percentage of negative response (75–87.5%) was given by MTT assay when male or female worms were used in the test whereas very small number (12.5–25%) of compounds showed negative response in the motility assay with male or female parasites as targets. When male and/or female adults plus mf were used as targets, both the assays increased the detection rate (positivity rate) to a great extent (up to 87.5%). The present study also reveals that those compounds that show LC_{100} at or below 62.5 μg/ml in *in vitro* assay systems can be considered as potential agents for follow-up in *in vivo* assay system.

In conclusion, the predictive value of the *in vitro* systems using the human parasite *B. malayi* was found to be very high as all (100%) the synthetic and plant products that were active in the *B. malayi–M. coucha* model were also found active in the *in vitro* systems. Moreover, 87.5% of the synthetic products and 9% of the plant products which were inactive in the *A. viteae–M. coucha* system were also picked up by the present *in vitro* systems as positive indicating that there are fewer chances of missing the activity of potential antifilarials in the *B. malayi in vitro* systems. These findings clearly show that the *B. malayi*-based MTT and motility assays can be employed as reliable prescreens for evaluating antifilarial activity of new products.

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Myxobacterial diversity of Indian soils – How many species do we have?

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Myxobacteria of tropical soils is an under-explored bacterial group. We report here the results of sampling in Pune district of Western Ghats. A number of novel morphotypes were found in forest as well as urban/semi-urban soils. There was a high level of floral dissimilarity between habitats. The morphotypes detected in Pune district also differed from the northern Indian species recorded earlier. Using a species individual curve on the Pune, Lucknow and pooled data, we try to estimate the number of species that are likely to be present in India. A plausible estimate is several fold higher than the species recorded worldwide so far.

MICROBIAL diversity is one of the difficult areas of biodiversity research. A number of studies[1–8] have made it clear that the number of species of bacteria known to science is only a tip of the iceberg. Majority of bacterial species have not been studied and described, either because they are unculturable by the conventional methods, or because we just have not explored enough. There is evidence that a large number of bacterial species are

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'unculturable[1,2,8], but the second possibility also cannot be ignored. Tropical field and forest soils, fresh and salt water, marshlands and other ecosystems are likely to be rich in a large number of unrecorded species that can be cultured. Here, we discuss the number of species of myxobacteria Indian soils are likely to possess based on data collected by earlier workers as well as our own sampling in the Western Ghats of Pune district.

Commonly cultured bacteria such as those found in air, water, soil or human body' which are presumably 'r' selected species, are ubiquitous. Nearly the same species are found all over the world. Higher organisms, on the other hand, both animals and plants, have substantial number of species endemic to small geographic areas. If the general relationship that more complex organisms have a greater tendency to be endemic is true, myxobacteria known for their remarkable complexity among prokaryotes, should exhibit relatively more endemicity. We suspect, therefore, that the tropical soils have a greater variety of locally abundant species of myxobacteria.

Myxobacteria are unique amongst the prokaryotes in displaying a wide range of social adaptations[9]. They have a complex life-cycle involving co-operative behaviour of a large aggregation of cells. Majority of myxobacteria feed on other bacteria, dead or alive, and also scavenge on complex organic matter in soil. Some are strongly cellulolytic. These gram negative bacteria exhibit gliding movement on moist surfaces and move slowly in large groups digesting any live or dead microorganisms on the way. Under unfavourable conditions, the cell aggregates undergo complex differentiation steps resulting in formation of fruiting bodies of characteristic shapes. Cells within a fruiting body differentiate to produce myxospores or microcysts. Fruit bodies of some species have a simple or branched fibrous stalk embedding many cells. The cells in the stalk do not form spores. Even in species that do not form a stalk, the fruit body formation is preceded by suicidal autolysis of a large number of cells in the aggregate[10]. This altruistic behaviour of myxobacteria is an interesting sociobiological problem[9].

Myxobacteria are also known to be a source of a wide variety of antibiotics, bacteriocins, cell wall lytic enzymes, lipases, nucleases, polysaccharides and proteases[11]. A species of interest today is *Sorangium cellulosum* which produces epothilone A having an action similar to taxol, a cytotoxic drug used in cancer treatment. Epothilone A is shown to be 2000–5000 times more active than taxol *in vitro*[12]. Tropical myxobacteria might have a hidden treasure of a still larger number of biologically active molecules.

