

# Re-Description and Morphometric Analysis of *Eucalyptus* Gall Wasp, *Leptocybe invasa*

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## ABSTRACT

Gall wasp, *Leptocybe invasa*, (Hymenoptera: Eulophidae), is a devastating pest of *Eucalyptus* plantations and nurseries. The pest causes galls on the midribs, petioles, and stem of tender shoots. In the present study detailed morphometric analysis with re-description, using twenty-six characters of gall wasp (female & male) have been given, which are distinctly supporting characters for the taxonomy and identification of *Eucalyptus* gall wasp.

## Highlights

- ① *Eucalyptus* gall wasp is major threat to the plantation throughout India and the responsible pest was studied in details.
- ② Morphometric analysis with re-description was carried out to identify the taxonomically correct species.

**Keywords:** *Eucalyptus*, Eulophidae, *Leptocybe invasa*, morphometric analysis.

The gall wasp *Leptocybe invasa* Fisher & La Salle, originating from Australia (Mendel *et al.* 2004), is a recent example of a serious threat to the various susceptible species of *Eucalyptus* in nurseries and plantations. Gall wasp inflicts the serious damage by producing galls, stunted the plant growth (Nyeko 2005). In its native environment, the population of *L. invasa* remains under check and balance, due to the presence of natural enemies. During the recent outbreak, its establishment in the Middle East (Mendel *et al.* 2004) and the Mediterranean region in 2000 (Protasov *et al.* 2008). Its presence was also reported from Kenya (Mutitu 2003), Uganda (Nyeko *et al.* 2005), South Africa (Neser *et al.* 2007), Zimbabwe (Ministry of Environment & Natural Resources Management, December 2010) and Mozambique (Tree Protection News, December 2010). Worldwide, *L. invasa* *Eucalyptus* plantations as well as small-scale farmers in Africa (Nyeko *et al.* 2009), South-East Asia (Thu *et al.* 2009), India (Kumari *et al.* 2010), China (Thu *et al.* 2009) and

South America (Wilcken *et al.* 2010). *L. invasa* has spread within a period of 10 years to all continents where *Eucalyptus* are grown.

In India, an attack of *L. invasa* was first reported from district, Mandya in Karnataka and later in 2002 at Marakkanam in Villupuram district of Tamil Nadu since and later on, the infestation has been spread rapidly throughout the country. India is a second highest area of *eucalyptus* plantation in the world; which is a potential destination for expansion of such invading pests of these invaders. Annually, invasive species cost millions of rupees in control measures and crop damage. Being an economically important insect pest of *Eucalyptus*, its detailed study on morphometrics & additional characters are helpful in its correct identification and it will also be useful for the researchers and workers on biological control of *L. invasa*.

In present study description of *L. invasa* has given in detail, based on taxonomically important morphological characters, including body length,



length & width of head, thorax, fore and hind wings, gaster and components of the genitalic capsule. These characters are of great taxonomic significance and can play an important role in the identification of *L. invasa*. Preliminary taxonomy, as well as basic aspects of its biology, was recorded by Mendel *et al.* (2004).

## MATERIALS AND METHODS

**Specimens:** Eucalyptus gall wasp, *L. invasa* was reared from galls on Eucalyptus leaves, petioles and twigs, collected on 08.09.2017 from eucalyptus plantation at district, Karnal, Haryana, India. Galls infested twigs were collected from well-branched seedlings, kept in the glass jar, covered with transparent fine muslin cloth, tighten with rubber band. Emerged adults of *L. invasa* (80 females & 5 males, ratio 16:1) were collected and preserved in 70% alcohol. In present study, these 15 specimens of gall wasp, 10 females and 5 males for dissection and their detailed morphometric, different characters were taken. After series of dehydration with alcohol, specimens were dissected in clove oil (Taylor 2002) under stereo zoom binocular microscope (AARK-zoom Star-VI). Dissected parts were kept in a drop of Canada balsam, oriented to the required positions, and permanently mounted with coverslips (Platner *et al.* 1999).

**Measurements & photography:** Twenty-one taxonomic characters (Head, antenna, mesosoma, forewings, metasoma, hind tibia, genitalia, ovipositor etc.) were measured with oculometer of different magnifications power (10x, 20x, 40x) in the Leitz labor luxs (Leica made in Germany) and different dissected body parts of specimens mounted on slides were photographed with Auto-montage 3D microscope with attached camera.

