

33 crucial steps for developing effective management practices and preventing the further spread of this pest to other
34 regions.

35 **Keywords:** *Polianthes tuberosa*, *Dysmicoccus brevipes*, Anona mealybug, Gulchadi, Ranjanigandha, *Solenopsis*
36 *geminata*

37 Tuberose *Agave amica* (Medikus) Thiede & Govaerts (Syn: *Polianthus tuberosa*; Asparagaceae) is
38 widely cultivated across various Indian states for its loose flowers and floral spikes. Tuberose, popularly known as
39 ‘*Rajanigandha* (The Fragrance of the Night) or *Sungandharaja* (King of Fragrance) is considered as the most
40 popular flower crop by small and marginal farmers as it provides consistent and high returns throughout the year¹.
41 Moreover, it is a hardy crop, capable of withstanding various biotic stresses, and adaptable to a wide range of soils
42 and climatic conditions². Severe outbreaks of this mealybug species were observed in 2021 in the tuberose fields
43 of ICAR-DFR, Pune, as well as in Yavat and the surrounding areas of Pune district, which is a major tuberose-
44 growing region in Maharashtra. Mealybug colonies were found developing on underground parts and basal region
45 of tuberose plants. The affected plants were observed to be completely dried out and drooping, with the infestation
46 being so severe (up to 100%) that farmers were compelled to destroy their entire fields. This mealybug species,
47 including its field characteristics, appeared to differ from the striped mealybug, *Ferrisia virgata* Cockerell
48 (Hemiptera: Pseudococcidae), which is known to damage tuberose crops in India^{3,4}. To confirm its identity,
49 mealybug specimens were preserved in 70% ethanol and slide-mounted in Canada balsam, following the
50 procedure described by Watson & Chandler⁵. The terminology for the adult female's morphological structures is
51 based on the definitions provided by the Williams and Granara de Willink⁶. The morphology of the slide-mounted
52 adult female was examined using 30 specimens mounted on 10 slides at the Division of Germplasm
53 Characterization, ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, India.
54 Morphological measurements were recorded using an eyepiece reticle on an Olympus BX 51 microscope,
55 calibrated with a stage micrometer. These measurements represent maximum dimensions, with setal lengths
56 including the base. Slide-mounted adult females were examined using a Nikon Digital Sight DSVI-1 on a Nikon
57 Eclipse 80i microscope, with measurements obtained from digital images using the M205 A Leica Application
58 Suite.

59 The mealybug species infesting tuberose plants was found to be grey pineapple mealybug, *Dysmicoccus*
60 *neobrevipes* Beardsley (Hemiptera: Pseudococcidae). Diagnostic characters of *D. neobrevipes* are depicted in Fig.
61 1. The morphological characters indicated in Fig. 1. are adapted from Williams and Granara de Willink⁶ and

