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# A new species of *Nesolynx* Ashmead, 1905 (Hymenoptera, Eulophidae) parasitizing potter wasp, *Delta pyriforme* (Fabricius, 1775) (Hymenoptera, Vespidae) in its nest from southern India

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**ABSTRACT:** *Nesolynx deltaphagus* **sp. nov.** parasitizing the potter wasp species *Delta pyriforme* (Fab.) (Hymenoptera, Vespidae) is newly described with illustrations from Kerala, India. This is the first report of parasitism of *Nesolynx* on Vespidae. A key for the Indian species of *Nesolynx* is provided along with the diagnosis of the new species with congeners. DNA barcode of the new species using universal primers of CO1 is also provided against accession number (Accession No: OK484482).


**KEY WORDS:** Chalcidoidea, Tetrastichinae, taxonomy, host record

## INTRODUCTION

*Nesolynx* Ashmead (Eulophidae, Tetrastichinae) is a small genus having widespread distribution in the Neotropical and Oriental regions (Noyes 2019). The genus is presently represented by 17 described species worldwide, nine from the Oriental region and five species namely *N. flavipes* Ashmead, *N. javanica* (Ferrière), *N. orientalis* Khan, Agnihotri & Sushil, *N. phaeosoma* (Waterston) and *N. thymus* (Girault) are recorded from India (Bouèek, 1976, 1988; Narendran, 2007; Noyes, 2019). The majority of *Nesolynx* species are gregarious primary parasitoids on pupae of Hymenoptera (Braconidae and Ichneumonidae), Lepidoptera (Gracillariidae, Limacodidae, Notodontidae and Psychidae, Pyralidae) or Hemiptera (Pseudococcidae) pupae and also act as hyperparasitoids through Tachinidae (Ferrière, 1939; Bouèek, 1988; Narendran, 2007; Noyes, 2019).

The potter or mason wasp species *Delta pyrforme* (Fab.) (Vespidae, Eumeninae) are solitary wasps preying and provisioning their developing immature mainly with caterpillars in excellently sculpted earthen incubation chambers for their developing immature (Segoli *et al*., 2020; Deshmukh, 2021). Even with the impregnable architectural finesse, the brood of the potter wasp are prone to attack. A strepsipteran parasite, *Stylops* sp. is found to attack *Eumenes petiolata* (=*D. pyriforme*) from India (Smith, 1859; Salt and Bequaert, 1929).

Members of eulophid genera *Elasmus* Westwood,

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*Melittobia* Westwood and *Kocourekia* Bouček are only known till date to attack aculeate Hymenoptera (Edwards and Pengelly, 1966; Krombein, 1967; Valentine, 1967; Bouček, 1977; Donovan, 1980; Dahms, 1984; Macfarlane *et al.,* 1984; Donovan and Macfarlane, 1984; Macfarlane and Palma, 1987; LaSalle, 1994; Okaba and Makino, 2008; Kim, *et al*., 2016; Cao *et al*., 2017; Noyes, 2019). A new species of *Nesolynx* attacking the pupa of *D. pyriforme* from southern India is described with illustrations.

## MATERIALS AND METHODS

Nest making by a female *D. pyriforme* was observed during the early hours 9th December 2020 on a branch of *Phyllanthus acidus* (L.) from Elathur (11°19'32.3"N ; 75°44'30.3"E, alt. 23 m above mean sea level) in Kozhikode district of Kerala, India. The potter wasp moved to and forth bringing in lumps of semi-solid mud within its mandibles held on by forelegs and carefully moulding the nest using its mandibles at an average of 12±3 seconds per lump. A single egg was placed after mass provisioning each cell at an average of 9±3 larvae per cell. Individual cells were closed with a narrow seal (through which the emerging wasps chew their way out). The entire nest with four chambers (three adjoining the branch and one exterior) was finished (6.5×3.5×2.5 cm) by late 14th December 2020. The nest was broken off from the branch after observing exit holes visible outside on 13th January 2021. On detaching, an intact pupal film was observed in one of the cells (Figs. 19, 20). Adult parasitoids emerged out of the film immediately and the nest along with emerging parasitoids were quickly transferred into a clear container. The adult potter wasp was identified by Dr. Girish Kumar (Vespidae expert, Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, Kerala).