One of the likely reasons why myxobacteria have been poorly studied in the tropics is because they are hard to obtain in axenic culture. The *Bergey's Manual* of *Systematic Bacteriology* only accounts the species that have been obtained in pure culture. There are likely to be a number of biotypes which are not listed since they have not been cultured axenically. In the absence of axenic culture, morphological differentiation of fruiting bodies obtained from the wild is possible. However, the fruit body morphology shows some level of variability within species. They may also undergo changes on subculture. The taxonomic literature on myxobacteria is tentative and confused making identification difficult[13–17]. The *Bergey's Manual of Determinative Bacteriology*[17] differs from the *Bergey's Manual of Systemic Bacteriology*[13] in recognizing genera. For example, the genera *Angiococcus*, *Corallococcus*, *Haploangium* and *Sorangium* are recognized by the former but not the latter. Only one account has been published so far on the taxonomy of Indian myxobacteria[16] which is based on extensive sampling on a small area in the vicinity of Lucknow. These authors observed 32 species, out of which 8 were novel. We compare Singh and Singh's

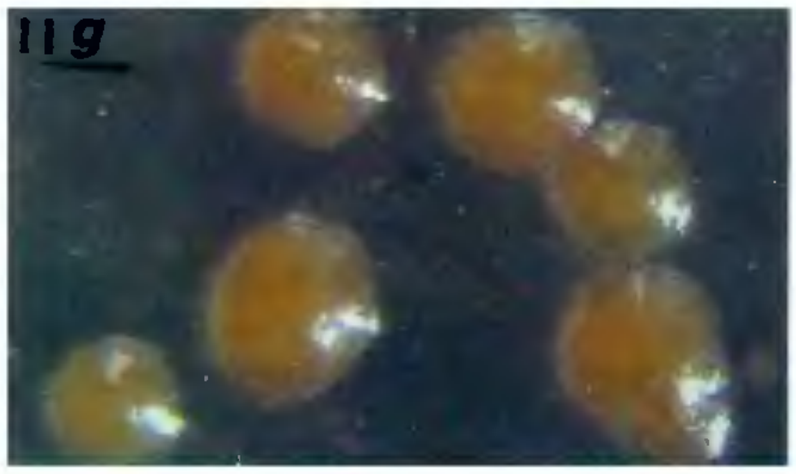
Table 1. Identified species of myxobacteria observed during sampling in urban Pune and the Pune district Western Ghats

Species	Habitat	Frequency of occurrence
Chondromyces apiculatus	Dead twig from forest soil, garden soil	2
C. sessile	Forest soils, goat dung, urban area	10
C. crocatus	Garden soil	1
C. pediculatus	Garden soil	1
Myxococcus xanthus	All habitats sampled	42
M. corraloides	Barren and playground soil, goat dung urban area	6
M. virescens	Urban soils	8
M. fulvus	Urban soils	2
Archangium species	Urban soils	2
Angiococcus brunneus	Garden soil	1
Chondrococcus macrosporous	Garden soil	2
Cystobacter species	Dead bark from forest area, urban soils	10

Table 2. Unidentified OTUs of myxobacteria recorded during sampling in urban Pune and the Pune district Western Ghats. All the 15 morphotypes have distinct differences from the recorded species in literature. The sizes refer to diameter of a sporangium

