Life History Traits of Three Cryptic Species Asia I, Asia II-1 and Asia II-7 of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Reconfirm Their Genetic Identities

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Life history traits of three cryptic species Asia I, Asia II-1 and Asia II-7 of *Bemisia tabaci* (Hemiptera: Aleyrodidae) reconfirm their genetic identities

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Abstract

The *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a pest of agricultural and horticultural crops. It is a species complex consisting of 34 cryptic species. For the distinction of these cryptic species molecular data is extensively used, but corroboration of these with life history traits has been inadequate. In the present study life history traits of 3 cryptic species Asia I, Asia II-1 and Asia II-7 were compared to verify whether biology data of these coincide with molecular data and genetic identities. The results revealed that developmental periods of Asia I, Asia II-1 and Asia II-7 groups ranged from 23.65 to 25.75 days and these were longer in Asia I than Asia II. Survivorships were nearly equal in all these varying from 68.23 to 69.12% with the variations being statistically insignificant. However, the durations of the preoviposition period, egg stage, fourth instar and longevity were observed to be significantly varying ($P \leq 0.001$). Multivariate analysis of the life history parameters through principal component analysis (PCA) revealed that the first 4 principal components (PCs) account for 49.5% of total variation. Separate clusters were observed for the Asia I, Asia II-1 and Asia II-7 with slight overlapping. Overall 70% of the classifications got correctly attributed through canonical discriminant analysis (CDA) and the clustering confirmed the groups revealed by principal component analysis (PCA). These clusterings were reconfirmed in the genetic identity of the 3 cryptic species Asia I, Asia II-1 and Asia II-7 determined through molecular characterization. Thus this study adds to the knowledge on the life history traits of the *B. tabaci* and its cryptic species complex in India.

Key Words: canonical discriminant analysis; cotton life history parameters; multivariate analysis; principal component analysis

Resumen

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) es una plaga de cultivos agrícolas y hortícolas. Su complejo de especies consta de 31 grupos genéticos o especies putativos. Para la distinción de los grupos genéticos de este complejo, los datos moleculares son utilizados ampliamente, pero la corroboración de estos con las caracteristicas de la historia de vida ha sido inadecuada. En este estudio se compararon las caracteristicas de la historia de vida de los grupos genéticos Asia I, Asia II-1 y II-Asia 7. El período de desarrollo de Asia-I, Asia II-1 y Asia II-7 fue desde 23.65 a 25.75 días, y fue mas largo en Asia I que en los grupos genéticos de Asia II. La sobrevivencia fue casi igual en todos los 3 grupos genéticos, que fue de 68.23 a 69.12% y la variación no fue estadísticamente significativa. Pero la duración del período de preoviposición, estadio de huevo, cuarto estadio y la longevidad de los 3 grupos genéticos observados fueron significativamente diferente ($P < 0.001$). El análisis multivariante de los parámetros de historia de vida a través de un análisis de componentes principales (ACP) reveló que los primeros 4 componentes principales (PC) representan el 49.5% de la variación total y se observaron grupos separados para los grupos genéticos Asia I, Asia II-1 y Asia II-7 con un poco de solapamiento. En general, el 70% de las clasificaciones se atribuyeron correctamente por el análisis discriminante canónico (CDA) y la agrupación confirmó los grupos revelados por el análisis de componentes principales (ACP). Estos grupos fueron idénticos a los grupos genéticos determinados por la caracterización molecular. Así, este estudio añade a los conocimientos sobre las características del ciclo vital de complejo de especies de *B. tabaci* y de sus grupos genéticos en la India.