Parasitoids were carefully aspirated, killed and stored in 70 percent ethyl alcohol, processed using standard Hexamethyldisiloxane treatment (Heraty and Hawks, 1998). Morphoanalysis of the specimens were done under Leica M205A Stereo zoom Microscope and images were captured using Leica DMC 2900 digital camera attached to the microscope. Measurements of the specimens were obtained using Leica LAS (Leica Application Suite V4.7.1) microsystems by Leica (Heerburg, Switzerland). Images at varying focal planes were stacked into a single image using Leica Automontage Software V4.2 and final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® CS5 (Version 12.0 x64). Molecular analysis was carried out using NucleoSpin® Tissue Kit (Macherey-Nagel) (DNA isolation) and PCR amplification of a 591 bp region near the 5' terminus of the CO1 gene following standard protocols and universal primers (Folmer *et al*., 1994). The amplified sequence was analysed using Geneious Pro v5.1 (Drummond *et al*. 2010), compared in online BLAST and uploaded to NCBI (Accession No: OK484482). The new species was also compared with the holotype images of *N. flavipes* Ashmead [USNMENT00802939] available in the entomological collection of National Museum of Natural History, Washington, U.S.A (NMNH, previously USNM).

The description of the new species is based on the type specimens deposited in the "National Zoological collections" of Zoological Survey of India, Western Ghats Regional Centre, Kozhikode (ZSIK).

**Terms and measurements**. The terms used are mainly those of Narendran (2007) unless noted otherwise. The nomenclature for cuticular sculpture follows Harris (1979). Abbreviations of terms used are as follows: AOL = distance between anterior ocellus and posterior ocellus; CC = costal cell; $fu_x$ = funicular number; $Gt_x$ = gastral tergum number; ML = median line or groove; MS = malar space; MV= marginal vein; OOL = oculo-ocellar distance, minimum distance between a posterior ocellus and eye; POL = postocellar distance, the distance between the two posterior ocelli; SMG = submedian groove; SMV = submarginal vein; STV= stigmal vein; UNC = uncus.

## RESULTS AND DISCUSSION

### Genus *Nesolynx* Ashmead, 1905

*Nesolynx* Ashmead 1905. 28: 966. Type species: *Nesolynx flavipes* Ashmead, by monotypy.

*Ceratotrastichus* Girault and Dodd in Girault 1913: 254. Type species: *Ceratotrastichus bisulcatus* (synonymy by Bouček, 1988).

*Omphalomomyia* Girault 1913: 174. Type species: *Omphalomomyia lividicaput* Girault (synonymy by Bouček, 1988).

*Aceratoneurella* Girault 1917: 7. Type species: *Aceratoneurella cinctiventris* Girault, (synonymy by Bouček, 1977).

**Diagnosis**. Head with vertex not collapsing; antenna short with funicular segments usually transverse; mesoscutum without ML; mid lobe of mesoscutum with dense and regular pilosity, each hair placed on small papillae; scutellum without SMG, anterior setae of scutellum well before middle; petiole very short, hardly visible.

**Note.** A key to Indian species is augmented from Narendran (2007) and modified to incorporate the new species, *N. deltaphagus*. Narendran (2007) states *N. orientalis* as a junior synonym of *N. javanica*, but this could not be validated in the present study due to lack of additional specimens and unavailability of type specimens on request. Hence, we retain the nominal status of the taxa and include the same in the Indian key.

**Key to the Indian species of *Nesolynx* Ashmead**

**1.** Mesosoma orange; metasoma orange, with dorsal margin black; vertex and frons black with metallic green reflections, lower face yellowish brown.....***thymus* Girault**

- Mesosoma black or dark brown with or without metallic tinge; metasoma mostly black or brown; vertex and frons black without metallic green reflections, lower face black or dark brown .....**2**

**2.** Mid lobe of mesoscutum with long semierect pilosity becoming larger posteriorly; wing without dense discal pubescence; flagellum pale yellow, brown on apical flagellomeres; metasoma longer than combined length of head and mesosoma; hind basitarsus shorter than second tarsomere ..... ***phaeosoma* (Waterston)**

