Евразиатский энтомол. журнал 22(6): 296–303
doi 10.15298/euroasentj.22.06.04

Identifying the host-associated morphological differentiation of the tobacco thrips *Thrips parvispinus* (Karny, 1925), an invasive thrips species

Морфологические отличия инвазивного вида табачного трипса *Thrips parvispinus* (Karny, 1925), связанные с особенностями кормового растения

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Key words: *Thrips parvispinus,* morphology, morphometry, principal component analysis.

Ключевые слова: *Thrips parvispinus,* морфология, морфометрия, анализ главных компонентов.

Abstract. *Thrips parvispinus* (Karny, 1925), a new invasive species of the order Thysanoptera (Terebrantia: Thripidae), was first discovered in Karnataka papaya crops in 2015. Since then, it has spread to many other states and crops, wreaking havoc in the chili industry. Despite quarantine limitations, the recent invasion of thrips into India rapidly is cause for concern, particularly given the globalisation situation. The current study documented the spread of the invasive *T. parvispinus* (Karny) pest on significant horticultural crops in Tamil Nadu. The species and its unique taxonomic traits are documented to aid identification. For further precise identification, the essential characteristics of *T. parvispinus* (Karny) were quantified. According to the principal component analysis (PCA) of the measurable components of each crop from which the species was derived, habitat alterations in precise, crop range expansion had the least impact on morphometric features (PC1 and PC2: 15.6 and 14.8 %, respectively).

Резюме. *Thrips parvispinus* (Karny, 1925) — инвазивный вид отряда Thysanoptera (Terebrantia: Thripidae), который впервые был обнаружен в посевах папайи штата Карнатака в 2015 году. С тех пор он распространился на многие другие штаты Индии, поражая другие культуры, нанося ущерб перцу чили. Несмотря на карантинные ограничения, недавнее стремительное вторжение трипсов в Индию вызывает беспокойство, особенно с учётом глобализации экономики. В представленном исследовании зафиксировано распространение инвазивного вредителя *T. parvispinus* (Karny) на важных садовых культурах в штате Тамил Наду. Вид и его уникальные таксономические особенности обсуждаются в статье для облегчения идентификации вредителя. Для точного определения вида проведена количественная оценка основных признаков *T. parvispinus* (Karny). По данным анализа главных компонент (PCA) при измерении признаков каждой культуры из которой были выведены трипсы, изменение среды обитания при установленном расширении ареала культур слабо влияло на морфометрические признаки (PC1 и PC2: 15,6 и 14,8 % соответственно).

Introduction

Invading species must engage in ferocious competition to survive in more recent biological systems. Several studies have demonstrated the competitive prowess an invading species enjoys by comparing its competitive abilities to those of the native species it is displacing. Rarely can invasive species live with native species for an extended time; usually, one species soon supplants the other. A few leading causes for expanding invasive insect species into new places include globalisation, the industrial revolution, and trade liberalisation [Haseeb et al., 2011].

Thrips are members of the Terebrantia and Tubulifera suborders of the Thysanoptera order. The approximately 6436 species of thrips in 788 genera act as pollinators, predators, pests, and plant virus vectors and remain economically significant in agriculture [Lewis, 1997; Pappu et al., 2009; Thrips Wiki, 2022]. Because adults can spread tospoviruses while larvae can acquire them, tospoviruses are unusual [Whitfield et al., 2005].

Thrips parvispinus (Karny, 1925) is a detrimental polyphagous pest that has decimated pepper (*Capsicum annum*) and other solanaceous crops during the last two decades [Vos, 1994; Murai et al., 2009; Sartiami et al., 2011; Johari, 2015]. This species belongs to the «*Thrips orientalis* group.» *T. parvispinus* (Karny) spread rapidly to other Southeast Asian states, Oceania, North America, Europe, Africa, and India due to increased plant material mobility under international trade practices [Waterhouse, 1993; Zhang et al., 2011]. The host range and geographic spread of *T. parvispinus* (Karny) have dramatically increased in recent decades. In Hawaii and other states in North America, *T. parvispinus* (Karny) severely damages papaya (*Carica papaya* L.) [Sugano et al., 2013], causing yield losses [Johari, Natali, 2018; NPPO, 2019]. In Malaysia, papaya developed a «bunchy top» deformity due to a saprophytic fungus called *Cladosporium oxysporum* was drawn to *T. parvispinus* (Karny) feeding damage [Lim, 1989].