No.	Fruiting body morphology	Spore morphology	Type of habitat	Result after subculture	Frequency of occurrence
1	Orange pear-shaped sporangia 100–150 µm without stalk, 15–20 in a bunch. Many have a pointed apex	Rod shaped with tapering ends 1 by 4–5 µm	Dead twig moist deciduous forest	Did not grow	1
2	Yellow circular with a distinctly granular surface without stalk, 50 µm	Spherical 1–2 µm	Dead bark deciduous forest	Did not grow	3
3	Light yellow branched stalks emerging from a single point each branch bearing a sporangiole of 70–80 µm, woolly surface	Rod shaped 1 by 2–3 µm	Dead twig from forest	Did not grow	1
4	Orange spherical woolly pompom toy-like sporangia with a white slightly spiral stalk 80–100 µm, sometimes sessile	Rod shaped 1 by 4–5 µm	Dead twig deciduous forest	Grew with swarms and consistent fruiting morphology	3
5	Bright orange spherical without stalk, granular surface 80–100 µm	Spherical 1–2 µm	Dead twig forest.	Did not grow	1
6	Orange irregular shaped without stalk, granular surface 130–360 µm	Spherical 1–2 µm	Dead bark from forest	Did not grow	1
7	Dark orange red spherical without stalk, in small clusters. 100–150 µm	Rod shaped 1 by 4–5 µm	Dead twig from forest	Did not grow	1
8	Orange, brown with a spiny surface clustered on a thick transparent stalk, 70–90 µm	Blunt ended rods 1 by 4–5 µm	Goat dung, urban area	Grew well with consistent morphology	3
9	Yellow-orange with white stalk, branched, each branch with a single spherical woolly sporangia giving a pompom toy-like appearance 80–100 µm. Stalk measures 50–80 by 150–200 µm	Blunt ended rods 1 by 3–4 µm	Dead bark forest soil	Swarm and fruiting bodies becoming dark orange red	2
10	Dark red to maroon, circular, clustered together like inverted bunch of grapes 20–25 µm	Spherical 2–3 µm	Goat dung, urban area	Transition in colour from dark red to orange and then to light yellow. Some turned pigmentless	2
11	Bright glistening yellow, circular disc like without stalk. Translucent gel like appearance.	Oval to small rods 2–4 µm	Goat dung, urban area	Growth with swarms, with fruiting bodies	3
12	Bright yellow, refractile. Sporangia 20–50 µm. On the dung pellet formed clusters which appeared to stand errect	Spherical 2–3 µm	Goat dung, urban area	Vegetative colonies obtained on agar which tended to digest agar surface in 4–5 days sinking slowly, no fruiting bodies	1
13	*Chondromyces* sp. with light pink sporangioles densely arranged with stalks. Tips of sporangioles swollen, transparent sometimes bifurcated	Rod shaped 1 by 5 µm	Dead twig from forest, garden soil	Grew alongside *C. apiculatus*. Could not be grown in pure culture	2
14	Resembling *Chondromyces pediculatus* but old fruiting bodies with additional stalks growing from the head giving a double-decker appearance	Rod shaped, 1 by 3–4 µm	Forest soil, dead bark	Consistent double-decker fruit bodies	7

data[16] with our sampling from Pune district to show that there is a large dissimilarity in myxobacterial flora between the two geographical areas and attempt to estimate the number of species Indian soils might have. Owing to the taxonomic tentativeness of this group, rather then focusing on species identification we treat clearly distinguishable morphotypes as separate Operational Taxonomic Units (OTUs) and attempt to answer quantitative questions related to myxobacterial diversity.



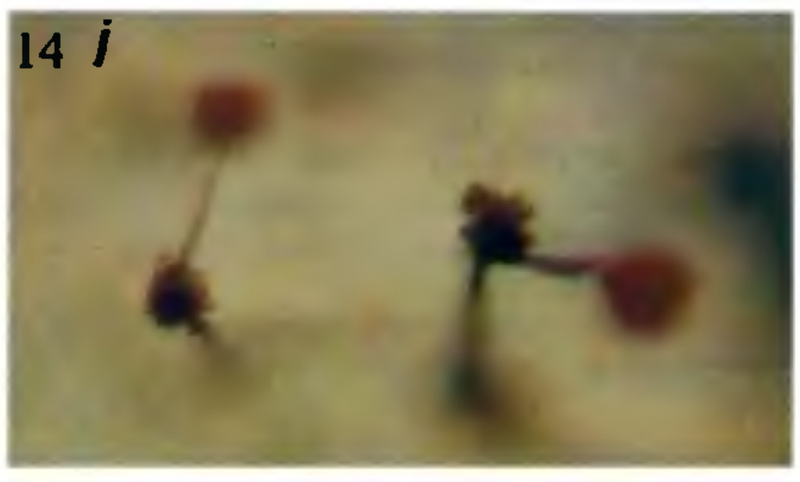


Figure 1 *a–j*. Fruiting bodies of some of the unidentified novel morphotypes. The numbers refer to those in Table 2. Fruiting bodies of 1 to 4 and 13 on tree bark, 8, 9 and 11 on *E. coli* agar; 14 (*i*): a young fruiting body on soil; 14 (*j*), old fruiting body of the same showing shrinkage of sporangia and the double-decker appearance.