Palabras Clave: análisis discriminante canónico; parámetros de historia de vida de algodón; análisis multivariante; análisis de componentes principales

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a haplodiploid, sap-sucking hemipteran pest in field crops of warm to hot climates between 30° N and 30° S of the equator. It is polyphagous and has been reported reproducing on more than 900 host plant species (Hsieh et al. 2006). In the last 2 decades, it has become a serious pest on short-lived herbaceous hosts including numerous dicotyledonous crops of agricultural and horticultural importance (Ahmed et al. 2010). The genetic complexity of *B. tabaci* was first recognized in the late 1950's (Bird 1957). Several populations were observed to be morphologically indistinguishable but differing in host range, host plant adaptability and

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their ability to transmit plant viruses (Perring 2001; Boykin et al. 2007). De Barro et al. (2011) stated that *B. tabaci* is a species complex of 11 well-defined high level groups containing at least 24 morphologically indistinguishable species. These were later concluded to consist of 34 cryptic species with differences in genetic structure, host plant preferences, bacterial symbionts and interbreeding capabilities (Hu et al. 2011; Liu et al. 2012; Boykin et al. 2012; Lee et al. 2013; Boykin, 2013; Boykin et al. 2013; Boykin & De Barro, 2014).

In India *B. tabaci* was first reported from cotton fields of Punjab in 1905 (Misra & Lambda 1929). Banks et al. (2001) reported the presence of invasive biotype B (now referred as Middle East Asia Minor 1, aka MEAM 1) from the fields of Kolar and Bangalore. Boykin et al. (2007) differentiated the Asian *B. tabaci* populations into Asia I, Asia II and China groups. Ahmed et al. (2011) reported Asia I, Asia II-1 and MEAM 1 from Punjab and Sindh province of Pakistan. Reddy et al. (2012) further resolved these and showed that Asia I, Asia II-1, Asia II-5, Asia II-7, Asia II-8 and MEAM1 groups are present in the Indian subcontinent and Asia.

Numerous studies elaborate the biology, ecology and developmental characteristics of *B. tabaci* and its biotypes, i.e., B biotype (Perring 2001; Brown & Czosnek 2002; Jones 2003; Horowitz et al. 2005; Liu et al. 2007), Cv biotype (Qiu et al. 2011), Q biotype (Drost et al. 1998; Muniz & Nombella 2001) and Asia II-1 and MEAM 1 cryptic species (Ahmed et al. 2014). Biological variations of *B. tabaci* had also been reported according to host plants, viz., cotton (Bethke et al. 1991; Thomas et al. 2011), sweet pepper (Muniz & Nombella 2001), eggplant and tomato (Tsai & Wang 1996), and soybean and garden bean (Mansaray & Sundufu 2009). But corroboration of life history traits with molecular data and genetic grouping had been largely inadequate. In the present study, we compare the life history traits of 3 cryptic species Asia I, Asia II-1 (formally referred to as ZHJ2 biotype) and Asia II-7 (formally referred to as Cv biotype) collected from the agroclimatic zones of India, and reared on cotton under controlled environmental conditions.

Materials and Methods

WHITEFLY POPULATIONS

Populations of *B. tabaci* were collected from 5 locations (Amravati, N 21°01'604" E 77°55'004", 367 m; Kalyani, N 23°37'36.2" E 87°44'09.4", 38 m; Ludhiana, N 30° 53'932" E 75 48'229", 240 m; Sriganganagar, N 29°45'205 " E 74°04'854 ", 171 m and Delhi, N 28°38'28.1" E 77°10'12.2", 213 m asl) in various agroclimatic zones of India from their host plants viz., cotton (*Gossypium* spp.; Malvales: Malvaceae), brinjal (*Solanum melongena* L.: Solanales: Solanaceae) and leucaena (*Leucaena* spp.; Fabales: Fabaceae). These were reared for 6 generations on line 'RCH138 BGII' of hybrid cotton in an insect proof climate control chamber at the Indian Agricultural Research Institute, New Delhi, India. The methodology for maintaining pure colonies followed was as described by Luan et al. (2008). Briefly each *B. tabaci* population was maintained in a separate insect proof climate control chamber in an acrylic cage (61 × 61 cm) on line 'RCH138 BGII' of hybrid cotton at 28 ± 2 °C, 60 ± 5% RH and 10:14 h L:D. The purity of each of the 3 cryptic species was monitored every alternate generation using mitochondrial cytochrome oxidase 1 (mtCO1) gene sequence analysis. The samples drawn from these cultures were used for biological studies on the above line of hybrid cotton as well as for molecular confirmation of their genetic identities.