- Mid lobe of mesoscutum with short and uniform pilosity; wing with dense discal pubescence; flagellum dark brown; metasoma as long as or little shorter than combined length of head and mesosoma; hind basitarsus longer than second tarsomere..... **3**

**3.** $Gt_1$ longest, at least reaching one-third of gaster, following tergites subequal; pedicel less than 2.0× as long as broad; MV 4–6× as long as STV; body shiny black or brown with slight metallic reflections on mesoscutum and metasoma; all legs yellow including forecoxa..... **4**

- All gastral segments almost equal; pedicel greater than 2× as long as broad; MV up to 4× as long as STV; body black without metallic reflection; all legs yellow with at least fore coxa black..... **5**

**4.** Frontovertex wide, 0.7× of total head width; mouth more than 2× broader than MS; POL 1.2× OOL; OOL greater than AOL; fore wing with decolourised area on parastigma..... ***deltaphagus* sp. nov.**

- Frontovertex 0.5× total head width; mouth slightly broader than MS; POL 3× OOL; OOL less than AOL; fore wing without decolourised area on parastigma..... ***flavipes* (Ashmead)**

**5.** Antennae situated at the level of ventral margin of eyes; $fu_2$ slightly longer than broad and $fu_3$ subquadrate; clava shorter than the combined length of two preceding funicular segments; scutellum with two pairs of setae; SMV with four setae; MV as long as SMV.....***javanica* (Ferriere)**

- Antennae situated well above the level of the ventral eye margin; $fu_2$ quadrate and $fu_3$ wider than long; clava longer than the combined length of two preceding funicular segments; scutellum with three pairs of setae; SMV with five setae; MV shorter than SMV..... ***orientalis* Khan, Agnihotri & Sushil**

***Nesolynx deltaphagus* sp. nov.** (Figs. 1–16)

LSIDurn:lsid:zoobank.org:act:2740AF9F-B79A-42FA-B4F8-FE8F1D2CB661

**Type material:** Holotype: ♀ India: Kerala, Kozhikode district, Elathur (11°20¹37ºN;

75°43¹6.74ºE, alt. 23m above mean sea level), 13.i.2021, Coll. C. Binoy, ex. pupa of *Delta pyriforme* (Fab.). Paratypes: 293♀, 35♂, same data as the holotype.

Depositories: Holotype ♀ [ZSIK] ZSIK Regd. No.ZSI/WGRC/IR/INV.21914, Paratype ♀

[ZSIK] ZSIK Regd. No. ZSI/WGRC/IR/INV.21915

**Diagnosis:** Body brownish black with metallic reflection on head and metasoma, all legs yellow with infuscations on fore and hind coxae, frontovertex wide, 0.7× total head width, POL 1.2× OOL, apical margin of clypeus bilobed, emarginated with deep median cleft; mandible with strong tooth and truncation, fore wing with dense discal setation, decolourised on parastigma (between SMV and MV), propodeum reticulated with distinct median carina, metasoma ovate, slightly shorter than combined length of head and mesosoma, $Gt_1$ longest, 0.3× as long as metasoma, smooth and shiny on anterior half, remaining terga reticulate dorsally.

**Description:** Holotype ♀ (Figs. 1–11) Body length 0.90 mm, length of fore wing 0.71 mm.

Body brownish black with metallic reflections on head, mesosoma and metasoma. The following parts variably coloured: eye and ocellus reddish brown; scape and pedicel pale yellow, rest of antennomeres dark brown; all legs yellow except fore coxa and base of hind coxa (yellowish brown), metatibial spur pale yellow, claws brown; frons and vertex dark metallic green; supraclypeal area yellowish brown, clypeus yellow with apical margin reddish brown; mandible yellow with ventral margin and apex reddish brown; maxillary palpi pale yellow; pronotum brown with slight metallic greenish lustre; mesoscutum and scutellum dark shiny brown; mesepimeron and mesepisternum brown, acropleuron and tegula pale yellow; metasoma brown with coppery lustre; all terga with slight metallic reflection on apical margin; wings hyaline with veins and setae pale brown (Figs. 1–11).

