DIFFERENTIAL DIAGNOSIS BETWEEN *TUNGA PENETRANS* (L., 1758) AND *T. TRIMAMILLATA* PAMPIGLIONE *ET AL.*, 2002 (INSECTA, SIPHONAPTERA), THE TWO SPECIES OF THE GENUS *TUNGA* PARASITIC IN MAN

PAMPIGLIONE S.*, TRENTINI M.**, FIORAVANTI M.L.* & GUSTINELLI A.*

Summary:

Of the ten currently known species of sand fleas, only two, *Tunga penetrans* and *Tunga trimamillata*, are known to be parasites of man, besides other warm blooded animals, most of which are peridomestic. The hosts of the other eight are limited to a few genera of wild mammals. *T. trimamillata* was only recently identified and differentiated from *T. penetrans* by features of the gravid female phase. In the present paper the different morphological characters of both for non-gravid females and males of the two species are described. In the non-gravid-females the distinguishing characters are: a) differences in the length of the first two segments of the maxillary palps (the first is the longest in *T. trimamillata*, whereas the second is the longest in *T. penetrans*), this feature is associated with another character *i.e.* the presence of short, thick spines in addition to the bristles on the surface of the segments only in *T. trimamillata*; b) the presence, only in *T. trimamillata*, of a row of spines on the antero-medial surface of the tibia of the 3rd pair of legs; c) the last abdominal spiracle protrudes in *T. trimamillata* but not in *T. penetrans*; d) the hood of the *hilla* in *spermatheca* is surrounded by a papilla only in *T. penetrans*. The following morphological characters differentiate males of *T. trimamillata* and *T. penetrans*: a) *T. trimamillata* has a row of spines on the antero-medial surface of the tibia of the 3rd pair of legs; b) the diameter of the abdominal spiracles of *T. trimamillata* is smaller than that of *T. penetrans* and the edges of the spiracles are more regular; c) the claspers and *aedeagus* of *T. trimamillata* are shorter than those of *T. penetrans*; d) the profile of the aedeagal apodeme differs between the two species.

KEY WORDS : differential diagnosis, *Tunga penetrans*, *Tunga trimamillata*, taxonomy, tungiasis.

Résumé : DIAGNOSE DIFFÉRENTIELLE ENTRE *TUNGA PENETRANS* (L., 1758) ET *T. TRIMAMILLATA* PAMPIGLIONE *ET AL.*, 2002 (INSECTA, SIPHONAPTERA), LES DEUX ESPÈCES DU GENRE *TUNGA* PARASITES DE L'HOMME

Parmi les dix espèces de puces-chiques décrites jusqu'à présent, Tunga penetrans *et* T. trimamillata *sont les deux seules reconnues comme pouvant parasiter l'homme, en plus de diverses autres espèces de mammifères, essentiellement synanthropes. Les huit autres sont inféodées à quelques genres d'animaux sauvages.* T. trimamillata *a été récemment identifiée et différenciée de* T. penetrans *seulement en tant que femelles gravides parasites. Les auteurs apportent ici des caractères morphologiques différentiels entre ces deux espèces, tant pour les femelles non gravides, que pour les mâles. Chez les femelles non gravides, les critères spécifiques sont les suivants : a) une longueur différente des deux premiers segments des palpes maxillaires, le premier étant plus long que chacun des autres chez* T. trimamillata, *tandis que chez* T. penetrans *c'est le deuxième; on note également, chez la première espèce, la présence d'épines courtes, épaisses, en plus des soies classiques; b) la présence, seulement chez* T. trimamillata, *d'une série d'épines sur la surface antéro-médiale du tibia III; c) le dernier spiracle de l'abdomen est en saillie, faisant une véritable protubérance chez* T. trimamillata *seulement; d) l'apex de la hilla forme une papille chez* T. penetrans, *mais non chez* T. trimamillata. *Chez les mâles, les caractères suivants ne sont présents que chez* T. trimamillata *: a) une série d'épines sur la surface antéro-médiale du tibia III; b) les spiracles abdominaux ont un diamètre plus réduit et un contour moins irrégulier; c) les claspers et l'*aedeagus *sont plus courts; d) le profil de l'apodème de l'*aedeagus *est différent.*

MOTS CLÉS : *diagnose différentielle,* Tunga penetrans, Tunga trimamillata, *taxonomie, puce-chique.*

INTRODUCTION

Of the ten known species of fleas belonging to the genus *Tunga* (Insecta, Siphonaptera, Tungidae), only two – *T. penetrans* and *T. trimamillata* – are known to be parasites of man (Westwood, 1840; Blanchard, 1890; Fioravanti *et al.*, 2003) and incidentally of other mostly peridomestic warm blooded animals. Hosts of the other eight species are restricted to a few genera of hosts, which are generally wild mammals (*Tamandua, Dasypus, Cabassous, Mus, Rattus, Akodon, Peromyscus, Neotoma,* etc).