DNA EXTRACTION

Genomic DNA was extracted from the whole adult female body as described by De Barro & Driver (1997). The PCR primers were employed to amplify a mtCO1 gene fragment (800-820) of *B. tabaci* (Frohlich et al. 1999) with the following PCR conditions: initial denaturation at 95 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 47 °C for 40 s and 72 °C for 1 min with final extension of 72 °C for 10 min. The PCR products were purified and sequencing was performed by Scigenomics Pvt. Ltd. (Cochin, India).

The mtCO1 sequences obtained here were compared with those of the *B. tabaci* species complex from GenBank (Dinsdale et al. 2010; Hu et al. 2011). The sequences were aligned with the CLUSTAL W algorithm (Thompson et al. 1994) and distances were calculated with the Kimura 2-parameter model of MEGA5 (Tamura et al. 2013). The NJ (Neighbour-Joining) program available in MEGA6 (Tamura et al. 2013) was used to infer phylogenetic relationships, using *B. afer* (Priesner & Hosny) as an outgroup. One thousand bootstrap replicates were performed for each analysis.

DEVELOPMENT

To determine the duration of the various developmental stages, males and females were confined on the abaxial surface of a fully expanded leaf in micro-cages, as described by Zang et al. (2005) and Xu et al. (2010). The eggs laid were observed at 24 h intervals. Transitions not directly observed were inferred from changes in morphology and size, or from the presence of exuviae. A Leica ES2 stereozoom microscope was used to observe the life cycle stages and observations were replicated 20 times for each genetic group.

SEX RATIO AND PREOVIPOSITION PERIODS

To evaluate the sex ratio, the adults emerging from the puparia were collected cage by cage and anaesthetized with carbon dioxide for 15–20 s; and their sexual gender was determined while these were inactive under the Leica ES2 stereozoom microscope taking into account their genitalia (Gill 1990). Twenty pairs of newly emerged adults (< 2 h) from each genetic group were randomly selected and confined in 20 clip cages for periodical observations on the preoviposition period.

LONGEVITY AND FECUNDITY

To analyse longevity and fecundity, newly emerged males and females were confined in pairs in clip cages having minute holes on both sides (3 × 3 mm), on leaves of 5 to 10 day-old plants. Longevities of males and females, and numbers of eggs laid were recorded daily from 20 such pairs.

CLUSTERING OF CRYPTIC SPECIES

Univariate one way single factor ANOVA was performed individually for all the observations on life cycle stages to find out the significant variables (Kalaisekar et al. 2012). After this the pattern of clustering was analyzed using multivariate statistical approaches (Tabachnick & Fidell 2007). The Principal Component Analysis (PCA; SAS procedure; PRINCOMP; SAS version 9.1.3, SAS Institute Inc., Cary, NC, USA) was used without any prior assumption of grouping, and which assesses the components for total variation among the specimens by calculating linear combinations of variables that explain the maximum of the total variation. Canonical Discriminant Analysis (CDA; SAS procedure; CANDISC) was used for calculating the linear combinations of variables that maximize the separation of means of previously defined classes. Contributions of the variables best summarizing the differences between classes are revealed by this technique. Since, Discriminant Func-

tion Analysis (DFA; SAS procedure; DISCRIM) maximizes the variation among groups, it was used to separate cryptic species of *B. tabaci* based on life history data.

Results

GENETIC IDENTITY

The PCR amplification of mtCO1 gene sequence was edited to remove PCR primer sequences, which yielded a ~750-bp fragment for each *B. tabaci* populations from the Amravati (KF298442), Kalyani (KF298441), Ludhiana (KF298443), Sriganganagar (KF298439) and Delhi (JQ023501) and these were compared with sequences of assigned members of *B. tabaci* species complex. Analysis of mtCO1 revealed that these were assignable to the Asia I, and Asia II groups namely Asia II-1 (formally referred to as the ZHJ2 biotype) and Asia II-7 (formally referred to as the Cv biotype). The Amravati and Kalyani populations clustered with Asia I, those of Ludhiana and Sriganganagar with Asia II-1, while the Delhi populations clustered with Asia II-7 cryptic species complex. All these populations showed 100% similarity with their respective sequences of assigned members of *B. tabaci* species complex.

