ON SOME NEW SPECIES OF INDIAN TRICHOGRAMMA
(HYMENOPTERA : TRICHOGRAMMATIDAE)*

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ABSTRACT. Six new species of Trichogramma namely, T. hesperidis, T. agriae, T.
pallidiventris, T. plaseyensis, T. poliae and T. raai, from India are described. Their
similarity to other Indian or exotic species is discussed. A key to all known Indian
species is presented. Crossing experiments between the new species and those resem-
bling them most closely are summarized to show their limited genetic compatibility or
incompatibility as the case may be.

INTRODUCTION

In the course of biosystematic studies on Trichogramma, several new species
were encountered in India, in addition to those already described (Nagaraja
and Nagarkatti, 1969). A few of these are morphologically distinct, although
there is evidence of many sibling species.

Morphologically identical (or nearly identical) populations that are re-
productively isolated have frequently posed a problem to the taxonomist
(DeBach, 1959). With parasite species the biological control worker, who has
access to live material, is in a better position to study such populations and
differentiate them on the basis of the habitats, which they occupy or hosts
which they attack. The recognition of such sibling species is of great impor-
tance to biological control (DeBach, 1960; Hafez and Doult, 1954).

Six species are described in this paper. In addition, differences between
them are discussed and the results of crossing tests between these species and
those with which they are most likely to be confused are included. Also given
is a key to the known species of Indian Trichogramma. This study has
revealed that some species may have a wider distribution than earlier believed.
For example, T. semblidis Auriv., had previously been reported from Europe and
the U.S.A. only, but has recently been discovered in India (Nagarkatti, 1972).

MATERIALS AND METHODS

All cultures of Trichogramma obtained in the field were reared on eggs of
Coreyra cephalonica St. in the laboratory at 26.6°C (±1.1) and 50% R.H.

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of specimens were prepared as follows: dead, dry adults, cleared in glacial acetic acid for about 12 hours were transferred to liquified phenol for 20-30 minutes and mounted in a phenol-balsam mixture. The latter part of this method is described by Wirth and Marston (1968). The genitalia of males were dissected on slide in the phenol-balsam mixture. Average body measurements are based on ten females. Crosses were conducted as described by Nagarkatti and Nagaraja (1968). The descriptions of colour of species are based on specimens reared on *C. cephalonica* under the conditions already specified.

**Descriptions of New Species**

1. **Trichogramma hesperidis**, sp. nov.

   Length 0.5 mm; width across head 0.2 mm.

   **Male**: Light yellow, with blackish checks, the sides of thorax, scutellum, leg segments and abdomen. Antennal flagellum with about 35 hairs nearly blunt and of moderate length, the longest being two and a half times the maximum width of flagellum (Fig 1). Fore wing fringe on tornus about one-fifth the width of wing (Fig. 7).

   **Genitalia** (Fig. 13): DEG with a broadly rounded posterior extremity, extruding slightly beyond level of GF; constrictions present at base of DEG; CS apparently bilobed, almost reaching tips of GF; MVP inconspicuous; central ridges paired but coalescing anteriorly and extending nearly two-thirds the length of genitalia, following which is a median ridge reaching anterior margin of gonobase; aedeagus together with apodemes about three-fourths the length of entire genitalia (Fig. 13a) and about two thirds that of hind tibia (Fig. 19).

   **Female**: Colour same as male but for the lighter abdominal tip. Ovipositor longer than hind tibia (Fig. 19).

   **Holotype** ♂, **allotype** ♀, unidentified Hesperiid eggs on paddy leaves at Kamalashile, South Kanara District, October 1969 (H. Nagaraja Coll.). Holotype (with dissected genitalia) and allotype deposited in the U.S. National Museum, Washington, D.C., Reg. No. 72529.

   **Paratypes** in the British Museum (Natural History), London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi (RRS Nos. 33/73, 34/73).

