripes and spots, 1.5–3 cm long	Perianth tube deep purple, 3.5–5 cm long	Perianth tube greenish, 5–7 cm long
Falls bearded, 4–5 × 1.5–2.5 cm; blade obovate–oblong, lilac–purple, mottled with darker blotches	Falls bearded, 6.2 × 2.5 cm, blade obovate, dark purple–lilac, mottled with deeper shade	Falls bearded, 4–5 × c. 2 cm; blade oblong, lilac–purple, with purple veins and blotches
Standards erect, uniformly purple, 3.5–4 × 1.2–1.5 cm	Standards diagonally erect, pale mauve, faintly mottled at base, c. 5 × 2 cm	Standards erect, uniformly lilac, c. 4 × 1.5 cm
Ovary green, 1.2–1.8 cm long; style branches c. 2 cm long	Ovary green, c. 2 cm long, mottled and faintly purple-striped; style-branches c. 2.5 cm long	Ovary greenish, 0.8–1 cm long; style branches c. 3 cm long
Capsule obovate to ellipsoid, 3.5–5 × 1.2–1.8 cm	Refuses fruit formation	Capsule ovoid to subglobose, 2–2.5 × 1.5–1.8 cm
Seeds pyriform, 5.5–6 × 3.5–4 mm, wrinkled, with a disc-shaped aril	Seeds do not set	Seeds pyriform, 5–6 × 3–3.5 mm, wrinkled, with an inconspicuous disc-shaped aril

monographic publications on *Iris* cultivation, including that of Mathew[15], Kohlein[16] and Service[14] have no report of its subsequent cultivation in England or elsewhere after its publication by Dykes in 1913.

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ACKNOWLEDGEMENTS. I thank Dr M. Sanjappa, Director, Botanical Survey of India, Kolkata and Prof. S. P. Rath, Utkal University, Bhubaneswar for guidance and suggestions, and Dr H. J. Noltie for sharing his field experience on Sikkim.

Received 1 July 2005; accepted 22 August 2005

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Fishmeal extract agar – a medium to inhibit swarming of *Proteus* spp.

Swarming on appropriate solid medium is characteristic of *Proteus* spp. It is the result of migration of a group of cells (swarm cells) from the edge of the developing micro-colony to an uninoculated area of the medium producing thin films of concentric rings of growth[1]. The swarming of *Proteus* may make it difficult to isolate in pure culture other pathogens which may be present in clinical specimens. Several anti-swarming agents have been described. Many of these inhibit the growth of certain pathogenic bacteria[1]. Fishmeal-based media were used for growing *Entamoeba histolytica*[2] and for the isolation and antibiotic susceptibility testing of medically important bacteria[3,4]. We report here the use of fishmeal extract agar to inhibit swarming of *Proteus* spp., without affecting the growth of other pathogenic bacteria which may be present along with *Proteus* in clinical specimens.

Fishmeal extract agar (FMEA) was prepared as previously described[2]. Five grams of fishmeal was boiled in 100 ml of distilled water and filtered through Whatman no. 1 filter paper. The volume was made up to 100 ml and pH was adjusted to 7.4. Agar (Hi Media) was added to a concentration of 2.0%, the medium was sterilized by autoclaving at 121°C for 15 min and poured into sterile petri plates. The plates were dried at 37°C for 1 h before inoculation. FEMA with sodium chloride was prepared by incorporating 0.5% sodium chloride into the former.

Clinical isolates of 25 *P. mirabilis* and 15 *P. vulgaris*, together with 40 swarming strains of *Proteus* spp. were inoculated on FMEA and nutrient agar (NA). The latter served as control. The swarming strains of *Proteus* were also inoculated on FMEA incorporated with 0.5% sodium chloride. Smears were prepared from growth on FMEA, FMEA with 0.5% sodium chloride and NA after 2 and 4 h, stained by Gram's stain and microscopically examined.

All 40 strains of *Proteus* showed swarming on NA. None of these swarmed on FMEA and FMEA incorporated with 0.5% sodium chloride (Table 1). Microscopy of growth on FMEA and FMEA with 0.5% sodium chloride after 2 and 4 h did not reveal swarm cells. Similar examination of growth on NA showed swarm cells.

The swarming of *Proteus* may make it difficult to isolate in pure culture, other bacterial pathogens in clinical specimens from polymicrobial infections. Many ways of inhibiting swarming have been described. Restricting movement of *Proteus* cells by increasing agar concentration to 3–4%; preventing formation and interfering with structure and activity of flagella by incorporating into media polyvalent H antisera; ethanol, boric acid, bile salts, detergents or by retarding the growth rate by incorporating growth inhibitors such as sulphonamides, neomycin, chloral hydrate, barbiturates, sodium azides or purine bases. Many of the anti-swarming agents are toxic and prevent the growth of delicate pathogens. Others may interfere with the colonial morphology of the organisms or lyse red blood cells in the medium, making the recognition and detection of hemolytic organisms difficult[1].

Our study showed that *Proteus* spp. does not swarm on FMEA. It is known that the omission of sodium chloride from the medium prevents spreading of *Proteus* colonies[5]. Inhibition of swarming of *Proteus* spp. on FMEA was regardless of in-