**Statistical analysis:** Different statistical techniques (standard deviation, average) were used during the present study using Microsoft Excel.

## Morphological abbreviations

The following abbreviations are used in the text:

CL<sub>1</sub>- CL<sub>2</sub>= Clavalsegments 1 & 2; CC = Costal cell; F1-F4 = Funicular segments 1,2,3 & 4; MS = Malar sulcus; MV = Marginal vein; ML = Median line, groove; OOL = Ocellar-ocular length; PMV = Post marginal vein; POL = Post ocellar line; OD = Ocellar

diameter; SLG = Sub lateral line; SMG = Sub median line; SMV = Sub marginal vein; STV = Stigmal vein; aa = aedeagal apo-demes; di = digitus; ds = digital spines; lp = length of phallobase; ps = parameral setae; pv = penis valve; vs = volsellar setae.

## RESULTS AND DISCUSSION

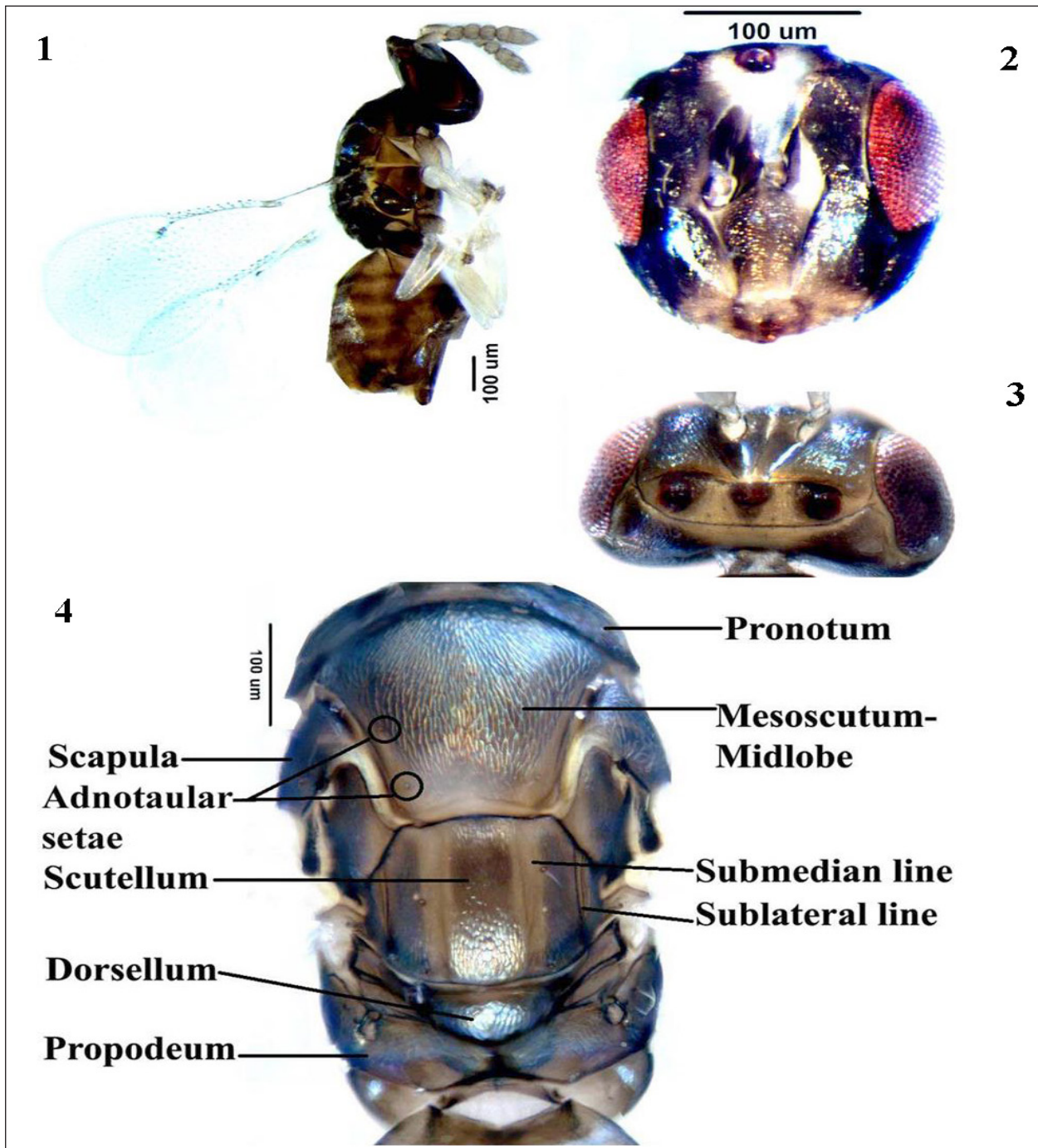
**Diagnosis:** Head color brown to dark bluish with metallic shine; mouth margins light brown to yellow; distinct groove and a weak area around the ocellar triangle. Midlobe of mesoscutum distinctly curved with 2-3 pairs adnotaular setae present on lateral side; scutellum with sub-median line and sub-lateral line; propodeum with raised lobe of the callus that partially overhangs the outer rim of the spiracle; dorsellum longer than propodeum medially. Antenna with scape yellow (darkened apically) and slightly expanded ventrally; pedicel conical; funicle (3 segments) roughly quadrate and longer than scape.