DEVELOPMENT

The developmental periods of the 3 cryptic species of *B. tabaci* are shown in Table 1. The total developmental periods of Asia I, Asia II-1 and Asia II-7 ranged from 23.80 to 25.75 days. This period was slightly longer in Asia I than in the Asia II. Statistical analysis showed a significant difference in the total developmental period ($F = 6.6246$, df = 4, $P < 0.001$). The developmental periods of the egg, and the first, second, third and fourth instars were longer in Asia I than in Asia II, among which the durations of the egg and the fourth instar periods were significantly varying ($F = 14.6818$, df = 4, $P < 0.001$; $F = 4.8298$, df = 4, $P < 0.001$ respectively).

SEX RATIO AND PREOVIPOSITION PERIOD

Results given in Table 1 indicate that females are always more numerous than males regardless of cryptic species. The highest sex ratio was recorded in Asia II-7 followed by Asia II-1 and lastly by Asia I, however the differences were not significant ($F = 1.8496$, df = 4, $P = 0.05423$). The preoviposition period, i.e., the period between adult emergence and egg deposition was the longest in Asia II-7 followed by Asia II-1 and shortest in Asia I, and these differences were statistically significant ($F = 3.8298$, df = 4, $P < 0.001$).

Table 1. Development parameters of the genetic groups of *Bemisia tabaci* (mean ± SE in days, $n = 20$).

Developmental stage/ Component	Asia I	Asia II-1	Asia II-7
Egg (days)	8.25 ± 0.18	7.35 ± 0.13	6.65 ± 0.21
First instar (days)	4.80 ± 0.16	4.65 ± 0.13	4.25 ± 0.12
Second instar (days)	3.45 ± 0.11	3.55 ± 0.13	3.85 ± 0.10
Third instar (days)	3.60 ± 0.17	3.30 ± 0.11	3.20 ± 0.09
Fourth instar (days)	5.65 ± 0.16	5.20 ± 0.09	5.85 ± 0.15
Total dev. period (days)	25.75 ± 1.60	24.05 ± 2.10	23.80 ± 0.99
Preoviposition periods (h)	62.3 ± 0.03	65.9 ± 0.00	68.2 ± 0.08
Longevity (male) (days)	13.5 ± 0.05	12.3 ± 0.07	13.9 ± 0.07
Longevity (female) (days)	17.6 ± 0.05	16.7 ± 0.05	16.5 ± 0.06
Fecundity (no. of eggs)	54.04 ± 3.40	62.3 ± 4.20	64.3 ± 2.40
Survivorship (%)	68.49 ± 1.02	68.94 ± 0.59	69.12 ± 0.56
Sex ratio (male: female)	1:2.3	1:2.8	1:3.1

LONGEVITY, FECUNDITY AND SURVIVORSHIP

The longevity and fecundity data of the cryptic species are shown in Table 1. Male longevity was in the following declining order: Asia II-7 > Asia I > Asia II-1, and the differences were statistically significant ($F = 8.4029$, df = 4, $P < 0.001$). The female longevity was in the following declining order: Asia I > Asia II-1 > Asia II-7, and these differences too were statistically significant ($F = 5.7036$, df = 4, $P < 0.001$). Mean fecundity was in the following declining order: Asia II-7 > Asia II-1 > Asia I. However, variations in fecundity among the 3 cryptic species were statistically insignificant ($F = 1.8496$, df = 4, $P = 0.1257$). The mean percentage of survivorship was found nearly equal in all the 3 cryptic species, and these ranged from 68.49 to 69.12% and the variations were statistically insignificant ($F = 2.4651$, df = 4, $P = 0.1638$).

CLUSTERING OF CRYPTIC SPECIES

Life history data such as developmental periods of eggs and instars, longevity, fecundity, preoviposition period and sex ratio were subjected to PCA, which resulted in a reduction of dimensions and in the identification of the sources of variation. The first 4 principal components (PCs) each with an eigenvalue >1 were observed to account for 49.5% of total variation (Table 2). PC1 explained about 25% of the total variation and had a positive loading for 3 variables, i.e., egg, and first and third instars. PC2 explained about 19% of total variation having positive loading for 2 variables, i.e., second instar and the longevity of the male. The plot for first 2 PCs i.e., PC1 and PC2 shown in Fig. 1 brings out the grouping of the cryptic species. These reveal that separate clustering was observed for Asia I, Asia II-1 and Asia II-7 with slight overlap.