T. parvispinus (Karny) was found in papaya for the first time in India [Tyagi et al., 2015] and has since spread to other host plants, including tobacco (*Nicotiana tabaccum* L.), bitter gourd (*Momordica charantia* L.), bottle gourd (*Lagenaria siceraria* Standl.), brinjal (*Solanum melongena* L.), chilli (*C. annuum* L.), cotton (*Gossypium hirsutum* L.), cucumber (*Cucumis sativus* L.), lablab (*Lablab purpureus* L.), mango (*Mangifera indica* L.), onions (*Allium cepa* L.), papaya (*C.papaya*), pigeon pea (*Cajanus cajan* L.), potatoes (*Solanum tuberosum* L.), sweet pepper (*C. annuum* L.), strawberries (*Fragaria annanasa* Duchesne), tamarind, *Chrysanthemum* sp., and *Dahlia rosea.* It also feeds on weeds, including *Ageratum* sp., *Alternanthera* sp., *Amaranthus* sp., *Axonopus* sp., *Parthenium hysteroporus,* and *Thunbergia* sp. [Nagaraju et al., 2021; Roselin et al., 2021; Sridhar et al., 2021; Rachana et al., 2022]. *T. parvispinus* (Karny) damages chili leaves and blooms under Indian field conditions, causing a 70–100 % decrease in production.

The first step in creating any pest risk assessment program is identifying the pest species accurately. Thrips are tiny insects (0.2 to 5 mm in length). As a result, a competent taxonomist is required to identify a species correctly. Poor identification caused *Thrips palmi* Karny, a potential pest, to be mistaken for *Thrips flavus* Schrank, a naturally occurring non-pest species, in India [Singh, Krishnareddy, 1996]. In the Netherlands, *T. parvispinus* (Karny) was recognised as *Thrips* (*Isoneurothrips*) *taiwanus* Takahashi [Mound, Collins, 2000]. For more than 250 years of contemporary taxonomic study, the number of currently recognised species has constantly been increasing. Nonetheless, our grasp of biodiversity must be thorough, especially concerning pest and vector species. Because of their minute size, cryptic behaviour, high degree of similarity of various developmental stages, polymorphism, intraspecific variations, and complex morphology, morphological characteristics are of limited use to non-specialists for accurate and quick identification [Ananthakrishnan, 1993; Asokan et al., 2007].

The study of many unweighted traits is the foundation of phenetic taxonomy; the number of variables that may be analysed for a plant or animal species is so huge that a mathematical tool is necessary to arrange them into units corresponding to taxa. Given the conditions, the current study employed chaetotaxy to characterise *T. parvispinus* (Karny), record its frequency on various crops and plants, investigate its occurrence on various host plants, and map its geographical distribution. The quantitative parameters of *T. parvispinus* (Karny) were assessed. Following the quantifiable traits, principal component analysis was performed to evaluate the species' differentiating attributes and the degree of change associated with different crop ranges.

Materials and methods

During 2020–2022, a location survey was done in six different agro-climatic zones of Tamil Nadu (North Western, Western, Southern, Cauvery-delta, High Rainfall, and Hilly Zone) to monitor and collect species from various crops. Surveys were carried out in several districts within these agro-climatic zones. Beans (*Phaseolus vulgaris* L.), bhendi (*Abelmoschus esculentus* L.), bitter gourd (*M. charantia*), Black nightshade (*Solanum nigrum* L.), bottle gourd (*L. siceraria*), chillies (*C. annuum*), capsicum (*C. annuum v. annuum*), carrot (*Daucus carota* L.), coriander (*Coriandrum sativum* L.), coccinia (*Coccinia grandis* L.), cowpea (*Vigna ungiculata* L.), cucumber (*C. sativus*), lablab (*L. purpureus*), moringa (*Moringa oleifera* Lam.), nerium (*Nerium oleander*), potato (*S. tuberosum*), ridge gourd (*Luffa acutangula* L.), sweet potato (*Ipomea batatus* (L.) Lam.), temple tree (*Plumeria rubra* L.) and yellow bell flower (*Tecoma stans* L.) were the most common crops found in the regions surveyed at each site. *T. parvispinus* (Karny) samples were identified from all these crops and utilised for further processing and analysis.