62 Beardsley⁷. *D. neobrevipes* is taxonomically close to the pink pineapple mealybug, *D. brevipes*⁶, and several
63 morphological traits of both species closely resemble each other. Recently, Choudhary et al.⁷ described the
64 morphological characteristics of *D. brevipes* specimens collected from garden pea fields, which show a significant
65 similarity to some of the characters observed in *D. neobrevipes* females collected from tuberose (Fig. 1a to Fig.
66 1m). These characters include the shape of the adult female's body, which is oval to broadly oval in shape, with 17
67 pairs of cerarii (Fig. 1a). The ventral surface of each lobe with a quadrate sclerotized area (Fig. 1b). Each antenna
68 typically consists of 8 segments (Fig. 1c). The legs are well developed, with a stout claw that lacks a denticle (Fig.
69 1d). Translucent pores are absent from the hind coxa (Fig. 1e), but are abundant on the posterior surfaces of the
70 hind femur (Fig. 1f) and hind tibia (Fig. 1g). The circulus is irregular or shaped like a coffee bean, divided by an
71 intersegmental line (Fig. 1h). The ostioles are well developed, featuring sclerotized inner lips along with small,
72 stout setae and trilocular pores around the periphery (Fig. 1i). Each anal lobe cerarius contains 2 enlarged conical
73 setae and 1 or 2 auxiliary setae (Fig. 1j), along with a compact group of trilocular pores, all located on a more or
74 less circular sclerotized area. The penultimate cerarii (Fig. 1k) are slightly smaller than those on the anal lobes,
75 each comprising 2-4 conical setae, 3-7 auxiliary setae, and a compact group of trilocular pores. The cerarii on the
76 head (Fig. 1l) each have 3-6 conical setae. The anal ring is having 6 setae (Fig. 1m). Other characteristics of
77 female *D. neobrevipes* collected from tuberose are detailed in Fig. 1n to Fig. 1v. The dorsal surface is scattered
78 with short, stiff setae throughout the surface (Fig. 1n). Similar short stiff setae present on surface anterolateral to
79 anal ring (Fig. 1o). Large discoidal pores, each larger than a trilocular pore, present conspicuous medially on the
80 dorsum of the abdomen, particularly on segments VI-VIII (Fig. 1p), each with reticulated surface. Smaller
81 discoidal pores with plain surface, always 1-3 present adjacent to each eye (Fig. 1q). Ventral surface with long
82 flagellate setae around vulva (Fig. 1r) and normal shorter flagellate setae present throughout venter. Multilocular
83 disc pores (Fig. 1s) present around the vulva, at the anterior edge of abdominal segment VII, and the posterior
84 edge of segment VI, in the median areas only. Trilocular pores (Fig. 1t) are evenly distributed, being less
85 numerous on the venter than on the dorsum. Oral collar tubular ducts come in two sizes: a small type (Fig. 1u) is
86 sparsely distributed across the abdominal segments and median area of the thorax, while a larger type (Fig. 1v) is
87 found in groups along the margins of the posterior abdominal segments.

88 Originating from pantropical regions, *D. neobrevipes* has now spread to several countries worldwide,
89 including India. This species was first described by Dr. J. W. Beardsley in 1959 from mealybug specimens
90 collected on tuberose (*Polianthes tuberosa*) in Hawaii, USA⁸. *D. neobrevipes* is a polyphagous species associated
91 with plant species across 67 genera in 40 plant families⁹. Although various main host plants of this species have

92 been documented in different countries, pineapple, banana¹⁰, and sisal (*Agave sisalana*)¹¹ are recognized as major
93 hosts. In these crops, *D. neobrevipes* has established itself in the fields and caused significant economic damage.
94 *D. neobrevipes* has been reported in many countries across the Americas, Africa, Europe, Asia, and Oceania. This
95 species is frequently intercepted on various hosts at U.S. ports of entry, including tuberose from Taiwan, and
96 originating from different countries¹². In Asia, severe outbreaks of *D. neobrevipes* have been observed on various
97 crops in Malaysia, the Philippines, Thailand¹³⁻¹⁵ and China¹¹. In India, *D. neobrevipes* has been documented on
98 various crops, including banana¹⁴. Although this species was originally described from specimens collected from
99 tuberose in the USA, and tuberose is considered as its main host in some countries, there is still a lack of basic
100 information including its natural history on tuberose plants. Given the destructive nature of *D. neobrevipes*, it is
101 imperative to obtain this fundamental information. To address this need, we conducted a detailed investigation and
102 present here an illustrative morphological diagnosis of *D. neobrevipes*, along with essential information on its
103 field establishment, impact on tuberose crops, nature of the damage, and observed field symptoms.