Head in dorsal view transverse, 2.6× as broad as long, vertex shiny metallic, finely reticulate with scattered setigerous pits; ocelli arranged in about obtuse angled triangle; POL 1.2× OOL, OOL 1.7× AOL, POL 2.1× AOL (Fig. 6); in frontal view head 1.2× as wide as its maximum length, sculpture same as that of vertex, setae arising from each pit; toruli inserted at ventral eye margin (Fig. 3); mouth 2.5× broader than malar space; clypeus bilobed, strongly emarginated, with a deep cleft medially; mandible bidentate with strong tooth and truncation (Figs. 3–5); eye pubescent, height of eye in profile 2× as long as malar space; malar sulcus distinct, curved at base; malar space finely reticulate, no setigerous pits (Fig. 4); antenna with two distinct annelli, three-segmented funicle and three segmented clava (11233); scape and pedicel with short adpressed setae; long sensillae and numerous adpressed setae on remainder of antennomeres; scape not reaching median ocellus, reaching only 0.7× of frons, 3.2× as long as wide, 2.1× as long as pedicel; pedicel 1.8× as long as wide, 1.7× as long as $fu_1$; all funicles subquadrate; $fu_3$ 1.2× longer than $fu_1$, 1.1× longer than $fu_2$; clava as long as combined length of all funiculars, 2.6× as long as wide, tapering to apex, distinct terminal spine present; relative length: width of antennomeres: scape = 75:23.5, pedicel = 41:22, $fu_1$ = 23.8:24.7, $fu_2$ = 24.8:26.5, $fu_3$ = 27.7:30.3, clava = 80:31 (Fig. 2).

***Mesosoma:*** Pronotum and mesoscutum distinctly pilose with setae arising from well-arranged rows of pits; surface transversely reticulate similar head; pronotum subconical with raised projecting spiracle on either side and five pairs of long setae near apical margin; mesoscutum 1.5× as long as wide with well-marked notauli and a pair of longer setae near posterior margin of mesoscutum; posterior part of lateral lobe of mesoscutum bare; axilla with similar sculpture as mesoscutum (Fig. 7); scutellum longitudinally reticulated with distinct sublateral grooves, 1.4× wider than long, having two pairs of long suberect setae, anterior setae placed towards anterior margin of scutellum; axillula reticulated without conspicuous setae; propodeum and dorsellum with wider reticulations; dorsellum 4× as long as wide, half-length of propodeum; propodeum short, 4× as long as broad, median carina distinct (Fig. 9); propodeal spiracle large, peritreme exposed, nearest to anterior margin, distance from anterior