### Re-description with additional morphometric of important characters

**Female:** Adult wasp is 1249.26  $\mu\text{m}$  ( $\pm 93.18$ ) long (Fig. 1); head slightly wider than long in facial view; eye dark reddish in color. Antenna with 4 anelli; 3 funicular segments and 3 club segments. Scape more than three times as long as wide. Pedicel long, slightly more than half the length of scape; funicle 3 segmented distinctly longer than other antennal segments; clava 3 segmented, all segments are almost subequal in length.

**Head:** Wider than long in facial view (Fig. 2), clypeus weakly bilobed, the lower margin of clypeus entire, antennal torulus inserted about half margin of eyes; MS convex anteriorly and 0.5 $\times$  of the eyes length. Slightly collapsed with a deep sulcus around the ocellar triangle, POL 2.3 $\times$  OOL, OOL as long as OD (Fig. 3). **Antennae:** (Fig. 9) with antennal formula 11433; 4 segmented anelli, 3 funicular segments, 3 club segments. Scape slightly expanded ventrally and  $(3.3 \pm 0.29) \times$  as long as wide, pedicel long and about  $(2.3 \pm 0.23) \times$  as long as wide, slightly more than half the length of scape (73: 128), funicle (3 segmented), longer than other antennal segments and  $(3.1 \pm 0.39) \times$  as long as wide; clava 3 segmented, about  $(2.3 \pm 0.25) \times$  as long as wide.

**Mesosoma** (Figs. 4 & 5): 1.48 $\times$  as long as broad. Pronotum short; midlobe of mesoscutum surface



**Figs. 1-4:** *Leptocybe invasa* (Female): 1. Adult; 2. Head (facial view); 3. Head (dorsal view); 4. Mesosoma (dorsal view)

curved, smooth and shiny without median line, ( $0.9 \pm 0.08$ )  $\times$  as long as its anterior width and  $1.5\times$  as long as posterior width, with 3-5 pairs adnotaular setae on either side; scutellum ( $0.9 \pm 0.07$ )  $\times$  as long as wide, sub-median line and sub-lateral lines or grooves present, with 2-3 pairs of dorsal setae, anterior pairs in middle of the proximal half and near to SML of scutellum; dorsellum clearly visible in dorsal view,  $1.4\times$  as long as median length of

propodeum; propodeum without median lines or lateral carinae, surface faintly reticulate, raised lobe of callus present, spiracular depression open to anterior margin of propodeum. *Forewings* (Fig. 8): ( $2.1 \pm 0.10$ )  $\times$  as long as wide; sub-marginal vein generally with 2-3 setae (dorsal); PMV short less than  $0.25\times$  length of stigma vein; basal vein without setae; speculum area less sub cubital line of setae, speculum closed behind by cubital line and cubital