104 Extensive surveys were conducted in major tuberose-growing localities of Maharashtra during August
105 2022. Observations on mealybug incidence, damage symptoms, and severity (%) were recorded by counting the
106 number of damaged plants in relation to the total number of plants in 100 sqm area in each field. A total of 62
107 tuberose fields in Yavat and 10 adjoining villages were examined for mealybug (*D. neobrevipes*) infestation.
108 Among them, 54 (87.09%) tuberose fields were found to have mealybug incidence, with infestation levels ranging
109 from 68% to 100%. Because *D. neobrevipes* hides underground and remains concealed during the early stages of
110 infestation, farmers struggled to control it effectively, even with the use of chemical pesticides, which were often
111 non-systemic. Consequently, many farmers with infested fields were prepared to destroy their entire crops to
112 eliminate the mealybugs.

113 Numerous mealybug nymphs were observed feeding primarily on the sap of underground parts, leaves
114 and shoots of basal region, which resulted in stunted plant growth. Severely infested plants showed symptoms of
115 drooping, complete blackening of leaves and shoots, and eventually died (Fig.2a to 2c). *D. neobrevipes* impairs
116 the plant's photosynthetic capacity by excreting sugary honeydew, which fouls plant surface and fosters the
117 growth of sooty mold. This mold blocks sunlight and air from reaching the leaves, further hindering
118 photosynthesis. Additionally, mealybug colonies were found on the spikes of infested plants, leading to poor-
119 quality flowers and reduced yield. Damaged plants often appeared drooped, with rotting underground portions.
120 The infested tuberose fields very often need to be destroyed, as *D. neobrevipes* attacks newly emerging shoots,
121 starting from the underground parts and progressing upward until the shoots die (Fig. 3a). Moreover, *D.*

122 *neobrevipes* can spread from the field to storage through infested bulbs during harvesting, where they continue to
123 develop and damage the stored tuberose bulbs, making them unsuitable for planting (Fig. 3b & 3c). In infested
124 tuberose fields, the large numbers of fire ants (*Solenopsis geminata*) were observed attending mealybug colonies
125 at nearly all surveyed locations including in stored bulbs. Rarely, but *Camponotus compressus* was also recorded
126 attending colonies of *D. neobrevipes* in few locations. These ants not only protect *D. neobrevipes* from natural
127 enemies but also facilitate its spread by transporting the mealybugs to new plants¹⁶.

128 *D. neobrevipes* reproduces sexually¹³ and is known to cause wilt disease in pineapple in Hawaii. It is
129 closely related to the mealybug *D. brevipes*, though these two species can be differentiated by specific taxonomic
130 features. Besides morphological differences, *D. neobrevipes* primarily targets the aerial parts of plants, while *D.*
131 *brevipes* feeds on the roots and basal portions of host plant⁸. However, we observed that *D. neobrevipes*
132 infestations initially start in the underground portions of tuberose plants, with mealybug colonies predominantly
133 found on the basal parts. These colonies can also be found on the aerial parts of the plant, including the shoots,
134 leaves, and even spikes during flowering and post flowering stage. In the case of pineapple, *D. neobrevipes* is
135 known to primarily attack the aerial parts of the plants, unlike its close relative *D. brevipes*, which mostly
136 develops in the underground portions⁸. Even when infesting aerial parts, *D. neobrevipes* tends to conceal itself in
137 protected areas of the pineapple, such as under bracts, making visual detection challenging¹⁸. This behavior
138 suggests that *D. neobrevipes* prefers to inhabit concealed areas of plant structures, which may not be available
139 before spike emergence in tuberose. Consequently, the initial infestation in tuberose were primarily found in the
140 underground and basal portions of the plant. Based on primary scientific literature, this study represents the first
141 scientifically confirmed report of *D. neobrevipes* as a significant pest of tuberose.