Sampling for myxobacteria was done from two major habitats in the Pune district. The first included garden, playground and agricultural soils, tree barks and animal dung from urban and suburban Pune. In these habitats the soils were typically exposed and free of leaf litter. The second comprised dry to moist deciduous forest soils of the Pune district, Western Ghats characterized by moderate to thick leaf litters and canopy cover. Myxobacteria were collected by screening for fruiting bodies on the surfaces of bark, leaf litter, twigs and dung. Any surface patch showing one or more types of fruiting bodies was collected and treated as a unit sample. Alternatively, soil samples were collected and cultivation of myxobacteria attempted using the techniques described later in this article. In these cases each sample that showed the presence of one or more types of myxobacteria was treated as a sampling unit. For statistical analysis both types of sampling units are considered together. Each of the soil samples was plated out using the following two methods simultaneously.

In the soil dung pellet method, a petri plate was filled two-thirds by the soil sample keeping adequate moisture level. Fresh goat dung pellets were sterilized by autoclaving, dipped in 100 mcg/ml cycloheximide for 30 min and placed on the soil. The plates were incubated at room temperature in diffused daylight.

In the bacterial lawn method, plain water agar plates were prepared. A thick paste of *E. coli* or *Klebsiella* cells, live or autoclaved, was spread on the agar surface and 10–20 mg of soil samples were placed on the bacterial lawn. The first evidence of myxobacterial growth was often observed to be a patch of clearing in the lawn from which a swarm of gliding cells moved out very slowly. Fruiting body formation usually followed swarm expansion.

For subculturing fruiting bodies directly obtained from the wild or those obtained on primary culture by either of the above, the following methods were used.

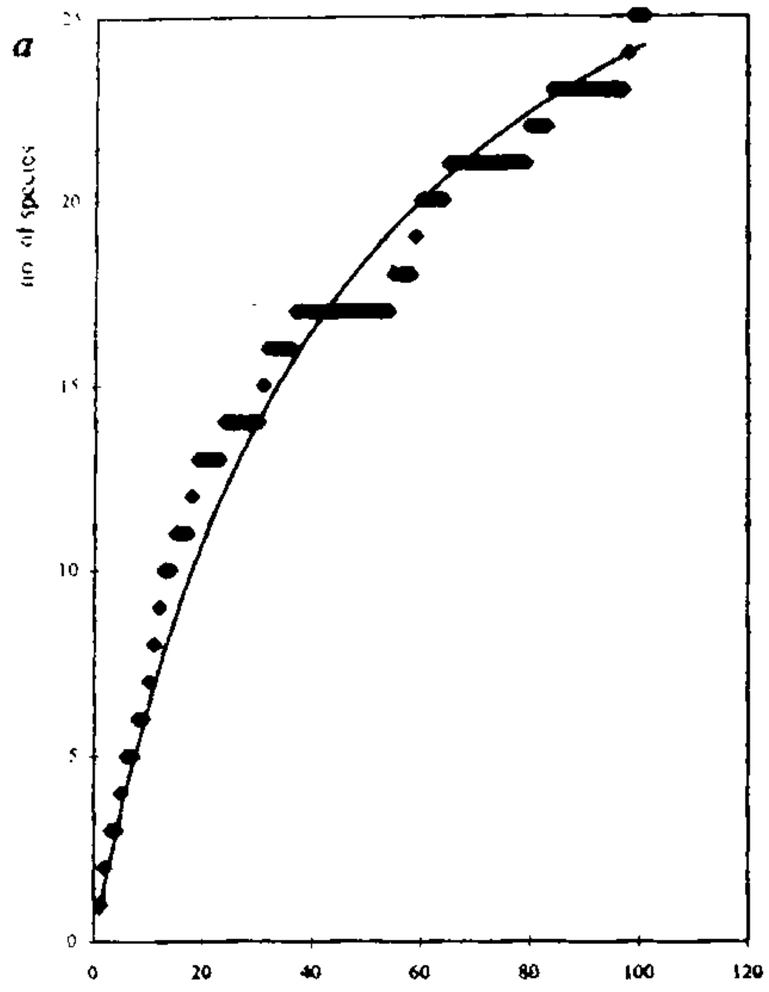
In the agar well method, wells were made on the water agar plate and filled with *E. coli* cells. A fruiting body from the source material or previous culture was inoculated in a well. This method was particularly useful for subculturing and maintaining isolates, particularly the stalkless forms.