**Table 1:** Morphometrics of important characters of female, *Leptocybe invasa*

| Body Parts | Characters (female)    | Measurements ( $\mu\text{m}$ ) $\pm$ SD |                  | Ratio (L/W) $\pm$ SD |
|------------|------------------------|---|------------------|----------------------|
|            |                        | Length                                  | Width            |                      |
| Body       | Body length            | 1249.26 $\pm$ 93.18                     | —                | —                    |
|            | Facial View            | 292.1 $\pm$ 51.2                        | 324.8 $\pm$ 49.9 | —                    |
| Head       | Malar Space            | 128.5 $\pm$ 16                          | —                | —                    |
|            | Eye                    | 149.09 $\pm$ 24.6                       | —                | —                    |
| Antenna    | Scape                  | 128.2 $\pm$ 10.3                        | 39.1 $\pm$ 3.7   | 3.3 $\pm$ 0.29       |
|            | Pedicel                | 73.2 $\pm$ 6.7                          | 32.3 $\pm$ 3.3   | 2.3 $\pm$ 0.23       |
|            | Funicle                | 118.9 $\pm$ 13.6                        | 38 $\pm$ 2.6     | 3.1 $\pm$ 0.39       |
|            | Clava                  | 108.8 $\pm$ 6.4                         | 47.1 $\pm$ 4.2   | 2.3 $\pm$ 0.25       |
|            | Fore wing              | 909.6 $\pm$ 45.1                        | 427.2 $\pm$ 24.7 | 2.1 $\pm$ 0.10       |
|            | Marginal Fringe        | 65.4 $\pm$ 15.2                         | —                | —                    |
|            | Stigmal Vein           | 70.3 $\pm$ 3.3                          | —                | —                    |
| Mesosoma   | Sub Marginal Vein      | 195.5 $\pm$ 15.9                        | —                | —                    |
|            | Marginal Vein          | 271.1 $\pm$ 26.2                        | —                | —                    |
|            | Mid lobe of Mesoscutum | 226.2 $\pm$ 29.6                        | 245.3 $\pm$ 18.7 | 0.9 $\pm$ 0.08       |
|            | Scutellum              | 163.7 $\pm$ 14.8                        | 192.4 $\pm$ 21.4 | 0.9 $\pm$ 0.07       |
|            | Dorsellum              | 59.9 $\pm$ 5.7                          | —                | —                    |
|            | Propodeum              | 42.1 $\pm$ 2.3                          | —                | —                    |
|            | Hind tibia             | 259.1 $\pm$ 20.2                        | —                | —                    |
| Metasoma   | Gaster                 | 521.2 $\pm$ 29                          | —                | —                    |
|            | Genital capsule        | 367.7 $\pm$ 30.7                        | —                | —                    |
|            | Ovipositor Sheath      | 74.4 $\pm$ 8.9                          | —                | —                    |

**Table 2:** Morphometrics of important characters of male, *Leptocybe invasa*

| Characters (male) | Measurements ( $\mu\text{m}$ ) $\pm$ SD |                  | Ratio (L/W) $\pm$ SD |
|-------------------|---|------------------|----------------------|
|                   | Length (L)                              | Width (W)        |                      |
| Body              | 1290.12 $\pm$ 64.17                     | —                | —                    |
| Scape             | 113.74 $\pm$ 3.64                       | 43.08 $\pm$ 3.43 | 2.65 $\pm$ 0.164     |
| Pedicel           | 68.75 $\pm$ 3.06                        | —                | —                    |
| Funicle           | 170.35 $\pm$ 6.30                       | —                | —                    |
| Antennal club     | 119.52 $\pm$ 6.67                       | 51.19 $\pm$ 3.69 | 2.40 $\pm$ 0.182     |
| Flagellar hair    | 89.02 $\pm$ 5.24                        | —                | —                    |
| Genitalia         | 173.76 $\pm$ 5.85                       | 76.06 $\pm$ 3.36 | 2.29 $\pm$ 0.057     |

row of setae not extending to basal vein; MV 3.9x as long as STV; marginal fringe little shorter than STV and 0.15x long as wing width.