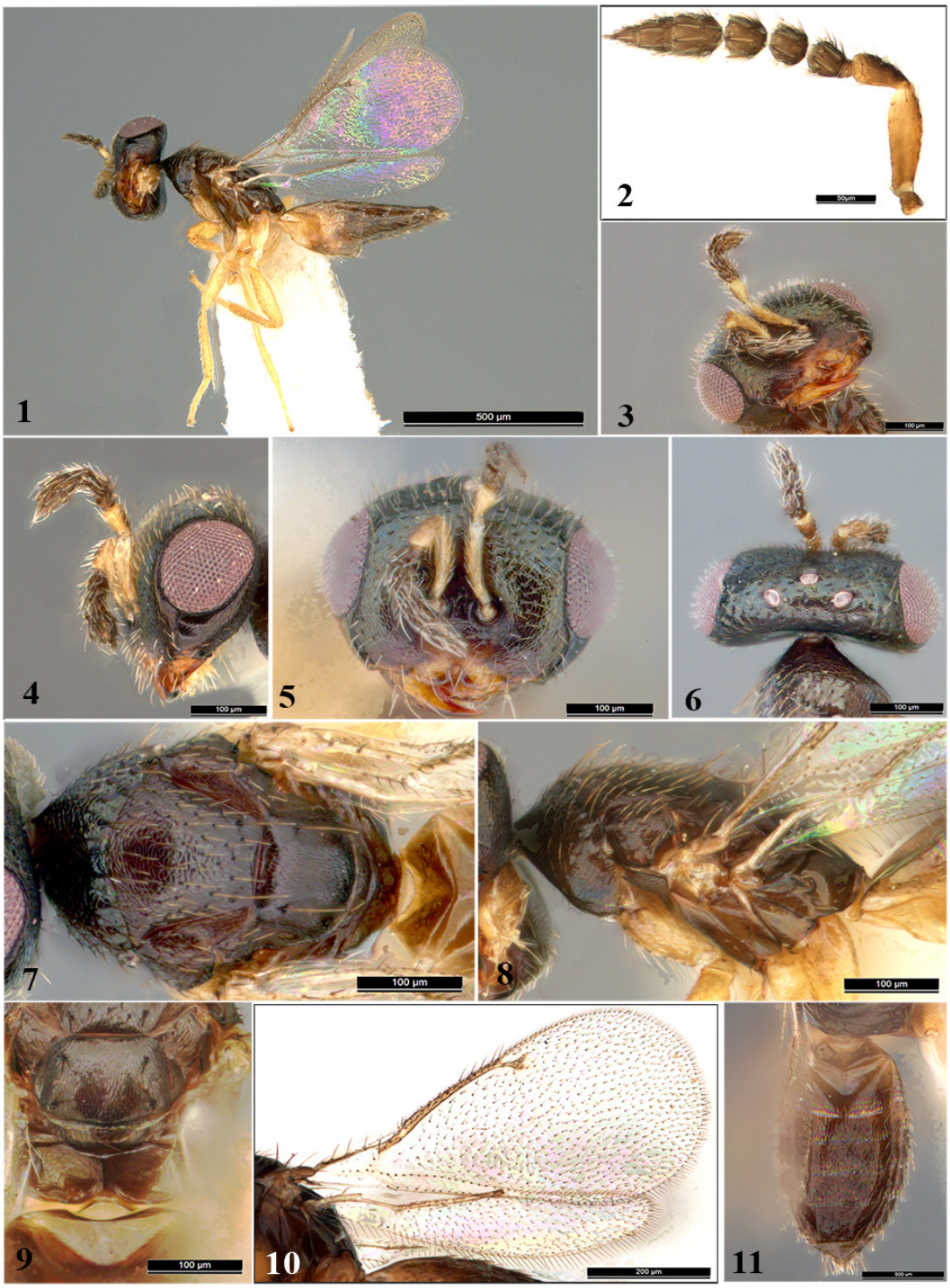


**Fig. 1-11** *Nesolynx deltaphagus* **sp. nov.** Holotype ♀. 1, habitus, lateral view; 2, antenna; 3, lower face (clypeus), frontal view; 4, head, lateral view; 5, head, frontal view; 6, head, dorsal view; 7, mesosoma, dorsal view, 8, mesosoma, lateral view, 9, propodeum, dorsal view, 10, fore wing, 11, metasoma, dorsal