Specimen preparation

Thrips adults and various life stages were collected in the field and kept in AGA fluid (60 % ethanol: glycerine: acetic acid in 6:1:1 ratio) for permanent slide preparation. By the process of maceration (clearing), the thrips samples washed with 60 % ethanol (24 h) and 5 % NaOH (1 h) solution for clarity. And then, the samples dried by immersing them in an ethanol series (From 60 % (24 h) to 70 % (1 h) to 80 % (20 min) to 95 % (5 min) and 100 % (5 min)) before being stored in clove oil for further use. Specimens in clove oil being mounted on slides using Hoyer's medium and dried for 5–7 days in 40 °C. The prepared slides were identified using relevant chaetotaxy and other taxonomy keys. A total of 20 specimens from each crop were further examined.

Morphometric studies

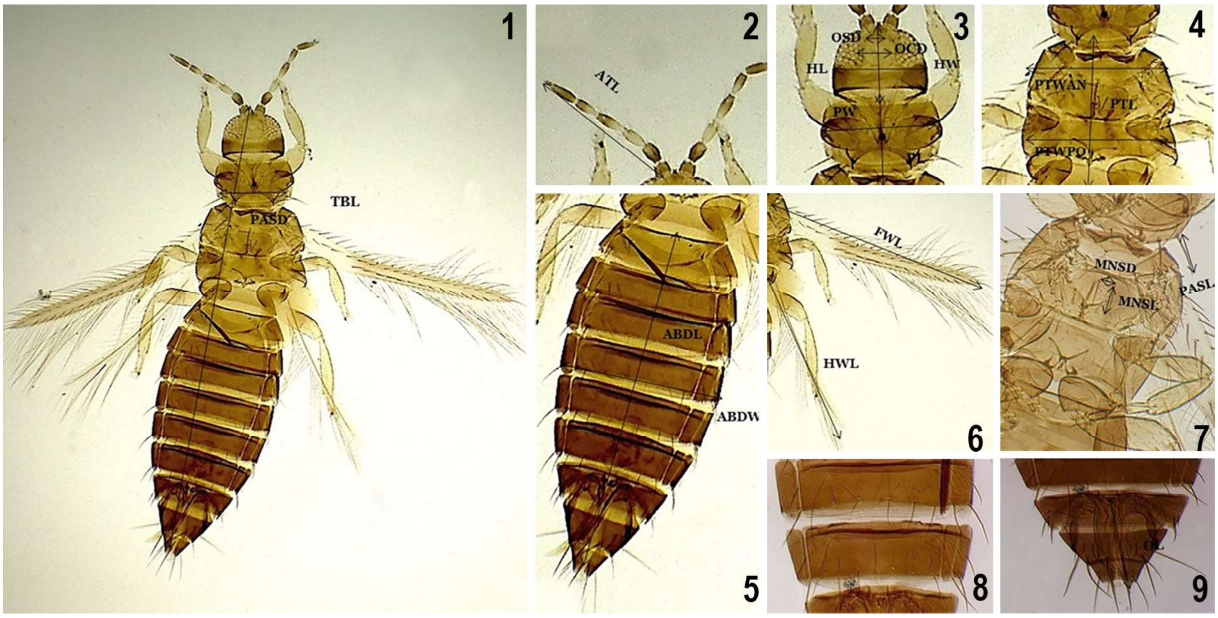
A compound microscope (Euromex iScope, The Netherlands) was used to analyse 21 morphological characteristics (Table 1; Figs 1–9) of the thrips body for each taxon specimen as described by Palmer et al. [1990]. The quantitative morphological characteristics studied were total body length (TBL), head length (HL), head width (HW), total antenna length (ATL), prothorax length (PL), pronotum width (PW), pterothorax length (PTL), pterothorax width — anterior (PTWAN), pterothorax width — posterior (PTWPO), ocellar setae distance (OSD), ocelli distance (OCD), forewing length (FWL), forewing width (FWW), hindwing length (HWL), abdomen length (ABDL), abdomen width (ABDW), ovipositor length (OL), postero marginal setae length (PASL), postero marginal setae distance (PASD), metanotal setae length (MNSL) and between the metanotal setae distance (MNSD). The majority of the attributes listed are often used for thrips identification. The quantitative characteristics were assessed as linear distances on digital pictures of slide-mounted specimens using the Euromex iScope microscope and image analyser software (Euromex iScope, The Netherlands).

