

## Rare observation of sea anemone *Calliactis polypus* on carapace of spider crab, *Doclea muricata* at the Covelong fish landing centre, Chennai, India

Observing the mutualistic relationship between sea anemone and hermit crab as well as gastropod shells is common. In fact, it is a good example of symbiosis in the marine environment. However, it is not common to find sea anemone on the true crab carapace. Few reports are available from the Indian waters<sup>1</sup>. During our routine visit to Covelong fish landing centre (12°47'31"N, 80°15'04"E), Chennai, for marine biodiversity assessment, on 29 August 2021, we observed a spider crab, *Doclea muricata* (Herbst, 1788) with a sea anemone on its carapace (Figure 1). Upon careful exami-

nation, it was identified as *Calliactis polypus* (Forsskål, 1775). According to the fisherfolks, the specimen was collected at ~5 m depth within 2 km from the Covelong coast. The sea anemone weighed 2.58 g and had a basal disk diameter of 1.65 cm. The crab was identified as a male, having carapace length and width of 4.9 and 4.4 cm respectively, and body weight of 33.44 g. The crab and sea anemone exhibit a mutualistic association. The crab provides substrate for sea anemone attachment and mobility for feeding, while the sea anemone provides protection

from predators<sup>2</sup>. Since not much information exists on the association of the reported crab and anemone species, it is difficult to infer that *D. muricata* and *C. polypus* are under commensal or mutualistic relationship. In the future, we will try to collect additional data to understand their relationship. In case we get live samples, we will carry out behaviour studies as well.

**Conflict of interest:** The authors declare no competing interest.



**Figure 1.** Sea anemone *Calliactis polypus* (Forsskål, 1775) on the carapace of spider crab *Doclea muricata* (Herbst, 1788).

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## Phylogenetic relationship between two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa*

The genus *Drosophila* is a unique and good biological model. It exhibits extensive diversity at the species level. At global level, there are more than 1500 species, including about 500 species from the Hawaiian Islands. In India, about 150 species are known to occur which includes both new species and new records. Further, the species which have been studied genetically also present variable degree of genetic di-

versity. In 1942, Mayr<sup>1</sup> gave the concept of sibling species which are morphologically similar but reproductively isolated. Since then, a large number of sibling species have been reported in the animal kingdom. However, they are more common in insects. In *Drosophila*, there are about hundred sibling species occurring in pairs/groups. The first pairs of sibling species reported in *Drosophila* are *D. mela-*

*nogaster* and *D. simulans*, as well as *D. pseudoobscura* and *D. persimilis*.

*D. ananassae* was described for the first time by Doleschall<sup>2</sup> in 1858 from Indonesia. It is a cosmopolitan and domestic species, but mainly circumtropical in distribution. Futch<sup>3</sup> studied speciation in South Pacific populations of *D. ananassae*, and reported light (brownish-yellow) and dark (blackish-brown) forms of this species.

Interestingly, they are morphologically similar in spite of the differences in pigmentation and lack post-mating reproductive isolation. They exist side by side and thus are sympatric and remain separate as reproductively isolated units. Later, these two forms, light and dark, were separated into different species by Bock and Wheeler<sup>4</sup>: the dark form as *D. ananassae* – polytypic cosmopolitan and domestic species and light form as *D. pallidosa* – localized as endemic species occurring in South Central Pacific Islands of Samoa and Fiji. Taxonomically, they were separated just on the basis of ethological isolation and differences in the number of sex comb teeth. Futch<sup>5</sup> studied ethological isolation between the species and designated them as a pair of sibling species. Singh<sup>6</sup> reviewed the cases of sibling species in the genus *Drosophila* showing evolutionary significance. *D. ananassae* and *D. pallidosa* are a unique pair of sibling species which have identical male genitalia and lack post-mating reproductive isolation<sup>7,8</sup>. Singh and Singh<sup>9</sup> reviewed the results obtained in this pair of sibling species with particular reference to their evolutionary relationship and suggested that *D. pallidosa* is a species in *statu nascendi*. Here, the recent published work pertaining to mate discrimination, the effect of age on mating propensity, sexual isolation, crossability, sex ratio, productivity, mating preference and initiation of sterility in hybrid males is summarized in the context of their phylogenetic relationship. In Figure 1, females, males and sex combs of both the species are shown<sup>8</sup>.