Canonical discriminant analysis (CDA) was carried out with prior grouping and use of the life history data as classification variables. The statistics namely Wilks' λ, Pillai's trace, Hotelling-Lawley Trace and Roy's greatest root (Table 3) were found to be significant at $P < 0.0001$. These statistics clearly depict the significant contribution towards the model with a lower Wilks' λ (0.2900), and which held true for all of the other statistics. The projection of biological data onto the first 2 canonical discriminant axes is shown in Fig. 2. The analysis was able to extract differences between the 3 cryptic species with slight overlap among them. The first canonical root clearly discriminates the cryptic species with the main contributions from the egg stage duration and the longevity of the female, while second canonical root was able to discriminate from the fourth instar and the longevity of the male (Table 2). This clustering obtained from CDA confirmed the grouping brought out by PCA. A cross validation of group membership was performed identifying the misclassification of specimens and assessing the utility of selected observations. Overall 70% of the classifications were correctly attributed with a relatively less misclassification. The result of cross validation accurately classified 85% of Asia II-1 populations followed by 75% of those of Asia I and 50% of Asia II-7 (Table 4).

Discussion

The range of host plants and host plant suitability are considered as 2 major factors affecting the spread and damage of *B. tabaci*. Host plant suitability and biology had been earlier studied in detail (Bethke et al. 1991; Lin & Ren 2005; Lida et al. 2009; Oriani et al. 2011). The life history traits of Asia I, Asia II-1 and Asia II-7 reported herein add to the existing knowledge on the variations in *B. tabaci* species complex. In the present study, we found significant variations in the total developmental time, egg period and duration of the fourth instar. These are similar to the results obtained on cotton earlier (Bethke et al. 1991; Thomas et al. 2011). The developmental time from egg to adult of the 3 cryptic species ranged from 23.80 to 25.75 days when these were

Table 2. Proportion of variation and variable coefficients for the life history data of *B. tabaci* species complex PC1, PC2, PC3 of PCA and standardized canonical coefficients (CDA)

Variable	Component 1	Component 2	Component 3	Canonical axis 1	Canonical axis 2
Egg	0.4503	-0.0627	0.3449	0.8932	-0.2077
First instar	0.5220	-0.3443	0.0831	0.0194	-0.0388
Second instar	-0.0685	0.4449	0.5260	-0.3127	0.3555
Third instar	0.5472	-0.0194	0.3980	0.1413	-0.2016
Fourth instar	0.1675	-0.1238	0.5355	0.2161	0.7661
Longevity♂	0.0578	0.6077	0.0878	0.3331	0.7829
Longevity♀	0.3083	0.4238	-0.2949	0.4076	-0.0950
Fecundity	-0.3069	-0.3391	0.2400	-0.2617	0.0011
Eigen values	2.0491	1.5247	1.1416		
Proportions of variation	25%	19%	14%		

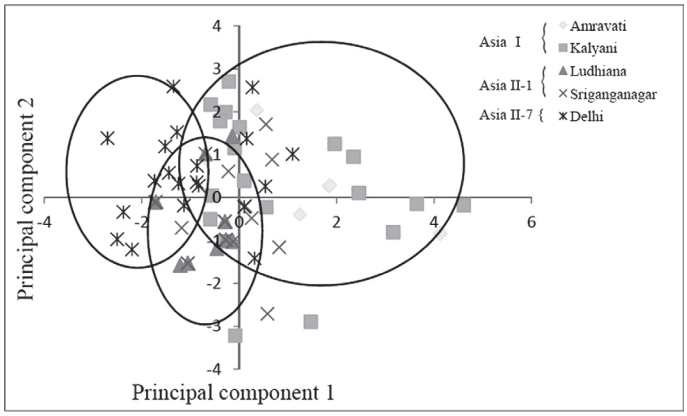

Fig. 1. Component analysis (PCA) showing the clustering of genetic groups of *Bemisia tabaci* species complex.