   **Remarks**: This species is almost identical to *T. flandersi* Nagaraja and Nagarkatti in its general appearance and the two should be considered sibling species, since they showed total reproductive isolation in laboratory crossing tests. The structure of the male genitalia differs slightly, the posterior extremity of the DEG being broader in the "waist" region just before the spatulate structure is formed and the less acute GF. The spatulate structure is also less rounded than in *T. flandersi*. 
Individuals appearing to belong to this species have also been obtained from the eggs of an unidentified lepidopteran on paddy at Kamalashile and from Hesperiid eggs at Bangalore, as well as at Motipur (Bihar), indicative of its wide distribution.

2. Trichogramma agriae, sp. nov.

Length 0.55 mm; width across head 0.2 mm.

Male: Black except for dull yellow inter-sclerital regions of thorax, anterior part of abdomen, tibiae and tarsi. Antenna with long, tapering hairs, longest of which is nearly three and a half times the maximum width of flagellum (Fig. 2). Fore wing with fringe on tornus about one-seventh the width of wing (Fig. 8).

Genitalia (Fig. 14): DEG highly sclerotized, triangular, with slight constrictions at base and blunt apex located below level of MVP; CS slightly below level of GF, the latter gently curving inwards, MVP small but distinct, located far below level of CS; CR apparently paired, running close together anteriorly, extending to nearly two-thirds the length of genitalia; a slight constriction present at suture between GB and rest of genitalia; aedeagus longer than apodemes, both together slightly shorter than hind tibia (Fig. 20).

Female: Same as male in pigmentation. Ovipositor long, nearly one and a half times the length of hind tibia (Fig. 20).

Holotype ♂, allotype ♀, Agrius convolvuli L. on Colocasia antiquorum; Devanahalli, near Bangalore, May 1969 (H. Nagaraja Coll.). Holotype (with dissected genitalia) and allotype mounted on slides in the U.S. National Museum, Washington, D.C., Reg. No. 72528. Paratypes in the British Museum (Natural History, London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi (RRS Nos. 31/73 & 72/73).

Remarks: This is the darkest of the species described in this paper and has the longest ovipositor. It somewhat resembles T. rojasi Nagaraja and Nagarkatti from Chile and T. plasseyensis, sp. nov. (vide infra) from India, but the short DEG and other characteristics such as long antennal hairs and ovipositor set this species apart from others.

3. Trichogramma pallidiventris, sp. nov.

Length 0.6 mm; width across head 0.2 mm.

Male: Dull yellow with black vertex, thoracic sclerites and abdomen. Antenna with long, tapering hairs, longest of which is 4 times the maximum width of flagellum (Fig. 3). Fore wing with fringe on tornus about one-fifth width of wing (Fig. 9).

Genitalia (Fig. 15): DEG horse-shoe shaped at base and slightly wider than rest of genitalia; MVP apparently absent; aedeagus distinctly longer than apodemes (Fig. 15a), both together longer than hind tibia (Fig. 21).
Female: Same as male in colour dorsally, except for whitish ventral aspect of thorax. Ovipositor long, about one and a half times as long as hind tibia (Fig. 21).

Holotype ♂, allotype ♀, Tryporyza incertulas (Walk.) on paddy at Kamalashile (South Kanara District), October 1969 (H. Nagaraja Coll.). Holotype (with genitalia dissected) and allotype mounted on slides, deposited in the U.S. National Museum, Washington, D.C., Reg. No. 72530. Paratypes in the British Museum (Natural History), London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi (RRS No. 35/73, 36/73).

Remarks: This species is morphologically almost identical to T. japonicum Ashm., the only difference being the whitish ventral aspect of thorax in females and a somewhat more extruded ovipositor than in the latter. The two species are, however, reproductively isolated as shown by crossing experiments. Therefore, T. pallidiventris and T. japonicum can be considered sibling species. Unlike T. japonicum, which is easily reared on Corcyra eggs, T. pallidiventris could be reared only with difficulty on the eggs of Corcyra and the culture could not also be maintained on this host fore more than a few generations.