Schug *et al.*<sup>10</sup> and Grath<sup>11</sup> quantified the level and pattern of mate discrimination as well as genetic differentiation using mitochondrial DNA polymorphism in 18 populations of cosmopolitan species *D. ananassae* from its entire geographical range and its sibling species *D. pallidosa* having restricted geographical distribution in the South Pacific Island. It was found that mate discrimination shows considerable variation throughout the range of species and maximum in South Pacific. These authors have suggested that colonization and genetic divergence may have an effect on the evolutionary origin of mate discrimination<sup>10,11</sup>. Interestingly, they also suggested the ancestral relationship of these populations from the South Pacific which shows strong mate discrimination and lends support to the conclusion that they may be in the early stages of cladogenesis. Besides this, their results of behavioural

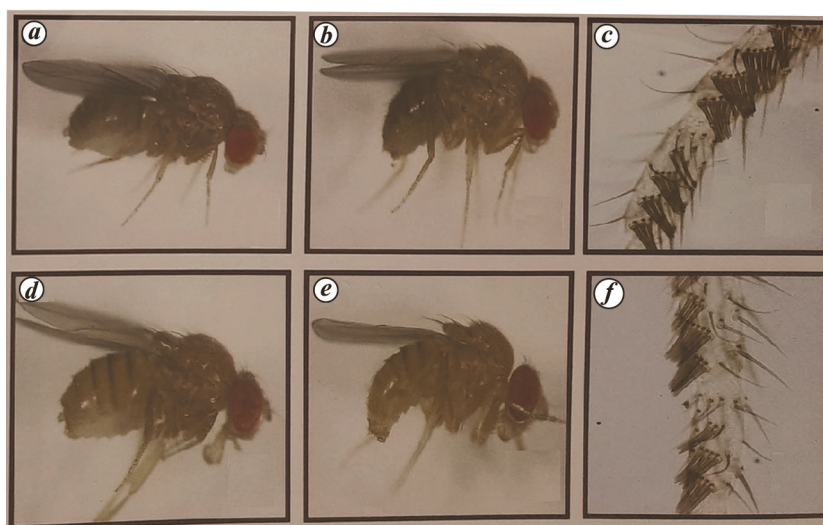
and genetic studies also demonstrate that the status of *D. pallidosa* as an independent species is doubtful and it cannot be designated as a ‘good species’.

Vishalakshi and Singh<sup>12</sup> studied crossability, productivity and sex ratio between these species and their hybrids, and discussed the results in context of their evolutionary divergence. They found that the degree of crossability and productivity was higher in matings of females and males of the same species than the reciprocal crosses. However, there was no sex ratio distortion in interspecific hybrids, indicating lower degree of genetic incompatibilities between these two sibling species<sup>12</sup>. It is known that higher the degree of evolutionary divergence between the hybridizing species, greater is the chance of the hybrids being developmentally unstable, which may be reflected in sex ratio distortion. Since 1 : 1 sex ratio was found in hybrids, it was taken as evidence for recent divergence between these two sibling species.

The effect of age on mating propensity was tested in this pair of sibling species by employing five wild-type strains of *D. ananassae* and three wild-type strains of *D. pallidosa*, all of different geographic origins<sup>13</sup>. Mating was directly observed in an Elens–Wattiaux mating chamber. For testing the effect of age, five groups of different ages were selected (4, 8, 12, 16 and 20 days) in every strain of both species. There was significant variation among the strains of both species. An increase in mating propensity was found up to 12 days, which then declined in both species. When

both the species were compared, mating propensity was higher in *D. ananassae* than *D. pallidosa*. This is expected because *D. ananassae* is a cosmopolitan and domestic species endowed with a high degree of genetic variability compared to *D. pallidosa* which is endemic to New Caledonia, Samoa, Tonga and Fiji Islands where it is sympatric and gene flow is prevented by ethological isolation. Both the species showed age-wise variation as well as strain-wise variation, but the former was more pronounced than the latter in *D. ananassae*. On the other hand, *D. pallidosa* showed opposite results. Both the species showed identical pattern as far as the effect of age was concerned, indicating that their divergence is a recent phenomenon<sup>13</sup>.