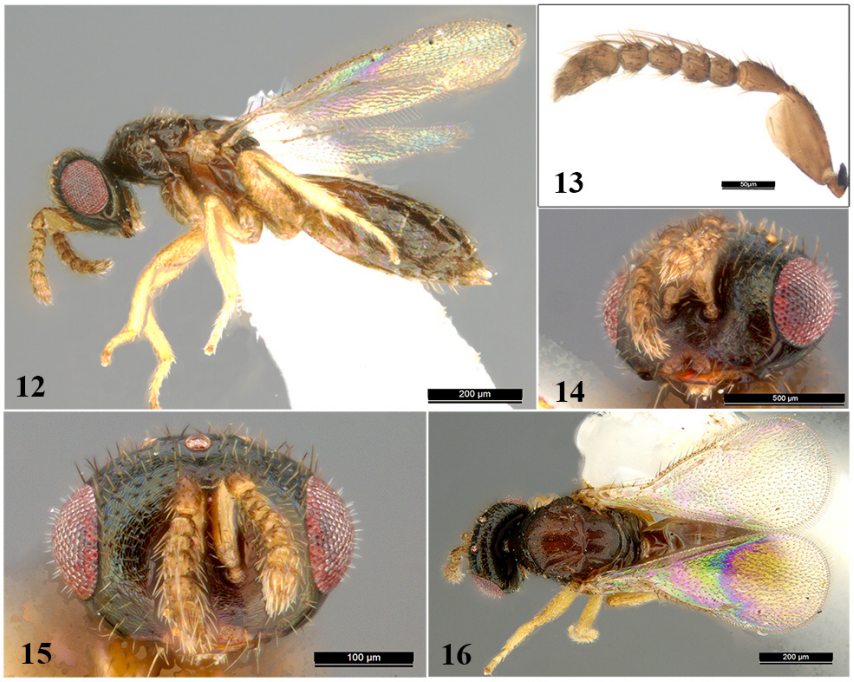


**Fig. 12-16** *Nesolynx deltaphagus* **sp. nov.** Paratype ♂. 12, habitus, lateral view; 13, antenna; 14, lower face (clypeus), frontal view; 15, head, frontal view; 16, habitus, dorsal view


**Fig. 17-20** *Delta pyriforme* (Fab.) Nesting and parasitisation. 17, ♀. *D. pyriforme* building nest on *Phyllanthus acidus* (L.) (December, 2019); 18, finished nest; 19, *N. deltaphagus* emerging out of pupal film of *D. pyriforme*; 20, emerged nest of *D. pyriforme* (arrow indicating the cell harbouring parasitised *D. pyriforme* pupa)

margin to spiracle less than diameter of spiracle; callus with three setae; lateral panel of pronotum and prepectus reticulate; acropleuron smooth; mesepisternum almost smooth, transepisternal sulcus present; upper mesepimeron and lower mesepimeron smooth and shiny, transepimeral sulcus almost straight; metapleuron weakly reticulate (Fig. 8).

***Wings:*** Fore wing hyaline, with short and dense discal setation, 1.9× as long as its maximum width; SMV with three strong semi erect dorsal setae; decolourised area on parastigma between SMV and MV; STV terminates in small rounded knob; cubital setal line wavy up to middle, remainder straight till apical wing margin; MV 1.8× as long as SMV; MV 5.3× as long as STV; UNC relatively long and slender, half-length of STV; costal cell 6.6× as long as broad, with single line of setae; basal cell having four small setae; basal vein with three setae, speculum closed below, marginal fringe on wing short; subcubital line nearest to the posterior wing margin; hind wing hyaline, 4.8× as long as wide, marginal fringe long, discal ciliation similar to that of fore wing (Fig. 10).

***Legs:*** Metacoxa setose, weakly reticulate, 2.4× as long as wide; hind femur pubescent, medially widened and tapering at both ends, surface reticulate, 2.4× as long maximum width; hind tibia densely setose, 1.1× as long as hind femur, 6.2× as long as wide; metatibial spur short, not reaching middle of basitarsus (Fig. 1).

***Metasoma:*** Petiole hardly visible in dorsal view; metasoma ovate, basal one third of $Gt_1$ smooth and shiny, remaining part reticulated with metallic reflections; densely setose, 1.2× longer than mesosoma, 0.1× shorter than combined length of head and mesosoma, 1.54× as long as broad (Fig.1); $Gt_1$ relatively large, posterior margin slightly emarginate, 0.3× as long as metasoma, $Gt_2$–$Gt_4$ subequal in length; $Gt_5$ 1.3× longer than preceding tergum; $Gt_6$–$Gt_8$ short, without metallic reflections; hypopygium reaching middle of metasoma; ovipositor slightly protruding; cercal setae unequal, longer one sinuate (Fig. 11).

**Male Description:** Paratype ♂ (Figs. 12–16). Body length 0.98 mm, length of forewing 0.63 mm. Surface sculpture similar to that of female. The following characters (other than usual sexual dimorphic states on number of antennomeres, size and terminal metasomal segments) may be considered in associating the male of *N. deltaphagus* **sp. nov.** from its female. Hind femur infuscated medially (Fig. 12); scape apically expanded into a plaque, 2.0× as long as maximum width; pedicel 2.2× $fu_1$; each funicle bearing a whorl of long bristle like setae, setae 2.5× longer than respective funicular length (Fig. 13); eye conspicuously setose (Figs 14, 15); metasoma 1.1× as long as the combined length of head and mesosoma (Figs. 12, 16).