Statistical analysis

The non-transformed data (ratio variables) were used for Principal Component Analysis in the statistical programme SPSS (IBM SPSS Statistics 27) using the dimension reduction approach [Polaszek et al., 2004]. A multidimensional system of variables is projected onto a low-dimensional map of components using the factoring technique. The solutions were subjected to a Varimax rotation in the directions having the highest variation in the dataset to avail the scree plot.

Author contribution statement

M.M. designed the principal research aims, supervised all the processes, and evaluated the manuscript.



Figs 1–9. Taxonomic characteristics of *Thrips parvispinus* (Karny) dorsally (1, 5–7, 9) and ventrally (2–4, 8). 1 — external appearance; 2 — antennae; 3 — head and thorax; 4, 8 — thorax; 5 — abdomen; 6 — wings; 7 — setae on abdominal ventrites; 9 — apical ventrites of abdomen. Designations: TBL — total body length, PASD — postero angular setae distance, ATL — antenna total length, OCD — ocelli distance, OSD — ocellar setae distance, HL — head length, HW — head width, PL — prothorax length, PW — prothorax width, PTL — pterothorax length, PTWAN — pterothorax width (anterior), PTWPO — pterothorax width (posterior), ABDL — abdomen length, ABDW — abdomen width, FWL — forewing length, FWW — forewing width, HWL — hindwing length, PASL — postero angular setae length, MNSD — distance between the metanotal setae, MNSL — metanotal setae length, OL — ovipositor length.

Рис. 1–9. Характеристика таксономических признаков *Thrips parvispinus* (Karny) в положении сверху (1, 5–7, 9) и снизу (2–4, 8). 1 — внешний вид; 2 — усики; 3 — голова и грудь; 4, 8 — грудь; 5 — брюшко; 6 — крылья; 7 — щетинка на вентритах брюшка; 9 — апикальные склериты брюшка. Обозначения: TBL — длина тела, PASD — расстояние между щетинками на задних углах, ATL — длина усика, OCD — расстояние между глазками, OSD — расстояние между щетинками глазков, HL — длина головы, HW — ширина головы, PL — длина переднегруди, PW — ширина переднегруди, PTL — длина птероторакса, PTWAN — ширина птероторакса спереди, PTWPO — ширина птероторакса сзади, ABDL — длина брюшка, ABDW — ширина брюшка, FWL — длина переднего крыла, FWW — ширина переднего крыла, HWL — длина заднего крыла, PASL — длина задних угловых щетинок, MNSD — расстояние между метанотальными щетинками, MNSL — длина метанотальных щетинок, OL — длина яйцеклада.

Table 1. Quantitative characteristics of *Thrips parvispinus* (Karny) populations infesting polyculture tropical vegetable ecosystem of Tamil Nadu, India

Таблица 1. Количественные характеристики популяций *Thrips parvispinus* (Karny), поражающего многовидовую тропическую растительную экосистему в Тамил Наду, Индия

Characteristics for study	Abbreviation	Mean ± SE	Range
Total body length	TBL	1274.682 ± 39.254	1202.910–1332.400
Head length	HL	120.072 ± 3.273	114.540–124.760
Head width	HW	178.854 ± 4.899	166.500–184.540
Antenna total length	ATL	272.365 ± 5.177	263.760–283.630
Prothorax length	PL	122.683 ± 3.054	117.540–127.050
Prothorax width	PW	211.316 ± 4.479	203.990–220.200
Pterothorax length	PTL	269.627 ± 9.129	255.390–286.190
Pterothorax width (anterior)	PTWAN	263.196 ± 9.352	249.600–280.240
Pterothorax width (posterior)	PTWPO	280.536 ± 9.092	263.310–297.080
Ocellar setae distance	OSD	27.290 ± 2.112	23.820–31.850
Ocelli distance	OCD	41.624 ± 3.839	34.530–48.600
Forewing length	FWL	672.811 ± 10.117	654.350–697.480
Forewing width	FWW	36.449 ± 2.327	32.230–41.430
Hindwing length	HWL	586.582 ± 13.417	558.630–611.030
Abdomen length	ABDL	717.940 ± 26.087	646.940–763.840
Abdomen width	ABDW	270.251 ± 7.019	258.480–285.450
Ovipositor length	OL	203.213 ± 5.074	194.850–211.240
Posterior angular setae length	PASL	57.730 ± 2.642	53.590–62.300
Posterior angular setae distance	PASD	135.004 ± 4.357	127.750–141.210
Distance between the metanotal setae	MNSD	22.404 ± 2.045	18.340–26.520
Metanotal setae length	MNSL	39.959 ± 4.283	32.210–46.470

Notes: All the measurements were in μm; the measurements were taken from 20 slide-mounted specimens, and the mean & range were defined.