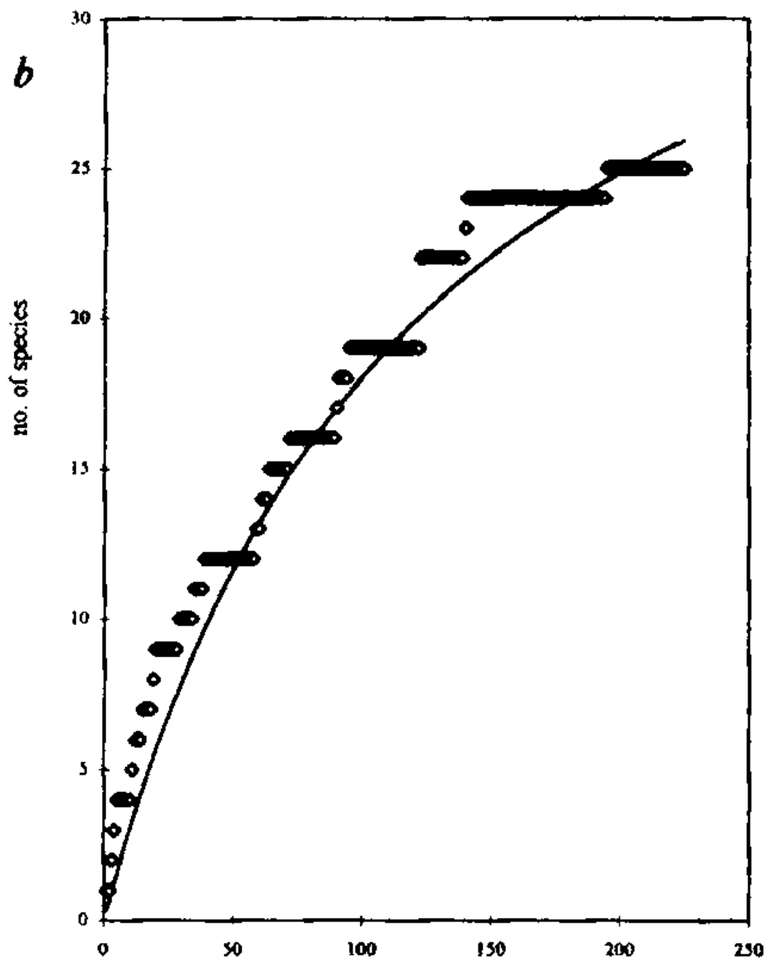
In the agar dung pellet method, autoclaved dung pellets were placed in molten plain agar. After solidification of the agar dung pellets got fixed, *E. coli* cell suspension prepared in cycloheximide (50 mcg/ml) was poured over them. A fruiting body was placed on each dung pellet. This was used extensively for subculturing stalked isolates.

Tables 1 and 2 show that 27 distinct OTUs were detected, out of which 14 could be identified to the genus level and 10 to the species level (Table 1). Table 2 describes the novel morphotypes detected which are illustrated in Figure 1. A substantial proportion of morphotypes distinguishable from the predescribed species in available literature including Singh's monograph on Indian species, indicates that the tropical soils are likely to have a large number of as yet undescribed species. The genera *Stelangium, Sorangium, Haploangium, Polyangium* and *Podangium* described from Lucknow were not detected in our samples. Out of the 32 species described by Singh and 27 by us, only 10 were common. The per cent similarity between the myxobacterial flora of Lucknow and Pune was only 19.4 and Jaccard coefficient on the presence/absence of data 0.263. The large dissimilarity between two distinct geographical regions of India indicates a large beta component of diversity. It also supports our hypothesis that this group of complex bacteria would exhibit greater endemicity of species.

In the Pune district sampling, a striking difference was present between the forest soils and urban/semi-urban soils. Out of the 16 morphotypes found in forest soils and 15 in urban/semi-urban ones, only 5 were common. The per cent similarity was relatively high (42.15) since the dominant species was shared by both the habitats. The Jaccard coefficient, however, was only 0.192. Thus the species turnover between habitats within a geographic area was also large. The forest soils showed a greater frequency of stalked species. In the forest soils, 21 out of 50 samples yielded stalked forms whereas only 1 out of 42 urban/semi-urban samples yielded stalked forms. The total number of stalked forms obtained from the two habitats were 8 and 4, respectively. The reason for the difference in the abundance of stalked forms can be ecological or socio-biological and needs further investigations.