**Distribution:** India: Kerala.

**Host:** Gregarious parasitoid on pupa of *Delta pyriforme* (Fabricius, 1775) (Figs. 17–20).

**Etymology:** The species epithet is derived from the host's genus name *Delta.*

**Remarks:** The new species resembles the Oriental species *N. javanica* (Ferrière) in the key to Oriental species of *Nesolynx* (Narendran, 2007) in having propodeum with median carina, fore wing with dense discal setation, but differs from the same in having OOL greater than AOL (*vs.* OOL less than AOL), POL 1.2× OOL (*vs.* POL 3× OOL), club as long as combined length of all funiculars (*vs.* club not longer than combined length of $fu_2$ and $fu_3$); 5.3× as long as STV (*vs.* MV 4.0× STV), metasoma 0.9× as long as combined length of head and mesosoma (*vs.* metasoma as long as combined length of head and mesosoma), $Gt_1$ longest (*vs.* gastral terga subequal in length), metasoma brown with slight metallic reflection (*vs.* metasoma without any metallic reflection).

*N. deltaphagus* **sp. nov.** is similar to *N. flavipes* Ashmead in having body black or brown with slight metallic on metasoma; $Gt_1$ longest and median carina on propodeum distinct. However it differs from *N. flavipes* in having: fore and hind coxae infuscate (*vs.* all legs yellow); fore wing with veins brown (*vs.,* fore wing with veins yellow); POL 1.2× OOL (*vs.* POL 3.5× OOL); OOL greater than AOL

(*vs.* OOL less than AOL); mouth 2.5× broader than MS (*vs.* mouth 1.2× broader than MS); mesoscutum coarsely reticulate with small setigerous pits (*vs.* mesosoma with large setigerous punctures); metasoma 0.9× as long as combined length of head and mesosoma (*vs.* metasoma as long as combined length of head and mesosoma).

*Nesolynx* is a group of gregarious parasitoids on pupae of various holometabolous insects and a few species are known to possess various desirable attributes of a biocontrol agent (Kumar *et al*., 1996; Narayanaswamy and Devaiah, 1998; Aruna and Manjunath, 2009). The potter wasp nest is mostly impenetrable to any foreign entity when it is completely moulded for incubation. The only report of a parasite on *D. pyriforme* is recorded by Smith (1859: 130) — *Eumenes petiolata,* ♀, India. The abdomen of the third segment with a female *Stylops* beneath it, at the fourth distorted by the pupa case of an escaped male — and subsequent confirmation by Salt and Bequaert (1929: 253). The present study forms the first report of any parasitic hymenopteran attacking the potter wasp *D. pyriforme*.

**Molecular analysis and need for a deeper probe**

Neighbour Joining trees (NJ trees) are quick visual summaries of degrees of specialization of a species, indications of taxonomic puzzles, variability in barcode length, BIN composition (the equivalent of sorting morphological look-alikes into unit trays), and data-checking and typically conspecifics grouped together in their own terminal clade (Sharkey *et al*., 2021). However, specimens with less than about 550 base pairs are more likely to be misplaced on the tree, often that requires other traits (morphology and/or host data in case of parasitoids). This leads to the placement of unrelated specimens to group together (lack of conserved base pairs). BLAST inventory of successfully barcoded conspecifics (irrespective of nomenclature) should also be present which is the primary requisite for any successful NJ tree preparation and if in absence, the resolution of such species complexes requires ecological, morphological, and a deeper genomic probe (Janzen *et al*., 2017 and references therein).

The CO1 genomic extracts of *N. deltaphagus* **sp. nov.** subjected to PCR amplification yielded a 591 bp (forward) and 377 bp (reverse) region near the 5' terminus standard protocols. The forward and reverse sequences were analysed using the online BLAST tool of NCBI for comparison with congeners. Because of the absence of any other related taxa in the database, the new taxa formed an outgroup to all deposited eulophid material and could not be represented on an NJ tree. Henceforth, this deposited sequence could be used in future reference of the *Nesolynx* taxa.

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