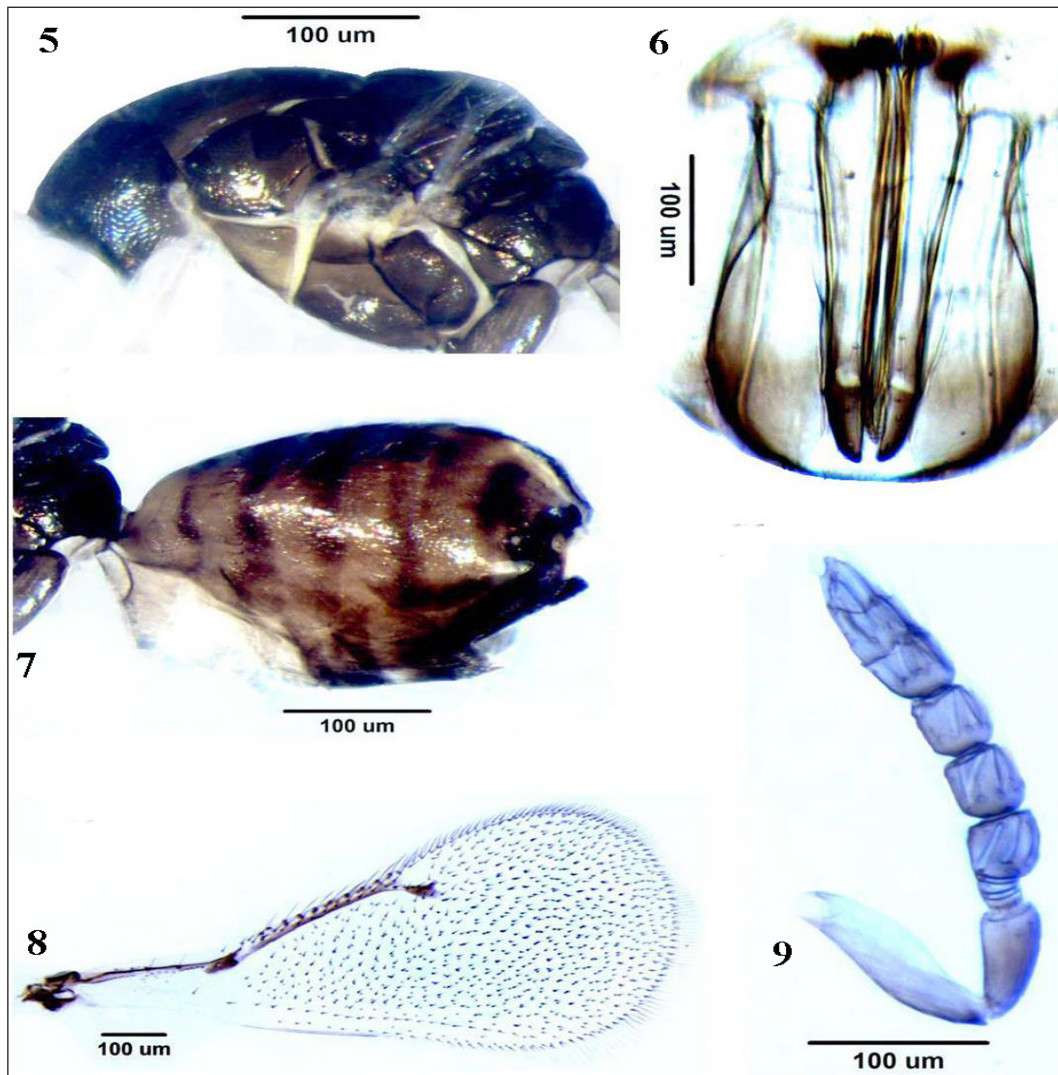
**Metasoma** (Figs 6 & 7): Sub sessile with very short transverse petiole. Shorter than mesosoma; hypopygium exceeding the half-length of gaster; ovipositor sheath short.

**Male:** (Fig. 10) 1290.12  $\mu\text{m}$  ( $\pm$ 64.17) long, wasp generally not observed due to the lytokous population, where females are produced from unfertilized eggs. All characters are similar to female except antennae (Fig.11) twelve segmented with

3 ring segments, 4 funicular, 3 claval, segments (11343). Scape with sensilla on lateral margin, (2.64  $\pm$  0.164)  $\times$  as long as wide; pedicle less than the half of funicle; funicle is longer than female funicles (170: 118); flagellum (119.52  $\pm$  6.67)  $\times$  as long as wide with long tapering flagellar hair, longest of which 1.74 $\times$  of the maximum width of flagellum.