Примечания: Все измерения в мкм; измерения были проведены на 20 образцах, экспонированных на предметном стекле, с определением средних значений и диапазона.

A.P. collected and analysed the insect sample, performed the experimental methods, analysed the results, and wrote the manuscript. B.V., C.N., and K.G. have helped with technical advice during research. P.L. contributed to samples from field collection. All authors read and approved the final manuscript.

The authors have no relevant financial or non-financial interests to disclose. The authors conducted no experiments with human participants or vertebrate animals for this paper.

The present work is registered in ZooBank (www.zoobank.org) under LSID urn:lsid:zoobank.org:pub:6059B06E-DA84-47D4-9982-AB921E432BA8.

Results

Thrips specimen collection

T. parvispinus (Karny) was found in five out of six (data not given) agro-climatic zones surveyed in Tamil Nadu. The species was found in beans, bhendi, bitter gourd, black nightshade, bottle gourd, chilies, capsicum, carrot, coriander, coccinia, cowpea, chrysanthemum, lablab, moringa, nerium, potato, ridge gourd, sweet potato, temple tree, and yellow bell flower.

Morphological characterization

T. parvispinus (Karny) was morphologically studied, and the following characteristics were observed (Figs 1–9). Both sexes have complete wings. The TBL range for *T. parvispinus* (Karny) was 1202.910 to 1332.400 μm, with a mean of 1274.682 ± 39.254 μm. The female has a brown body; head and thorax are lighter than the abdomen; the head is darker than the middle area; the legs are predominantly yellow; and the fore wings brown with a sharply pale base. The antenna has seven segments, with segments III and IV having forked sensing cones and segments III, IV, and V appearing yellow. The ATL was 272.365 ± 5.177 μm longer with a range of 263.760 to 283.630 μm. Ocellar setae pair III is short and appears on the ocellar triangle's front borders; postocular setae pair I & III are somewhat longer than ocellar setae III, and postocular pair II is minute. There are two pairs of long postero-angular setae on the pronotum and three pairs on the posterior border. The medial metanotum is reticulate, with reticles that vary in shape and may have internal sculptured patterns; the

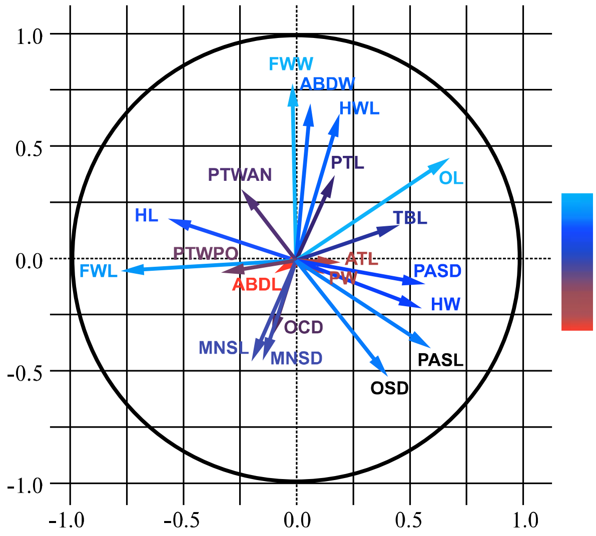


Fig. 10. Scatter plot of the variables concerning the first and second principal components. Designations as in Figs 1–9.

Рис. 10. График разброса переменных относительно первой и второй главных компонент. Обозначения как на рис. 1–9.

median setae are long and emerge behind the anterior border; and the campaniform sensilla are absent [Mound et al., 2010; CABI, 2018]. Twenty-one quantifiable morphological factors were chosen from the recorded ones for further investigation. Table 1 shows the mean and range of documented thrips attributes.