In order to estimate the total number of morphotypes that could be present at any site including the types not detected in the samples, we constructed species individual curves using simulated subsampling. Occurrence of a species in one sample was taken as one individual in spite of the number of sporangia produced. Individuals were drawn randomly from the data and a species accumulation curve plotted. We fitted the Michaelis–Menten equation to the curve in order to estimate the total number of species that are likely to be present including the ones that might have been missed during sampling[18]. This exercise was done separately for the Lucknow data and Pune data, as well as on the pooled data of both the locations. The estimate for the total number of species obtained for Lucknow was 41.1 (Figure 2 *a*) and Pune 34.8 (Figure 2 *b*). If the same degree of similarity is assumed for the sampled and unsampled species, a total of approximately 64 species should be present in the two geographic locations. This estimate is close to the one obtained from the pooled data using species individual curves (59.8) (Figure 2 *c*). There are a number of rea-





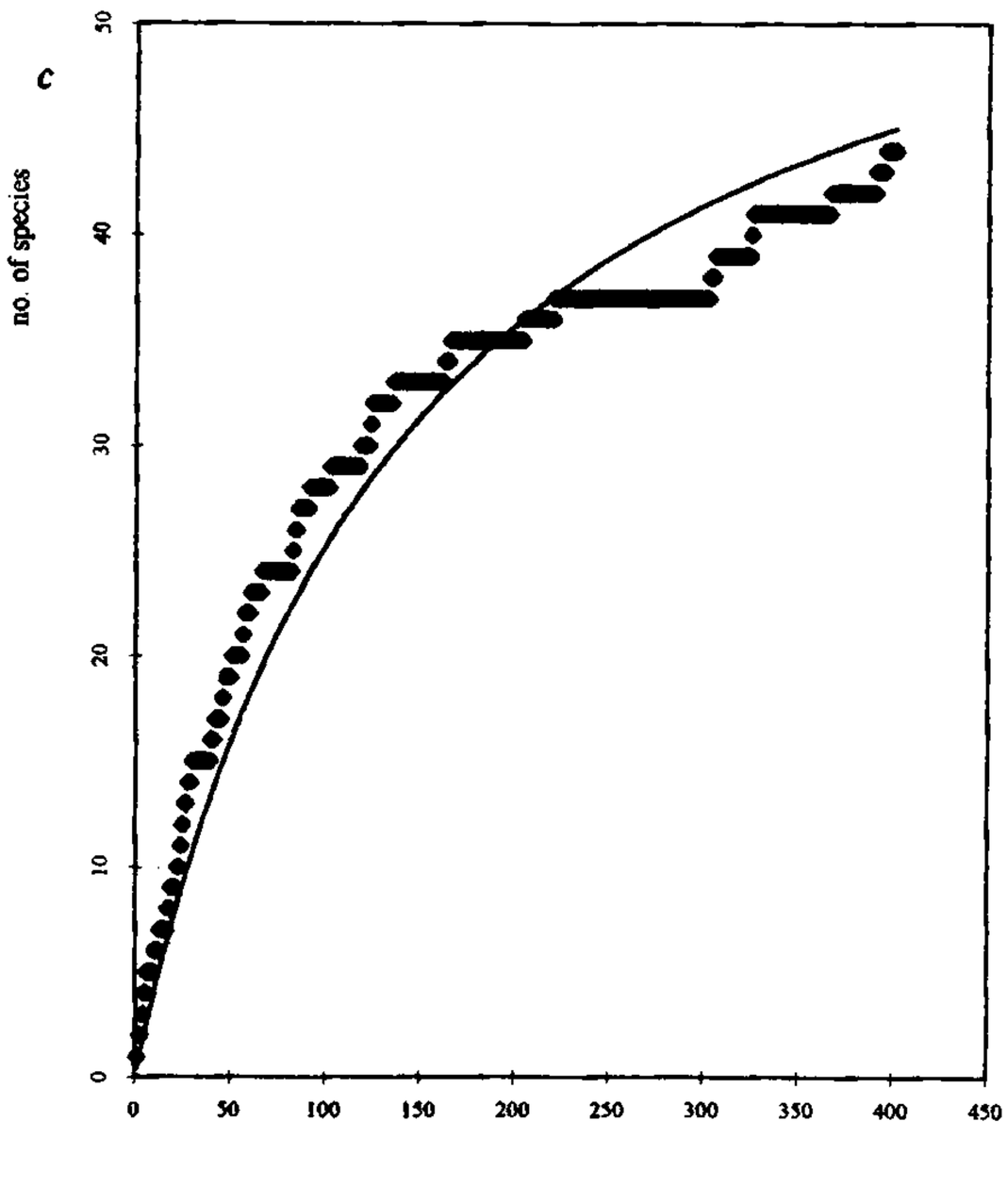


Figure 2. Species individual curves for myxobacterial species *a*, in Pune region; *b*, in Lucknow region; *c*, for pooled data from Pune and Lucknow. A Michaelis–Menten equation $S = S_{max}N/(K_m + N)$, where S is the number of species, N the number of individuals, S_{max} the maximum number of species that could be present and K_m the Michaelis–Menten constant, was fitted to the data. Estimated parameters for the best fit Michaelis–Menten equation in each case were *a*, $S_{max} = 34.8$, $K_m = 45$; *b*, $S_{max} = 41.1$, $K_m = 122$; *c*, $S_{max} = 59.8$, $K_m = 142$.

sons to believe that both of them would be gross underestimates. Paranjape and Gore[18] have shown that calculating species richness from species individual curves results in an underestimate for sample sizes comprising less than 6000 individuals. For samples of less than 1000 individuals the estimate can be less than 25% of the true number. The estimate reflects only two relatively small geographic areas of India and a limited va-

riety of habitats. Considering the habitat diversity of India and the observed floral differences between locations and between habitats, a conservative guess for the number of species in India should be a few hundreds and a liberal one up to a thousand. This is remarkable since the *Bergey's Manual of Systematic Bacteriology* lists only 40 species from all over the world.