**Genitalia** (Fig. 12): (176  $\pm$  5.85)  $\mu\text{m}$  long and (76.06  $\pm$  3.36)  $\mu\text{m}$  wide, narrow and elongated (2.29  $\pm$  0.057) $\times$  as long as wide; digitus 2 $\times$  as long as wide with single digital spine; parameral setae reaching up to the base of digital spine; volsellar ridge distinct





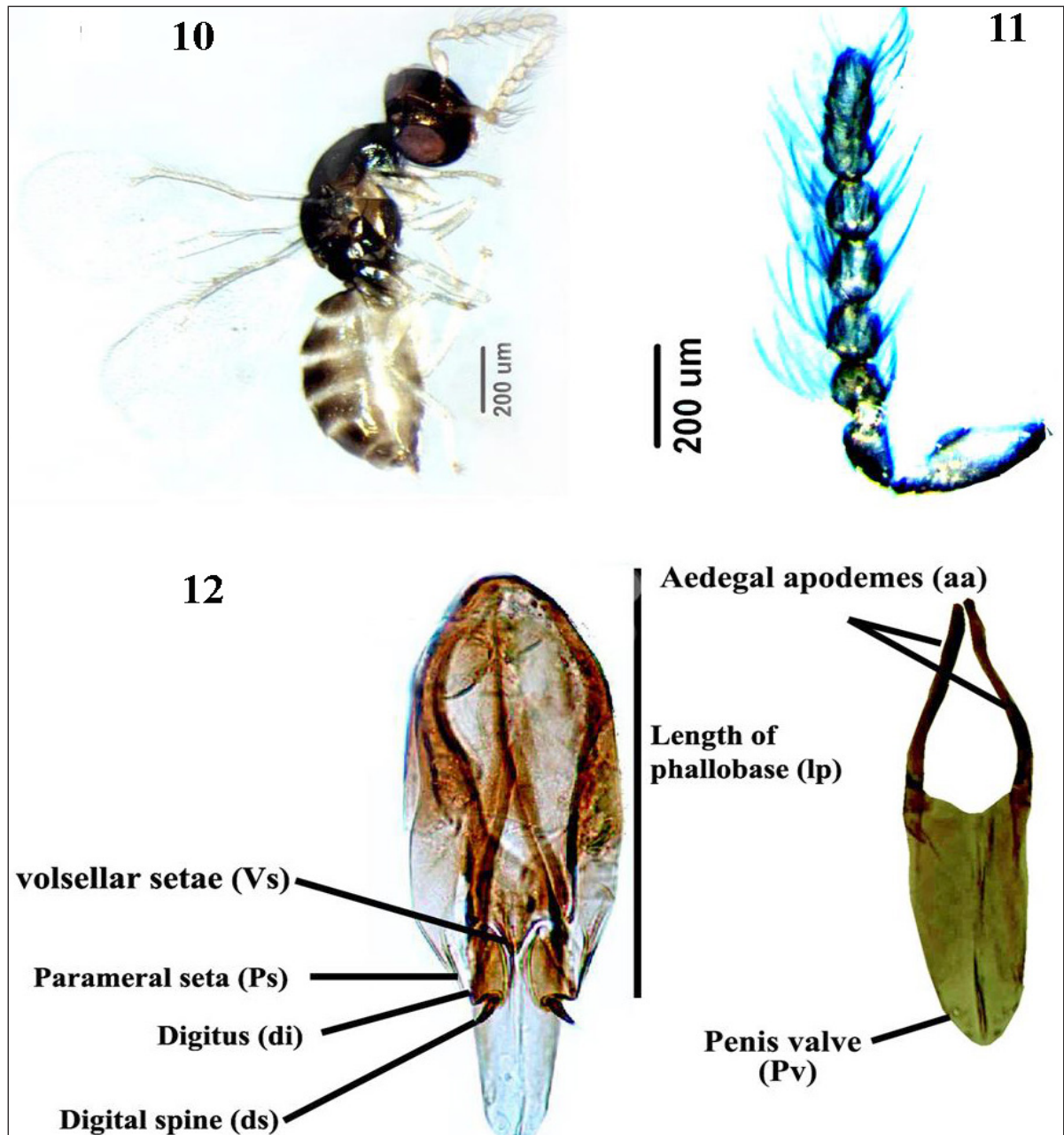
**Figs. 5-9:** *Leptocybe invasa* (Female): 5. Mesosoma (lateral view); 6. Genitalia; 7. gaster (lateral view); 8. Fore wing; 9. antenna

having two volsellar setae; penis valve slightly longer than aedeagal apodemes, both together longer than phallobase.