Non-transformed data was used to generate 19 components based on 21 variables and biplots of all the pairwise combinations of those analysed. Table 2 shows the eight primary components' Eigenvalues and weights (component score coefficients). Figure 10 depicts a scatter plot for the first and second primary components, computed using ratio variables. For thrips individuals, the cumulative variance of the two principal components (PC1 and PC2) reached 30.4 % of the overall variation (Fig. 10: Dim.1 and Dim.2). The scree plot of the Eigenvalues of the 21 variables is shown in Fig. 11. The

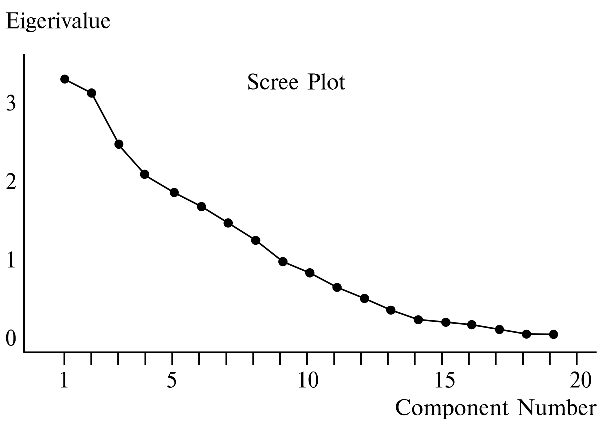


Fig. 11. Scree plot for the variability in the thrips quantitative characteristic data matrix as a function of the number of Eigenvalues.

Рис. 11. График изменчивости количественных показателей трипса в зависимости от количества собственных значений матрицы.

scree plot suggests that the first 10 components (PC1 to PC10) contribute 90 % of the total, with the Eigenvalues of the first 4 components (PC1, PC2, PC3, and PC4) contributing 50% of the total variables. The variables TBL, HL, HW, FWL, OL, PASL, and PASD exhibited the most noticeable influence on PC-I. The least significant impacts, on the other hand, were shown by ATL, PL, PTL, HWL, ABDL, ABDW, ABSL, and PASD (Fig. 12). The HL and FWL were negatively correlated, while TBL, HW, OL, PASL, and PASD had a positive effect on the population.

Other variables contributed similarly. The impacts of FWW, ABDW, OL, and PASL were the biggest along PC-II, whereas all other components had minor effects and contributed almost equally. When all other things were considered, size did not affect group prejudice. The correlation and covariance matrices PCA results were nearly identical (Fig.12) The classifications produced by the correlation matrices analysis were significantly more accurate, thus they were utilised for interpreting the results of PCA. The first and second significant axes accounted for 15.6 % and 14.8 % of the overall variance, respectively. Host variations and adjustments did not affect quantitative qualities due to the split of the components (PC1 and PC2).

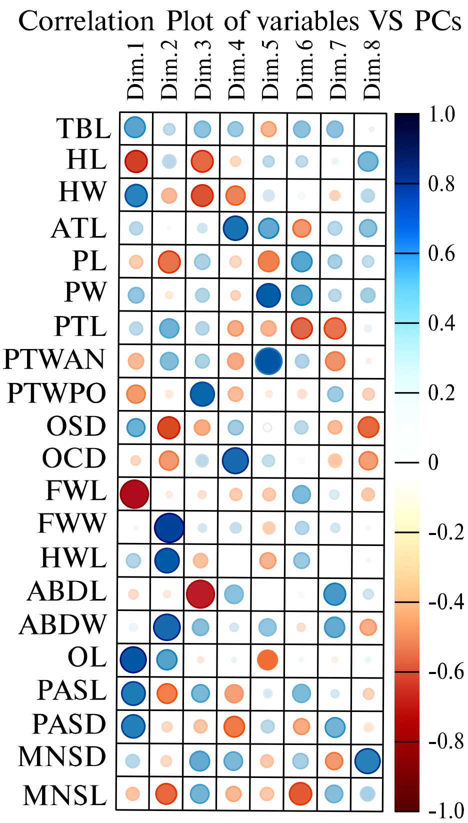


Fig. 12. Correlation plot between variables and Principal components (PCs). Designations as in Figs 1–9.

Рис. 12. График корреляции между переменными и основными компонентами (PC). Обозначения как на рис. 1–9.

Discussion

The field of taxonomy presently uses a variety of classification schemes and methods to categorise species and determine their relationships. The categorisation of Thysanoptera order is divided into two categories: phenetic (based on the physical traits of adult specimens) and phylogenetic (based on evolutionary connections) [Mound, 2010]. The crucial connections between traits impacting the primary sources of variation in a particular population reflecting a location are discovered using morphometric analysis as a numerical taxonomy technique. Only a complex interplay of qualitative and quantitative (morphometric) traits can reliably identify tiny or cryptic thrips. Morphology and other biological characteristics, such as observations of developmental phases and interactions with host plants, give additional information and may aid in understanding taxonomic relationships [Crespi et al., 1998; Mound, Morris, 2007].