4. Trichogramma plasseyensis, sp. nov.

Length 0.5 mm; width across head 0.18 mm.

Male: Brownish-yellow with blackish thoracic and abdominal sclerites. Antenna with 30-35 short, somewhat blunt hairs, the longest being about twice the maximum width of flagellum (Fig. 5). Fore wing with comparatively long fringe, that on tornus being about one-fifth the width of wing (Fig. 10).

Genitalia (Fig. 16): DEG highly sclerotized with slight constrictions, moderately bulging sides at base and acute apex extending beyond MVP but slightly below CS. CS slightly below level of GF; MVP small but distinct; CR paired, extending anteriorly only for a short distance (less than half the length of genitalia); aedeagus almost as long as or slightly longer than apodemes (Fig. 16a), both together slightly shorter than hind tibia (Fig. 22).

Female: Same as male in colour; ovipositor as long as or slightly longer than hind tibia (Fig. 22).

Holotype ♂, allotype ♀, Chilo infuscatellus (Sn.) at Polia farm, Plassey (West Bengal), September 1968 (K. Ramachandran Nair Coll.). Holotype (with dissected male genitalia) and allotype mounted on slides, deposited in the U.S. National Museum, Washington, D.C., Reg. No. 72531. Paratypes in the British Museum (Natural History), London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi (RRS No. 37/73, 38/73).

Remarks: This species is distinct in having small MVP, short antennal hairs and apex of DEG not reaching the level of CS but extending beyond MVP.
When crossed with *T. chilotraeae*, *T. plasseyensis* was found to be reproductively isolated from the latter (see under 'Crossing Experiments').

A few *Trichogramma* specimens later obtained from Dr. C. S. Li, formerly of Department of Agriculture, Stock and Fisheries, Bubia Loe, New Guinea, reared from *Chilo terrenellus* Pag. on sugarcane resembled *T. plasseyensis*. Crosses could not be conducted as live cultures were not available.

5. *Trichogramma poliae*, sp. nov.

*Length* 0.56 mm; width across head 0.18 mm.

*Male*: Light brownish-yellow with fuscous cheeks, thoracic sclerites and abdomen. Antenna with 30-35 tapering moderately long hairs, longest of which is about 3 times the maximum width of flagellum (Fig. 4). Fore wing with fringe on tornus about one-sixth the width of wing (Fig. 11).

*Genitalia* (Fig. 17): DEG with prominent narrow, broadly separated lateral lobes and apex almost reaching upper level of CS; CS below level of GF; MVP large, broad at base but below level of CS. CR paired, running anteriorly to less than half the length of genitalia; aedeagus longer than apodemes, both together shorter than hind tibia (Fig. 23).

*Holotype* ♂, *allootype* ♀, *C. infuscatellus* at Polia farm, Plassey (West Bengal), September 1968 (K. Ramachandran Nair Coll.). Holotype (with dissected genitalia) and allootype mounted on slides, deposited in the U.S. National Museum, Washington, D.C., Reg. No. 72532. *Paratypes* in the British Museum (Natural History), London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi. (RRS No. 39/73, 40/73).

*Remarks*: This species is almost identical to *T. australicum* morphologically. It differs however, from *australicum* in being slightly darker, having narrower lateral lobes of DEG which are also set far apart and fewer antennal hairs (30-35); in *australicum* this number exceeds 40. Moreover, *Corcyra* eggs parasitised by *T. poliae* turn brown instead of black as with *T. australicum*. These two species interbreed only to a very limited extent, indicating a high degree of reproductive isolation.

Among Trichogrammatids received from Dr. Tsong Hong Su, Taiwan Sugar Experiment Station, Tainan, Taiwan, reared from *Eucosma* sp. on sugarcane, two males and a female resembled *T. poliae*. Further material could not be obtained. It is, therefore, possible that *T. poliae* occurs in Taiwan.

6. *Trichogramma raoi*, sp. nov.

*Length* 0.45 mm; width across head 0.20 mm.