**Morphometric analysis:** Previously, such type observations on morphometric characters were not recorded and this study has been carried out for the first time. The observations are summarized in following tables (Tables 1&2).

In most of the taxonomic descriptions limited characters of taxonomic importance are given, in the original description of new species (Nagaraja 1973). Similarly, in case of original description of *Leptocybe invasa* Fisher & La Salle is very brief, and the details of male wasp was not given. Recently some workers have studied detailed morphometric of hymenopteran wasps. Burk and Heraty (2002) have studied morphometrics of four *Trichogramma*

spp. viz. *T. californicum*, *T. exigum*, *T. minutum*, *T. platneri* and found that species varies in order of *T. platneri* > *T. minutum* > *T. californicum* > *T. exigum*. They have separated the males of *T. californicum* and *T. exigum* from *T. minutum* using parameters, pertaining the ratio of maximum length of flagellar hairs and width of flagellum. Querino & Zucchi (2004) attempted the study on the morphometrics of six species of *Trichogramma*, using different morphometric parameters, pertaining the length of the posterior extension of dorsal lamina, length of the dorsal lamina, with genital capsule and length of the mesoscale posterior setae. Similarly, *T. chilonis* and *T. japonicum* was separated by the genital capsule length and width. Hassan and Yousuf (2007), Khan & Yousuf (2017) and (khan *et al.* 2018) separated two species of *Trichogramma* (*T. chilonis*



**Figs. 10-12:** *Leptocybe invasa* (male): 10. adult; 11. Antenna; 12. genitalia

and *T. japonicum*) using different characters for morphometric analysis.

## CONCLUSION

Fisher & LaSalle reported and species, *Leptocybe invasa* as new genus in 2004 and gave the description based on female characters while male was unknown. For filling the gap in our existing knowledge on *Leptocybe invasa* this species is re-described after incorporating its detailed morphometric analysis along with additional morphometric characters of both female and male. Following important

morphometric taxonomic characters of *Leptocybe invasa* have been studied for the first time:

Body length of female 1249.26 ( $\pm 93.18$ )  $\mu\text{m}$  and male 1290.12  $\mu\text{m}$  ( $\pm 64.17$ ); Head wider than long (facial view); POL 2.3 $\times$  OOL, OOL as long as OD. Scape 3.3  $\times$  as long as wide and slightly expanded ventrally, pedicel long and 2.3  $\times$  as long as wide, slightly more than half the length of scape (73: 128), funicle (3 segments), longer than other antennal segments and 3.1 $\times$  as long as wide; clava 3 segmented, about 2.3 $\times$  as long as wide. Midlobe of mesoscutum curved without median line, 0.9  $\times$  as long as its anterior

width and 1.5× as long as posterior width, surface smooth and shiny without median line, with 3-5 pairs adnotaular setae on either side; scutellum 0.9 × as long as wide, sub-median line and sub-lateral lines or grooves present. Forewings with PMV short less than 0.25× length of stigma vein; MV 3.9× as long as STV; marginal fringe little shorter than STV and 0.15× long as wing width. Ovipositor sheath short, not reaching the middle apical margin of the abdomen.

Male 1290.12 µm long (longer than female); antennae with Scape having sensilla on lateral margin, 2.65 × as long as wide; pedicel less than the half of funicle; flagellum 2.40 × as long as wide with long tapering flagellar hair, longest of which 1.74× of the maximum width of flagellum.