142 The present study indicates that *D. neobrevipes* has emerged as a new devastating pest of tuberose in
143 Maharashtra, India. Its concealed habit during the initial stages of infestation makes early visual detection
144 challenging. Since ants facilitate the spread of this pest and its protection from natural enemies, managing ant
145 populations should be a primary focus in controlling mealybugs. Additionally, *D. neobrevipes* can spread with
146 infested bulbs and they continue to develop in storage, rendering the bulbs unsuitable for planting. Chances of
147 spread of mealybugs through infested planting material will also be very high. Therefore, it is recommended to
148 thoroughly wash harvested bulbs with a 1% detergent solution and allow them to dry in the shade before storage.
149 Stored bulbs should be regularly inspected for mealybug colonies and washed again with 1% detergent before
150 planting to prevent initial field infestations. To tackle the issue of this emerging pest affecting tuberose, it is
151 essential to focus on increasing awareness among farmers and conducting regular monitoring in tuberose-growing

152 regions throughout India. Further efforts are needed to understand its field bio-ecology and natural enemies to
153 develop effective management practices

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159 **Statements and Declarations:**

160 Authors declare that this work has not been published previously or it is not under consideration for publication
161 elsewhere. The article's publication is approved by all the authors and tacitly or explicitly by the responsible
162 authorities where the work was carried out

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164 **Conflicts of interest statement:**

165 The authors declare that they have no known competing financial interests or personal relationships that could
166 have appeared to influence the work reported in this paper.

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168 **References:**

- 169 1. Singh, K. P., Tiwari, A. K. and Saha, T. N., AICRP on Floriculture, ICAR Database on Tuberose
170 (*Polyanthes tuberosa* Linn.) (2010-11 to 2013-14). ICAR-DFR Technical Bulletin No. 17. Director,
171 ICAR- Directorate of Floricultural Research, College of Agriculture Campus, Shivajinagar, Pune-411005
172 (Maharashtra), India, **2015**.
- 173 2. Sadhukhan, R., Chowdhuri, T. K. and Datta, S. K., Tuberose (*Polyanthes tuberosa* Linn. / *Agave amica*).
174 In *Floriculture and Ornamental Plants*. Singapore: Springer Singapore, **2021**, pp. 1-52;
175 https://doi.org/10.1007/978-981-15-3518-5_17
- 176 3. Mani, M. & Krishnamoorthy A., Field Efficacy of Australian ladybird beetle, *Cryptolaemus montrouzieri*
177 Mulsant in the suppression of stripped mealybug *Ferrisia virgata* (Cockerell) on tuberose. *J. Biol.*
178 *Control.*, **2007**, 21 (Special Issue):129-131.
179
- 180 4. Shanthi, M., Nalini, R., Rajavel, D.S., Murali Baskaran, R.K., Occurrence of mealybug, *Ferrisia virgata*
181 Cock on tuberose in Madurai, Tamilnadu. *Insect Environ.*, **2008**, 13(4): 149.
- 182 5. Watson, G.W., Chandler, L.R., Identification of mealybugs important in the Caribbean region. 2nd ed.
183 (revised). Egham, Surrey, UK: CABI Bioscience. **2000**.
- 184 6. Williams, D.J., Granara de Willink, M.C., Mealybugs of Central and South America. CAB International,
185 UK, 644 pp. **1992**.