*Male*: Brownish-yellow with fuscous sides of pronotum, mesonotum, pleurae, abdomen and coxae. Antenna with 35-40 long tapering hairs, longest of which is nearly 4 times the maximum width of flagellum (Fig. 6).
Fore wing with fringe on tornus slightly less than one-sixth the width of wing (Fig. 12).

Genitalia (Fig. 18): DEG very narrow, weakly sclerotized with acute posterior extremity which ends far below level of CS; CS slightly below level of GF; MVP barely visible; CR apparently single, extending half-way up to anterior margin of GB; aedeagus longer than apodemes, both together slightly shorter than the entire genitalia and about two-thirds the length of hind tibia (Fig. 24).

Female: Colour same as male but for yellowish tip of abdomen. Ovipositor as long as or very slightly longer than hind tibia (Fig. 24).

Holotype ♀ alleotype ♂, eggs of an unidentified lepidopteran on sugarcane leaves at Kamalashile, South Kanara District, Mysore, October 1968 (H. Nagaraja Coll.). Holotype (with dissected genitalia) and allotype deposited in the U.S. National Museum; Washington, D. C., Reg. No. 72533. Paratypes in the British Museum (Natural History), London; Academy of Sciences, Leningrad; California Insect Survey, Berkeley; and Indian Agricultural Research Institute, New Delhi (RRS Nos. 41/73, 42/73).

Remarks: The colour and male genitalia of this species closely resemble those of T. achaeeae. However, in the male genitalia, the DEG in raoi, is narrower at the base and the posterior extremity is far below level of CS and the latter do not reach the level of GF. Also, in achaeeae the MVP is present though inconspicuous while it is scarcely visible in raoi. The male antennal hairs are also much longer in raoi than in achaeeae. Moreover, raoi and achaeeae do not cross as can be seen in the section Crossing Experiments’.

A key to the presently known Indian species of Trichogramma

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DEG with expanded spatulate posterior extremity extending beyond level of GF</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>DEG not as above</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>Male genitalia with sharply converging GF; spatula of DEG rounded posteriorly with narrow “waist”</td>
<td>flandersi</td>
</tr>
<tr>
<td></td>
<td>Male genitalia with GF not as sharply converging as above; spatula of DEG broadly rounded posteriorly with broader “waist”</td>
<td>hesperidis*</td>
</tr>
<tr>
<td>3.</td>
<td>DEG horse-shoe shaped at base; MVP apparently absent; ovipositor about 1½ times (or more) the length of hind tibia</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>DEG not as above; MVP present (though very inconspicuous in achaeeae and raoi)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ovipositor not as long as above (except in T. agriae)</td>
<td>pallidiventris*</td>
</tr>
<tr>
<td>4.</td>
<td>Females with white ventral region of thorax; ovipositor extruding markedly beyond last abdominal segment</td>
<td>japonicum</td>
</tr>
<tr>
<td></td>
<td>Females without white pigmentation on thorax; ovipositor not extruding as markedly as in above species</td>
<td>*New species</td>
</tr>
</tbody>
</table>
5. Dimorphic males; alate males with normal and apterous males with gynecoic antennae... semblidis

Monomorphic males

6. DEG with prominent lateral lobes

DEG without prominent lateral lobes

7. Lateral lobes of DEG narrow and widely separated; less than 40 hairs on male antennal flagellum; adults somewhat dark pigmented; parasitised Corcyra eggs turning brown... poliae*

Lateral lobes of DEG broader and not as widely separated as above; more than 40 hairs on male antennal flagellum; adults lighter pigmented; parasitised Corcyra eggs turning black... australicum

8. DEG narrow at base, Without marked constrictions, narrow posterior extremity having nearly parallel sides; MVP extremity minute; aedeagus with apodemes shorter than entire genitalia

DEG broader at base with marked constrictions; posterior extremity broadly converging; MVP distinct though small in some species; aedeagus with apodemes as long as entire genitalia

9. CS and posterior extremity of DEG reaching level of GF; male antennal hairs nearly 3 times maximum width of flagellum... achaee