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## REFERENCES

- Burks, R.A. and Heraty, J.M. 2002. Morphometric analysis of four species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) attacking codling moth and other tortricid pests in North America. *Journal of Hymenoptera Research*, **11**(2): 167-187.
- Hassan, E. and Yousuf, M. 2007. First record of *Trichogramma plasseyensis* Nagaraja (Hymenoptera: Trichogrammatidae), from Central India, and its morphometrics and additional diagnostic characters, *Indian Journal of Entomology*, **69**(1): 58-62.
- Khan, S. and Yousuf, M. 2017. Morphometric analysis of *Trichogramma achaeae* Nagaraja and Nagarkatti, an important biological control agent of agriculture and forestry. *Journal of Biological Control*, **31**(20): 90-94.
- Khan, S., Yousuf, M. and Ikram, M. 2018. Morphometric studies of two species of *Trichogramma* (Hymenoptera: Trichogrammatidae), *Proceeding of Zoological Society* (<https://doi.org/10.1007/s12595-018-0260-4>)
- Kumari, N.K., Kulkarni, H., Vastrad, A.S. and Goud, K.B. 2010. Biology of eucalyptus gall wasp, *Leptocybe invasa* Fisher and La Salle (Hymenoptera: Eulophiae). *Karnataka Journal of Agricultural Science*, **23**: 211-212.
- Mendel, Z., Protasov, A., Fisher, N. and La Salle, J. 2004. The taxonomy and natural history of *Leptocybe invasa* (Hymenoptera: Eulophidae) gen & sp. nov., an invasive gall inducer on Eucalyptus *Australian Journal of Entomology*, **43**: 101-113.
- Ministry of Environment & Natural Resources Management, Republic of Zimbabwe, Zimbabwe's Fourth National Report to the Convention on biological Diversity. Available from: <http://www.cbd.int/doc/world/zw/zw-nr-04-en.pdf> (December 2010).
- Mutitu, K.E. 2003. A pest threat to Eucalyptus species in Kenya. *KEFRI Technical Report*, pp. 12.
- Neser, S. and Millar, I. 2007. *Blastopsylla occidentalis*: another new Eucalyptus pest in South Africa. *Plant Prot. News*, **72**: 2.
- Nyeko, P. 2005. The cause, incidence and severity of a new gall damage on eucalyptus species at Oruchinga refugee settlement in Mbarara district, Uganda. *Ugandan Journal of Agricultural Science*, **11**: 47-50.
- Nyeko, P., Mutitu, E.K. and Day, R.K. 2007. Farmers knowledge, perceptions and management of the gall-forming wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), on Eucalyptus species in Uganda. *International Journal of Pest Management*, **53**: 111-119.
- Nyeko, P., Mutitu, E.K. and Day, R.K. 2009. Eucalyptus infestation by *Leptocybe invasa* in Uganda. *African Journal of Ecology*, **47**: 299-307.
- Platner, G.R., Velten, R.K., Planoutene, M. and Pinto, J.D. 1999. Slide-mounting techniques for *Trichogramma* (Trichogrammatidae) and other minute parasitic hymenoptera. *Entomological News*, **110**: 56-64.
- Protasov, A., Doganlar, M., LaSalle, J. and Mendel, Z. 2008. Occurrence of two local *Megastigmus* species parasitic on the eucalyptus gall wasp *Leptocybe invasa* in Israel and Turkey. *Phytoparasitica*, **36**: 449-459.
- Querino, R.B. and Zucchi, R.A. 2004. Morphometric analysis in *Trichogramma* (Hymenoptera: Trichogrammatidae) species. *Neotropical Entomology*, **33**(5): 583-588.
- Thu, P.Q., Dell, B. and Burgess, T.I. 2009. Susceptibility of 18 eucalypt species to the gall wasp *Leptocybe invasa* in the nursery and young plantations in Vietnam. *Science Asia*, **35**: 113-117.
- Taylor, L.V. 2002. Four new species of *Trichogramma* Westwood (Trichogrammatidae) from Japan. Master's theses 2379 San Jose State University, 1-36.
- Tree Protection News. 2010. Newsletter of the Tree Protection Co-operative Programme and the DST/NRF Centre of Excellence in Tree Health and Biotechnology. December 2010, vol. 20.
- Wilcken, C.F., Soliman, E.P., de Sa, L.A.N., Barbosa, L.R., Dias, T.K., Ferreira-Filho, P.J. and Oliveira, R.J.R. 2010. Bronze Bug *Thaumastocoris peregrinus* Carpintero and Dellape (Hemiptera: Thaumastocoridae) on Eucalyptus in Brazil and its distribution. *Journal of Plant Protection Research*, **50**: 201-205.

