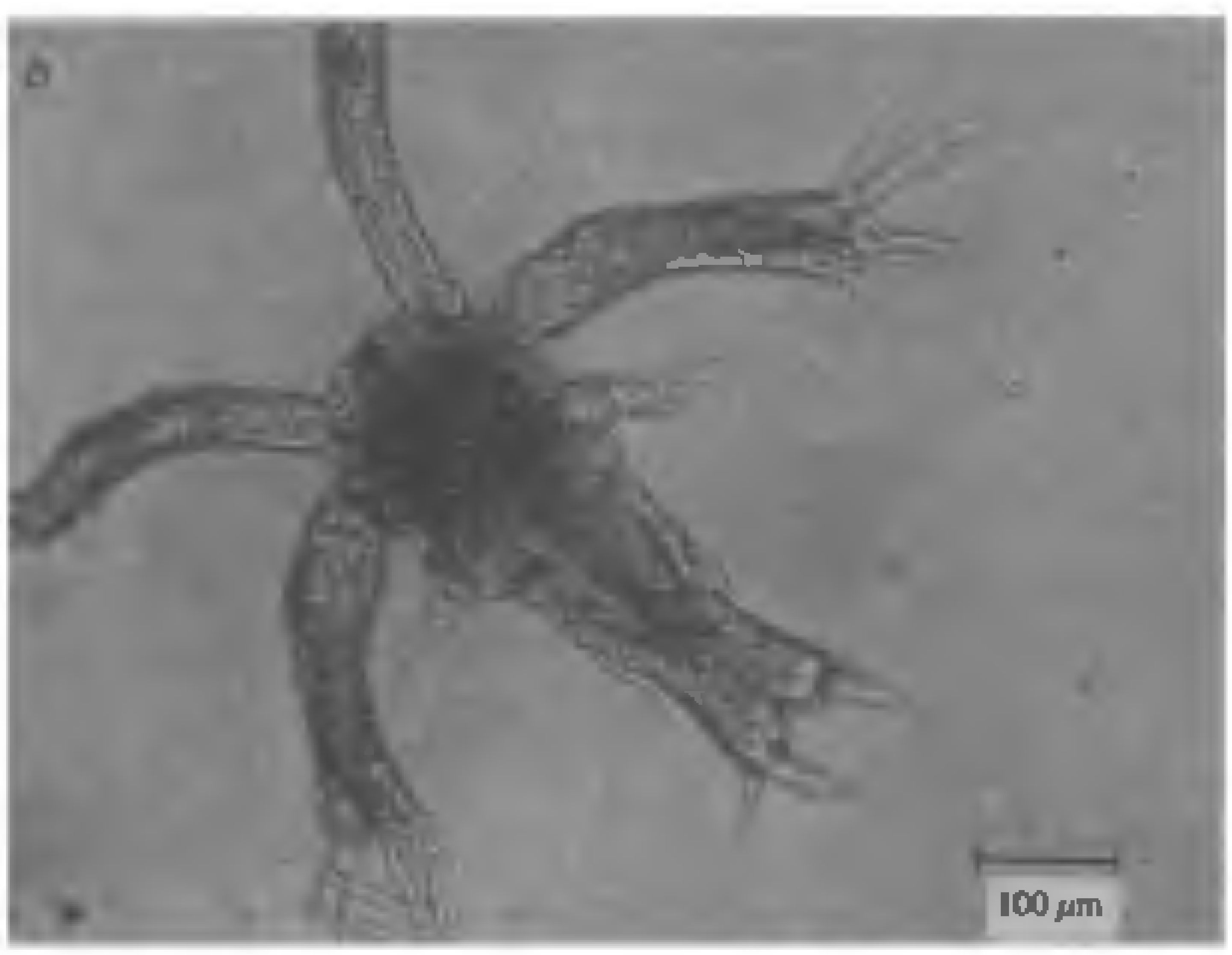

Figure 1. ***a***, Eversion (arrow) in vitrified stage II nauplius of *P. indicus* when the vitrification medium was diluted step-wise. ***b***, Stage VI nauplius of *P. indicus* frozen to −40°C using the slow cooling procedure and thawed at >300°C/min.

lar medium, thereby forcing the cell to first dehydrate by efflux of water and then equilibrate by influx of cryoprotectant. This exchange causes an intracellular build up of cryoprotectant, that continues until the freezing point of the cryoprotectant is reached, which is always, much below that of the freezable water. Rapid thawing, though helpful in preventing intracellular recrystallization, does not allow sufficient time for the cell to equilibrate with the surrounding medium, making slow dilution necessary. The high percentage of survival, 82% in nauplii frozen to −30°C and 63% in nauplii frozen to −40°C, (Figure 1,*b*) using 15% v/v ethylene glycol suggests that rapid thawing and slow dilution can be incorporated in the protocol for cryopreservation of nauplii of penaeid prawns (Table 1). However, it remains to be seen, and will be the focus of our immediate investigations, if slow thawing and rapid dilution will yield better results.