The current study attempted to identify distinguishing traits in *T. parvispinus* (Karny) specimens collected in various crops and invasive areas of the species' new biological habitat. The study's findings revealed no distinction in the species group owing to the impact of geographical regions. Table 2 lists the range of values for each taxonomic attribute. Upon analysis, each characteristic contributed equally to the data group regarding Eigenvalues. Eigenvalues demonstrate the relative contribution of each principal component in illustrating the general variability of sampled material in principal component analysis. The numerical value of an Eigenvalue is a direct measure of the weight of a single component in the overall variability of a set of data [Kucharczyk, Kucharczyk, 2009].

The first four primary components explained more than 51 % of the variation, each contributing to more than 1 %. The other variables contributed equally to the dataset, accounting for more than 49 % of the variation. The first ten of the twenty-one components studied accounted for about 90 % of the variance, while the other ten had Eigenvalues less than one, which may be discarded. Because numerous characteristics contributed equally to the variance, as shown by the Eigenvalue data separation, it can be concluded that changes in *T. parvispinus* (Karny) crop environment did not affect its quantitative qualities. The findings are consistent with those of Palmer, Wetton [1987], who utilised morphometric and PCA analysis to separate *T. hawaiiensis* Morgan, 1913 and *T. florum* Schmutz, 1913 into distinct groupings. Eigenvalues aided the first component of the PCA analysis, which distinguishes the species. The findings agree with Fekrat et al. [2009], who used two components to discretely group — to explain the higher contribution of variance [Fekrat et al., 2014]. Regardless of potential measurement errors, the morphometry data help distinguishes between related species [Kucharczyk 2010]. According to Sosiak, Barden [2020], variations in the fundamental components alter the characteristics of the entire colony.

Morphological traits are frequently used to differentiate taxa. However, not all species are suitable for this method, often due to a lack of phenotypic variety. The observation is that different species regularly live on the same plant and that most species are linked to several hosts. Furthermore, host adaptability is the primary path to host generalism. Every organism adapts to the newest range when faced with unexpected situations. *T. parvispinus* (Karny), an invasive species from Thailand, has the

Table 2. Eigenvalues of the principal components utilised in the analysis of 20 quantitative characteristics of *Thrips parvispinus* (Karny) populations infesting polyculture tropical vegetable ecosystem

Таблица 2. Значения основных компонентов, использованных при анализе 20 количественных характеристик популяций *Thrips parvispinus* (Karny), заселяющих многовидовую тропическую растительную экосистему

Principal component	Eigenvalue	Percentage of Variance	Cumulative Percentage of Variance
PC1	3.274	15.59	15.59
PC2	3.108	14.8	30.39
PC3	2.448	11.655	42.046
PC4	2.051	9.767	51.813
PC5	1.848	8.801	60.614
PC6	1.657	7.893	68.507
PC7	1.457	6.938	75.444
PC8	1.22	5.811	81.255
PC9	0.96	4.569	85.824
PC10	0.812	3.867	89.691
PC11	0.638	3.038	92.729
PC12	0.495	2.356	95.085
PC13	0.345	1.642	96.727
PC14	0.223	1.064	97.791
PC15	0.173	0.822	98.613
PC16	0.144	0.684	99.298
PC17	0.083	0.393	99.691
PC18	0.039	0.188	99.878
PC19	0.026	0.122	100

aptitude for adaptability and flexibility across the host range, making it invasive. However, colonising a new host may cause the organism to acquire more genetic differentiation. *T. parvispinus* (Karny), on the other hand, was recently invaded and exhibited no apparent morphological differences. Crespi et al. [1998] used COI gene sequences to analyse Australian gall-forming thrips species. They observed that each species looked like a pair of related species that could not previously be separated due to «long-term morphological stasis». Because the characteristic piercing-sucking feeding mode limits the ecological and morphological diversity options in this order, the recurrence of stereotypic morphologies does not always suggest sister connections. In that circumstance, the species can produce several morpho-types through other invasions and adaptations, as evidenced by the COI-based study of the species [Brunner et al., 2004].

Acknowledgments

The authors thank the Department of Agricultural Entomology, TNAU, Coimbatore, for their financial and practical assistance in collecting specimens and sharing resources while analysing insect samples in the lab.

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Поступила в редакцию 7.6. 2023