DEG below level of CS; CS slightly below level of GF; male antennal hairs nearly 4 times maximum width of flagellum... raoi*

10. CS markedly below level of GF; MVP long, nearly reaching level of CS; DEG without marked constriction and not pulging at base... chiloreaee

CS slightly below level of GF; MVP small, far below level of CS; DEG with marked constriction and bulging slightly at base

11. Male antennal hairs long, tapering, longest being 3½ times maximum width of flagellum

DEG with blunt posterior extremity, not reaching level of MVP; ovipositor nearly 1½ times the length of hind tibia... agriae

Male antennal hairs short, almost blunt, longest being about 2 times maximum width of flagellum; DEG with short posterior extremity, extending beyond level of MVP but below level of CS; ovipositor only slightly longer than hind tibia... plasseyensis*

**CROSSING EXPERIMENTS**

Crosses have been conducted between the new species and the species resembling them closely, such as (1) *T. poliae* and *T. australicum*, (2) *T. hesperidis* and *T. flandersi*, (3) *T. plasseyensis* and *T. chiloreaee*, (4) *T. pallidiventris* and *T. japonicum* (strains of the latter from India and the Philippines were used), and (5) *T. raoi* and *T. achaee*. Crosses between *T. plasseyensis* and *T. agriae* could not be conducted as the culture of *plasseyensis* is not now available. Details of these crosses are given in Table I.

It can be observed from the morphological descriptions that *T. poliae* and *T. australicum* are nearly identical. However, when crossed they produced only a small percentage of $F_1$ females in both directions (5.8 and 1.6) and this in one
TABLE I. RESULTS OF CROSSES BETWEEN THE NEW SPECIES OF Trichogramma AND THOSE RESEMBLING THEM MOST CLOSELY

<table>
<thead>
<tr>
<th>Rep. No.</th>
<th>Crosses</th>
<th>No. of parental females</th>
<th>F₁ Progeny</th>
<th>% Female progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>1</td>
<td>australicum</td>
<td>poliae</td>
<td>26</td>
<td>270</td>
</tr>
<tr>
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<td>poliae</td>
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<td>107</td>
</tr>
<tr>
<td>3</td>
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<td>poliae</td>
<td>15</td>
<td>113</td>
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<td>175</td>
</tr>
<tr>
<td>2</td>
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<td>australicum</td>
<td>15</td>
<td>93</td>
</tr>
<tr>
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<td>poliae</td>
<td>australicum</td>
<td>15</td>
<td>121</td>
</tr>
<tr>
<td>1</td>
<td>hesperidis (Hebbal)</td>
<td>flandersi</td>
<td>25</td>
<td>740</td>
</tr>
<tr>
<td>1</td>
<td>flandersi (Hebbal)</td>
<td>hesperidis</td>
<td>17</td>
<td>248</td>
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<tr>
<td>1</td>
<td>chilotraeae</td>
<td>plasseyensis</td>
<td>27</td>
<td>129</td>
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<tr>
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<td>chilotraeae</td>
<td>27</td>
<td>192</td>
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<td>1</td>
<td>pallidiventris</td>
<td>japonicum (Kamalashile)</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>1</td>
<td>japonicum¹ (Kamalashile)</td>
<td>pallidiventris</td>
<td>15</td>
<td>1</td>
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<td>pallidiventris</td>
<td>japonicum (Philippines)</td>
<td>16</td>
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<tr>
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<td>japonicum (Philippines)</td>
<td>pallidiventris</td>
<td>16</td>
<td>112</td>
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<tr>
<td>1</td>
<td>raoi</td>
<td>achaearae</td>
<td>27</td>
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<tr>
<td>1</td>
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<td>raoi</td>
<td>27</td>
<td>481</td>
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<td>1</td>
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<td>rojasi</td>
<td>8</td>
<td>13</td>
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<td>1</td>
<td>rojasi</td>
<td>agriae</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

1. Low progeny production indicates insemination reaction; although females lived for one day oviposition was not normal as indicated by the single male in the F₁ generation.

of the three replicates. Since they interbreed only to a negligible extent, these could be considered as sibling species. It seems possible that ethological factors limit gene exchange between the two in nature, though some interbreeding is possible when they are brought together in the laboratory.
Similarly, *T. hesperidis* and *T. flandersi* though morphologically almost alike, show total reproductive isolation when crossed. *T. hesperidis* and *T. flandersi* can also be considered as sibling species.