Table 1. Freezing of prawn nauplii—experimental protocol

Cryoprotectant	Ethylene glycol, 15% v/v
Addition	Two steps at 15°C
Equilibration time	15 min
Cooling rate	−1.5°C/min
Seeding	−6°C
Final temperature	−40°C
Thawing rate	Rapid >300°C/min
Dilution	Slow

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Effect of mixed inoculation with isogenic strains of *Bradyrhizobium* on total nodules of pigeonpea

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Interstrain competition is known to influence the proportion of nodules formed by rhizobia from a mixed population. Here we show that inoculation with mixed strains of *Bradyrhizobium* not only influences the proportion of nodules but also the total number of nodules formed on pigeonpea.

COMPETITION among strains of rhizobia for occupying infection sites on root system is a common phenomenon operating in soil[1]. This leads to formation of varying proportions of nodules by competing strains. Some non-nodulating strains of rhizobia may block nodulation by nodulating strains[2–4]. Instances of bacteria such as *Erwinia herbicola* affecting nodulation by *Rhizobium meliloti* are also known[5]. However, there is very little information about the influence of mixed strains of rhizobia on the total number of nodules. Such influence we report here.

A wild-type strain of *Bradyrhizobium* sp. (*Cajanus*)

Table 1. Actual and calculated values of number of nodules on pigeonpea plant due to inoculation with the wild type strain and its Azi^r mutants as single or mixed cultures. (Values mean of 20 plants in 10 leonard jars)

Single strain inoculation			Mixed strain inoculation		
				No. of nodules formed	
Strain	Genotype	No. of nodules formed	Strain	Actual	Calculated†
ARS39(w)	wild	12 8			
Azi5	mutant	41 5**	W+Azi5	12.5*	27.2
Azi30	mutant	22 0	W+Azi30	14.0	17.4
Azi36	mutant	12.5	W+Azi36	18.7	12.7
Azi50	mutant	27.3*	W+Azi50	12.8	22.1
Azi51	mutant	23.5	W+Azi51	19.4	18.2
Azi52	mutant	8.0	W+Azi52	32.0**	10.4

SEM = 7.1, LSD $P<0.05 = 14.4$, $P<0.01 = 19.2$.

†Values mean of nodules produced by each strain as single strain inoculant.
*&** Significant at $P<0.05$ and $P<0.01$ respectively.

ARS39 and its six spontaneous azide-resistant mutants were examined for their ability to nodulate pigeonpea cv. Pusa 33 as single cultures as well as in pairs with the wild-type strain. Mutants were isolated through different experiments by plating 0.1 ml stationary phase cell suspension (10^3–10^8 cells ml^{-1}) on yeast extract L-arabinose agar containing $>36\,\mu g$ NaN_3 ml^{-1}. Sporadic colonies, which appeared after seven days were checked for purity and resistance to higher concentrations of sodium azide. All the six mutant strains used in this study were resistant up to 200 μg NaN_3 ml^{-1} medium and retained this resistance even after the plant passage. The inoculum mixture contained equal number of cells of each strain and the total population was 10^8 cells ml^{-1}, equivalent to the population in single strain inoculum. The study was conducted in replicated Leonard jars[6], under bacteriologically controlled conditions in glasshouse during the monsoon season.

Examination of roots after 30 days of sowing revealed that the number of nodules formed by two mutants (Azi5 and Azi50) were significantly more than the nodules formed by the wild type strain (Table 1). Among mixed inoculation treatments, a combination of wild type strain and Azi52 produced significantly more nodules than formed by two strains individually. Since the ratio between wild type and mutant strains in the inoculum mixture was 1:1, theoretically each strain should get equal opportunity of infecting the roots and total number of nodules should be equal to the mean of number of nodules formed by two strains when inoculated individually. The significant deviations in nodule number by the mixture of wild type and Azi52, and wild type and Azi5 show that there was interaction between the two inoculant strains, which affected the total nodulation. Though the total number of nodules on the roots is controlled by the host plant[7], it appears that the cumulative effect of interactions between the strains in mixed population, also stimulate or inhibit the total nodulation on root system.

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An unusual giant pycnogonid (Pycnogonida–Colossendeidae) *Decolopoda qasimi* sp. nov. from Antarctic waters

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Five specimens of benthic pycnogonids collected from the Southern Ocean are described. Of these, two are identified as *Nymphon australis* (Hodgson) and two as *Ecleipsotherma spinosa* (Hodgson). One specimen under the class Colossendeidae, is described as new to science, *Decolopoda qasimi*, sp. nov.

THE pycnogonid fauna of the Southern or the Antarctic Ocean is reportedly richer than the Arctic and includes a large assemblage of the world's polymerous species[1,2]. Colossendeids and Nymphonid species are commonly known from this region and 14 genera and approximately 100 species have, so far, been reported[3–13]. Gigantism amongst the species and even in some