Singh and Singh<sup>14</sup> conducted experiments to study the fertility of hybrids males, testis morphology and protein content of testes in parental species and their hybrids. Interestingly, there was some indication of reduction in fertility of hybrid males, although it was not significant. Comparison of testis morphology between the two species and their hybrids showed no difference. However, protein content remained low in hybrid males compared to parental species. These results taken together demonstrate that the two species have not diverged much; rather the rate of speciation is slow, although they initially diverged 1.68 MYA. During this time other pairs have diverged much compared to *D. ananassae* and *D. pallidosa*<sup>15</sup>. Low level of sterility in hybrid males and similar testis morphology in parental species and hybrids



**Figure 1.** *Drosophila ananassae* and *Drosophila pallidosa*: two sibling species. **a**, Female of *D. ananassae*. **b**, Male of *D. ananassae*. **c**, Sex comb of *D. ananassae*. **d**, Female of *D. pallidosa*. **e**, Male of *D. pallidosa*. **f**, Sex comb of *D. pallidosa*<sup>8</sup>.

certainly provide evidence that the rate of speciation between these two sibling species is slow and they are still in the process of speciation (incipient species). Singh and Singh<sup>16</sup> also studied mating preference and productivity in these two species and their hybrids. It was found that females of both species were more discriminative for mating against alien males rather than conspecific males. This discrimination was stronger for *D. ananassae* females which are considered as ancestral and cosmopolitan species. Further, there was no decrease in the production of progeny or viable offspring compared to conspecific males. Although both hybrid sons were producing less number of progeny in comparison to all crosses, the difference was not significant statistically<sup>16</sup>.

Sexual isolation has been studied between light and dark forms of *D. ananassae* and also between the two species when they were designated as sibling species under laboratory conditions (for references, see Singh and Singh<sup>8</sup>). In fact, sexual isolation in the sympatric natural populations of these two species is the basis for their separation as independent species. In their most recent study, Singh and Singh<sup>17</sup> conducted sexual isolation tests between these two species by employing different strains of both species. They found evidence for both intra- and inter-specific sexual isolation in these two species. Interestingly, *D. pallidosa* being an endemic species showed greater degree of intraspecific sexual isolation compared to its sibling cosmopolitan species. On the basis of greater degree of sexual isolation within *D. pallidosa* than between the two species, Singh and Singh<sup>17</sup> suggested parallel evolutionary divergence within and between the species. It is likely that the rate of evolutionary divergence within the species may overtake the rate of divergence between these two sibling species. Using molecular techniques, it has been demonstrated that there is involvement of certain loci in mate discrimination and willingness of females to mate, which are located in 2L, XL and 3R (refs 18, 19).

Thus, sexual isolation between these two sibling species has a genetic basis. Singh<sup>20</sup> has also indicated that they have ancestral and derived relationship: *D. ananassae* has given rise to *D. pallidosa*. The degree and pattern of sexual isolation has been used to discuss the phylogenetic relationship and direction of evolution between the species<sup>21</sup>. These two sympatric sibling species remain isolated in natural sympatric situations through sexual isolation which is the basis of their separation as independent species<sup>4</sup>.

Futch<sup>3</sup> studied speciation in South Pacific populations of *D. ananassae* and reported the occurrence of light and dark forms. He found cytogenetical and behavioural differences between the two forms. In 1972, Bock and Wheeler<sup>4</sup> named the light form as a new species, *D. pallidosa*. So the dark form is *D. ananassae*, cosmopolitan and domestic species, and the light form is *D. pallidosa* which is endemic to certain South Pacific Islands and their distribution is sympatric. Their separation is based on sexual isolation and differences in male sex comb teeth number. Male genitalia are identical in both the species. In the laboratory, they are crossable producing fertile hybrids and thus lacking post-zygotic isolation. In 1973, Futch<sup>5</sup> designated them as sibling species. Singh<sup>8</sup> suggested that it is a unique pair of sibling species. The results of recent studies conducted on this pair of sibling species described here clearly show that there are similarities and differences between them. Even the status of *D. pallidosa* as an independent species is doubtful, and it has been suggested that it is not a good species<sup>10</sup>. It may be in *statu nascenti*<sup>7,9</sup>. It is concluded that *D. pallidosa* is still in the process of speciation and the divergence between these two species is a recent event. Further, they are phylogenetically very close to each other and have an ancestral-derived relationship.

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