*T. pallidiventris* and the Kamalashile population of *T. japonicum* do not interbreed, although they are sympatric and occur on the same host, that is, *Tryporyza incertulas*. There is also evidence of an insemination reaction as very few males emerged in the cross involving *pallidiventris* females and only one male emerged in the reciprocal cross. At the same time, *pallidiventris* when crossed with the Philippines population of *japonicum* not only yielded a fair number of $F_1$ progeny but also a small percentage of females in both directions (5.8 and 1.8). The two spatially separated populations of *japonicum* from the Philippines and Kamalashile however interbreed freely yielding normal percentage of females (76 and 77) and are also morphologically indistinguishable. This therefore appears to be a case of character displacement. Brown and Wilson (1957) observed that the ant *Lasius flavus* (L.) having a holarctic distribution differs both morphologically and ecologically from *L. nearcticus* Wheeler in north-eastern U.S. to which region the latter species is restricted. But elsewhere in its range *L. flavus* is rather similar to *L. nearcticus*. In the present case both *T. japonicum* and *T. pallidiventris* were collected only once from *T. incertulas* eggs at Kamalashile but they could be occurring on other hosts. It is also possible that the preferred host of *T. pallidiventris* is something other than *T. incertulas* and occurs on a different host plant in which case interspecific mating and hybridization in nature would be prevented. This aspect of study needs further investigations. According to Brown and Wilson (*loc. cit.*) when recently separated species become sympatric, selection favours strong differentiation which will reduce probability of wastage of gametes by matings of low fertility. This explanation probably holds good for the sympatric *japonicum* and *pallidiventris* which may be recently separated species.

**Conclusion**

Although the initial difficulties in identifying *Trichogramma* spp. have to some extent been resolved, the presence of numerous sibling species has added to the complexity of the problem. Rapid speciation is considered to occur amongst the Chalcidoidea (Askew, 1968) and is probably also true of the genus *Trichogramma*. Sympatric speciation appears as likely in this genus as allopatic speciation. Evidence of the former is borne out by the species *T. poliae* and *T. australicum* both of which occur in the same habitat and on the same hosts, are nearly identical morphologically, and yet exhibit reproductive isolation. That they may differ in physiology is evident from the fact that *Corcyra* eggs parasitised by *T. poliae* turn brown, while those attacked by *T. australicum* turn black (as with other species of *Trichogramma*). In this instance it is hard to understand how reproductive isolation has arisen in the absence of any con-
ceivable barriers. However, such sympatric evolution has been demonstrated in insects such as Maniola jurtina by Ford (1964) and may occur more commonly than we tend to believe. The same argument holds good for the reproductive isolation present between hesperidis and flandersi and between pallidiventris and japonicum.

Morphologically hesperidis and flandersi are nearly alike but show total reproductive isolation and attack different hosts. The former has been reared from P. mathias and the latter from C. infuscetellus. T. hesperidis has been collected at Bangalore, Kamalashile in Mysore State and Motipur in Bihar, while studies have so far indicated that T. flandersi is confined to the Mandya area in Mysore State. It is possible that T. flandersi is endemic to this area.

Similarly T. pallidiventris which has also not been recorded from other areas in India may be endemic to the Kamalashile area.

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REFERENCES


Diagrammatic sketch of male genitalia of *Trichogramma* with aedeagus separated out. Explanations to abbreviations: DEG-Dorsal expansion of gonobase; GF-Gonoforceps; CS-chelate structure, MVP-Median ventral projection; CR-Central ridges; GB-Gonobase.