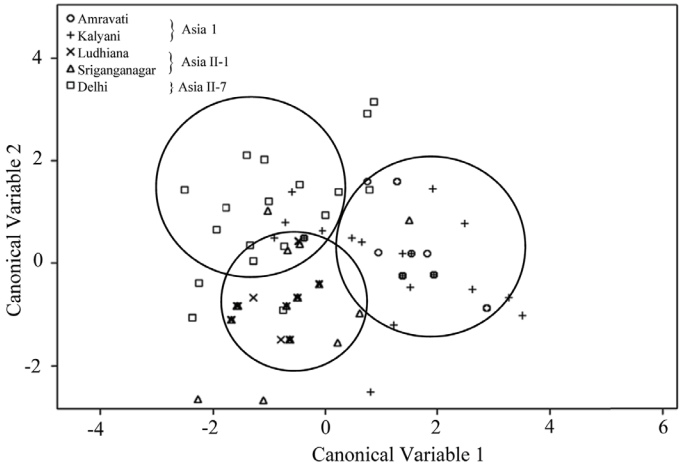

Fig. 2. Canonical discriminant functional analyses showing 3 genetic groups of *Bemisia tabaci* species complex.

reared on cotton, which was longer than those reported for Asia II-1 from Pakistan (Ahmed et al. 2014) and for B biotype (now referred as MEAM1) (Lida et al. 2009) on different hosts, but nearly similar to the Cv biotype (now referred as Asia II-7) on cucumber and tomato (Qiu et al. 2011). This variation might be due to host plant because time required for *B. tabaci* to complete development from egg to adult was influenced by the host plant on which it fed (Coudriet et al. 1985; Mansary & Sundufu 2009).

The largest sex ratio was recorded in Asia II-7 followed by Asia I and Asia II-1. The host plant was demonstrated to have a significant effect on the longevity and fecundity of *B. tabaci* (Qiu et al. 2003; Lin & Ren 2005; Qiu et al. 2011; Ahmed et al. 2014). Herein, we found that the longevity of both the male and the female varied significantly, ranging from 12.3 to 17.6 days despite being on the same host plant, i.e., cotton. The longevities of Asia I and Asia II-7 were similar to that of the Cv biotype on tomato, but differed from that of the B biotype (Qiu et al. 2011), which was in the range (10–15 days) reported by Gerling et al. (2001) for *B. tabaci* in the field at temperatures in the higher 20 °C.

However the longevity of Asia II-1 was lesser than that reported by Ahmed et al. (2014), which was 28.8 ± 0.5 days. The fecundity variations were statistically insignificant, and fecundity was less than the reported fecundity of B biotype and Cv biotype on other hosts (Qiu et al. 2011) and of Asia II-1 on cotton (Ahmed et al. 2014), but nearly similar to the fecundity of B biotype on laurel and Cv biotype on poinsettia (Qiu et al. 2011).

The clustering of populations revealed through the results of the PCA and CDA of the life history data was identical to the genetic identity of the 3 cryptic species Asia I, Asia II-1 and Asia II-7 determined by molecular characterization. The genetic diversities of *B. tabaci* populations shown through various molecular studies (Dinsdale et al. 2010; De Barro et al. 2011; Reddy et al. 2012; Lee et al. 2013) were found to

Table 3. Multivariate statistics and F approximations for the life history data of the *Bemisia tabaci* species complex.

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.29000	4.07	32	326.12	< .0001
Pillai's Trace	0.94127	3.50	32	364	< .0001
Hotelling-Lawley Trace	1.68882	4.58	32	219.69	< .0001
Roy's Greatest Root	1.00983	11.49	8	91	< .0001

Table 4. Cross validation matrix of the Discriminant Function Analysis (DFA) for life history data of the *Bemisia tabaci* species complex.

	Asia I	Asia II-1	Asia II-7	Total
Asia I	15	0	5	20
	75%	0	25%	100
Asia II-1	0	17	3	20
	0	85%	15%	100
Asia II-7	3	7	10	20
	15%	35%	50%	100

be in agreement with the present study. DFA with a higher classification (85%, 75% and 50%) values helps to establish the probable validity of these life history traits variations. Thus the biological data with the help of PCA and CDA might provide additional knowledge on the *B. tabaci* cryptic species complex known from India.

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