This quantitative argument is backed by the novelty of some of the morphotypes. On the one hand, we have morphotypes 13 and 14 (Table 2) which can be comfortably put into existing genera but perhaps require the status of a new species, on the other, there are unidentified OTUs such as 1, 2, 4 and 9 (Table 2) which are radically different from all existing groups of myxobacteria. The morphotypes 2, 4 and 9 perhaps form one close group since they resemble in spore morphology as well as the pompom toy-like woolly spherical sporangia, but differ in sporangial size and stalk. We have not seriously pursued the taxonomy of these types but if morphological novelty reflects phylogenetic novelty, this preliminary exploration of the Western Ghats indicates that many more surprises are likely to be witnessed on further exploration.

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Arbuscular mycorrhizal fungal diversity of stressed soils of Bailadila iron ore sites in Bastar region of Madhya Pradesh

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Arbuscular mycorrhizal (AM) fungi help in sustenance and conservation of tropical plant gene pool and diversity. Iron-stressed soils of Bailadila Iron Ore Project (BIOP), Bastar, Madhya Pradesh were studied for the occurrence, distribution and diversity of these crucial microbial components of bulk soil and rhizosphere. Eighteen soil and 10 plant samples were collected. Soil pH varied between 6.5 and 7.5; N content between 0.05 and 1.25% and P content between 2 and 13 ppm. Eighty-nine species of AM fungi scattered over 6 genera were recovered. *Glomus* species were the most dominant, constituting 56.82% of the total isolates followed by *Acaulospora* (21.35%), *Scutellospora* (15.73%) and *Gigaspora* (3.37%). *Entrophospora* and *Sclerocystis* were represented by a single species each. Amongst the various species, *S. pachycaulis*, *A. scrobiculata* and *G. intraradices* were the dominant forms in order of their appearance. Natural AM infection in plants collected from various sites varied between 25 and 90%. Spore population was strikingly low in the soils (0–2000 kg^{-1} soil). There was no direct relationship between soil nutrient status and percentage of infection or spore density. All the eight species of angiosperms and a single species of pteridophyte showed an average to high level of mycorrhizal infection (25–90%). The AM dependency of the host species in the iron-stressed habitats seems to be quite high and may play a significant role in establishment under metallotoxic conditions. The capacity of the native AM isolates to survive under iron stress may be instrumental in reclamation of disturbed sites.

ARBUSCULAR mycorrhizal (AM) fungi are ubiquitous in the terrestrial habitats invading over 80% of the land plants[1–3]. Taylor *et al.*[4] have demonstrated the establishment of this mutualistic relationship as early as Lower Devonian period. The structural and functional

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