- 186 7. Choudhary, J. S., Gotyal, B. S., Joshi, S., Ramya, R. S., Patil, J., Lekhana, N., Selvaraj, K., Shivakumara,
187 K. T. Pineapple mealybug, *Dysmicoccus brevipes* Cockerell (Hemiptera: Pseudococcidae): a new threat
188 to garden pea, *Pisum sativum* L. in India, 08 June 2023, PREPRINT (Version 1) available at Research
189 Square. **2023**. <https://doi.org/10.21203/rs.3.rs-3028765/v1>
- 190 8. Beardsley, J. W., On the taxonomy of pineapple mealybugs in Hawaii, with a description of a previously
191 unnamed species (Homoptera: Pseudococcidae). *Proc. Hawaii. Entomol. Soc.*, **1959**, 17(1): 29-37.
- 192 9. García Morales, M., Denno, B.D., Miller, D.R., Miller, G.L., Ben-Dov, Y., Hardy, N.B., ScaleNet: A
193 literature-based model of scale insect biology and systematics. Database. **2016**, doi:
194 10.1093/database/bav118. <http://scalenet.info> [26.08.2024].
- 195 10. Curry, C., *Dysmicoccus neobrevipes* (grey pineapple mealybug). Available from CABI, Wallingford, UK
196 Crop Protection Compendium **2022**, DOI:10.1079/CPC.20251.20210102445
- 197 11. Qin, Z., Wu, J., Qiu, B.L., Ren, S., Ali, S., Effects of host plant on the development, survivorship and
198 reproduction of *Dysmicoccus neobrevipes* Beardsley (Hemiptera: Pseudococcidae). *Crop Protec.*, **2011**,
199 30(9):1124-1128.
- 200 12. Miller, D., Rung, A., Parikh, G., Venable, G., Redford, A.J., Evans, G.A., Gill, R.J., ScaleNet Edition 2.
201 **2014**, <https://idtools.org/tools/1044/index.cfm?packageID=1113&entityID=3400>
- 202 13. Beardsley, J. W., Notes on the pineapple mealybug complex, with descriptions of two new species
203 (Homoptera: Pseudococcidae). *Proc. Hawaii. Entomol. Soc.*, **1965**, 19(1): 55-68.
- 204 14. Rohrbach, K.G., Beardsley, J.W., German, T.L., Reimer, N., Sanford, W.G., Mealybug wilt, mealybugs
205 and ants on pineapple. *Plant. Dis.*, **1988**, 72: 558–565.
- 206 15. Williams, D., Watson, G., The scale insects of the tropical South Pacific region. Part 2. The mealybugs
207 (Pseudococcidae). CAB International Institute of Entomology; London, UK. **1988**
- 208 16. Vazhacharickal, P., Mathew, J., Sajeshkumar, N.K., Sabu, M., Sreejith, P.E., *Common pest and diseases*
209 *affecting Banana in South-India: an overview*. Publisher: Prem Jose (ISBN: 9781713114451,
210 1713114453), **2019**. Access online: <https://books.google.co.in/books?id=oCnxDwAAQBAJ>
- 211 17. Beardsley, J.W., TH Su, F.L., McEwen, D., Gerling, D., Field investigations on the inter relationships of
212 the big-headed ant, the gray pineapple mealybug, and pineapple mealybug wilt disease in Hawaii. *Proc.*
213 *Hawaii. Entomol. Soc.*, **1982**, 24: 51–67.
- 214 18. Jahn, G. C., Beardsley, J. W., González-Hernández, H., A review of the association of ants with
215 mealybug wilt disease of pineapple. *Proc. Hawaii. Entomol. Soc.*, **2003**, 36: 9-28.
- 216

217

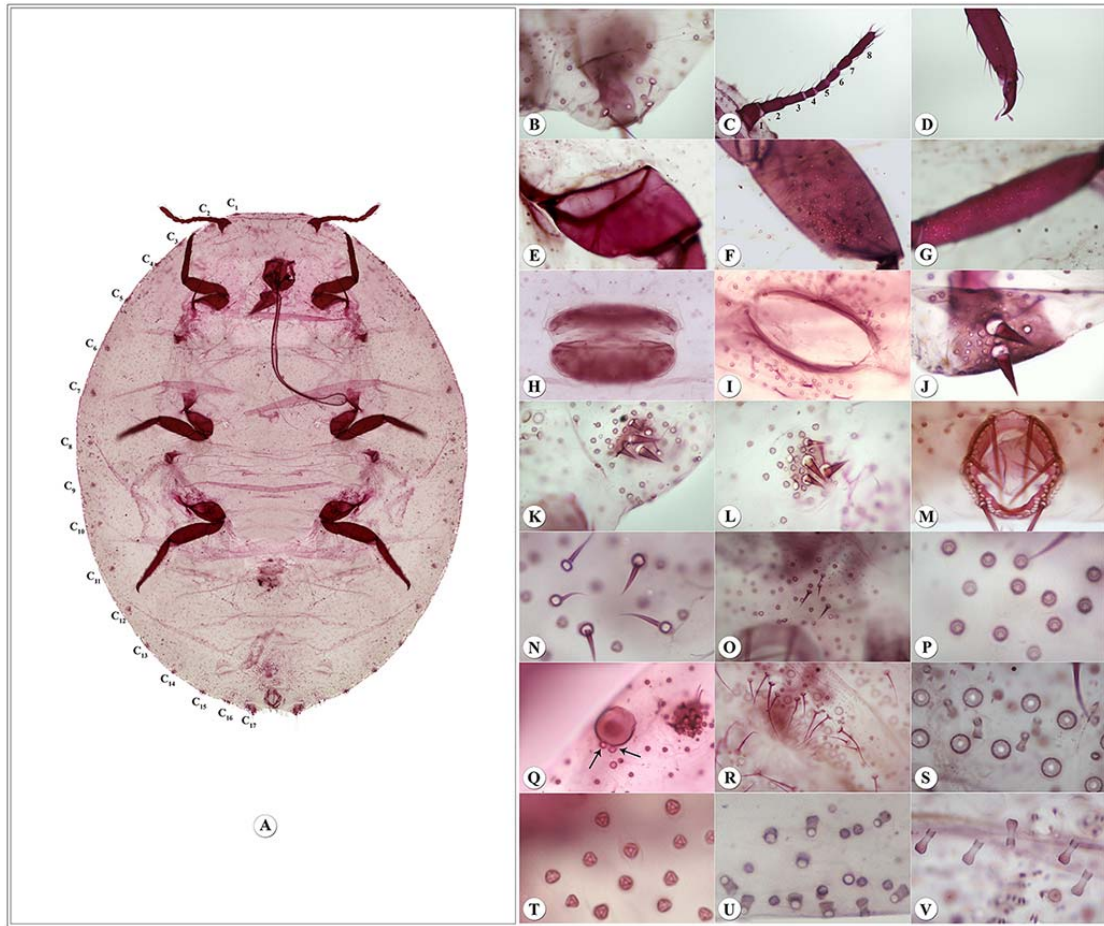


Fig. 1. Taxonomic characters of slide mounted female of *Dysmicoccus neobrevipes* Beardsley. Body derm showing 17 cerarii marked as C1 – C17 (a); Ventral surface of anal lobe (b); Antenna (c); Claw (d); Coxa without translucent pores (e); Femur with translucent pores (f); Tibia with translucent pores (g); Circulus (h); Ostiole (i); Anal lobe cerarius (j); Penultimate cerarius (k); Cerarius on head (l); Anal ring (m); Dorsal setae (n); Dorsal setae on abdominal segment VIII anterior to anal ring (o); Dorsal discoidal pores (p); Discoidal pores near eye (q); Ventral setae around vulva (r); Multilocular pores (s); Trilocular pores (t); Smaller oral collar tubular ducts (u); Larger tubular ducts (v)

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222 Fig. 2. Symptoms of mealybug, *D. neobrevipes* infestation to tuberose plants. Damage symptoms at initial phase
223 of *D. neobrevipes* infestation include a loss of vigor, downward drooping of leaves, and drying of leaf tips (a); *D.*
224 *neobrevipes* colonies developed on underground parts of the tuberose plants along with large number of ants,
225 *Solenopsis geminata* attending mealybugs (b); *D. neobrevipes* infestation started developing on basal portion and
226 above ground plant parts and excessive honeydew excretion by *D. neobrevipes* leading to blackening of leaves and
227 shoots (c)

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230 Fig. 3. Colonies of mealybug (*D. neobrevipes*) forming on newly emerging shoots, beginning at the underground
231 parts and spreading upward to the shoots and leaves (a); *D. neobrevipes* colonies developing on freshly harvested
232 bulbs (b); and completely damaged buds unsuitable for planting (c).