Since *T. trimamillata* has only recently been identified (Pampiglione *et al.*, 2002), most cases of human tungiasis, observed by physicians in the past, were attributed to *T. penetrans*, often without a careful morphological study. It seems probable that some cases were wrongly identified. In some of the accounts in the 18th and 19th centuries (Sloane, 1725; Juan & Ulloa 1748; Pohl & Kollar, 1832; Heusser & Clarez, 1860; Karsten, 1865; Bonnet, 1867; Guyon, 1870) mention was made of the possible existence of two or three different species, referring to opinions of indigenous

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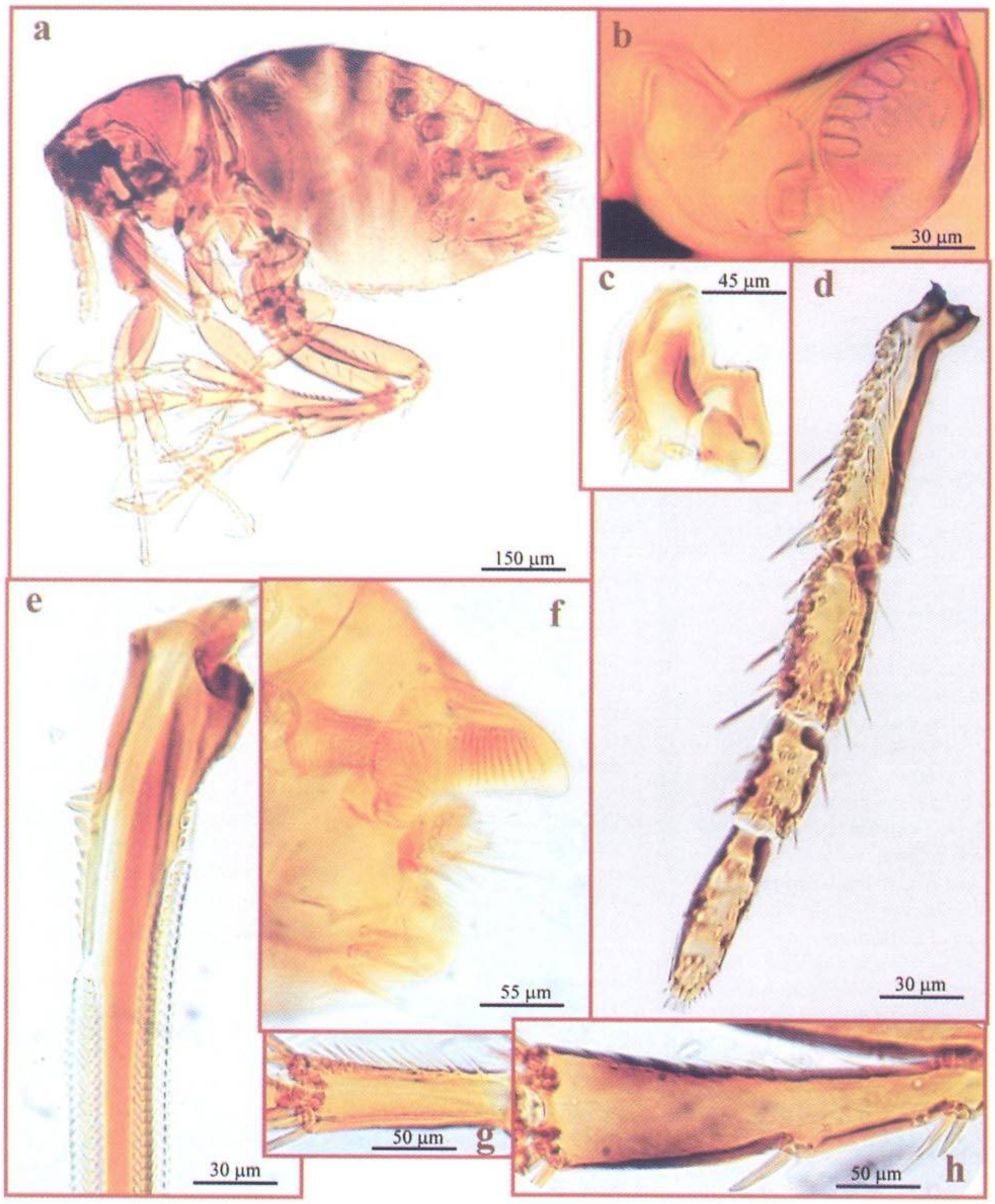

Fig. 1. – a) A non-gravid female of *Tunga trimamillata*. Some details: b) antennal club; c) *maxilla*; d) maxillary palp; e) *lacinia*; f) terminal segments of the abdomen; g) first tarsal segment of the 3rd pair of legs; h) tibia of the same leg.

peoples of South America. However no other species parasitic in man were discovered until this century. Recently, for the first time, we were able to collect numerous specimens of the two species, both males and non-gravid females. The aim of this study was to compare their morphological characteristics to see if these stages could be reliably identified. The differential characters of gravid females of both species were illustrated in a previous report (Pampiglione *et al.*, 2003).

MATERIALS AND METHODS

The specimens were collected in Ecuador using humans as bait or on sticky cardboards placed on the ground of pig sties or cow stalls, in the localities of Santa Isabel and Catacocha in the Andean province of Azuay and Loja. Fifteen males and 30 non-gravid females each of *T. trimamillata* and *T. penetrans* were examined. The specimens were fixed in 70 % ethanol and cleared in Hoyer's medium, directly after washing in tap water (females) or after immersion in 10 % NaOH for 24 hours (males). Some other specimens of both species were dissected and stained in either MIF (merthiolate, iodine, formaldehyde) or Lugol's jodine to highlight some features of the internal organs.

RESULTS AND DISCUSSION

After extraction of the chigoe, a striking morphological differences between gravid females of the two species is clearly visible with the naked eye: *T. trimamillata* has three anterior humps

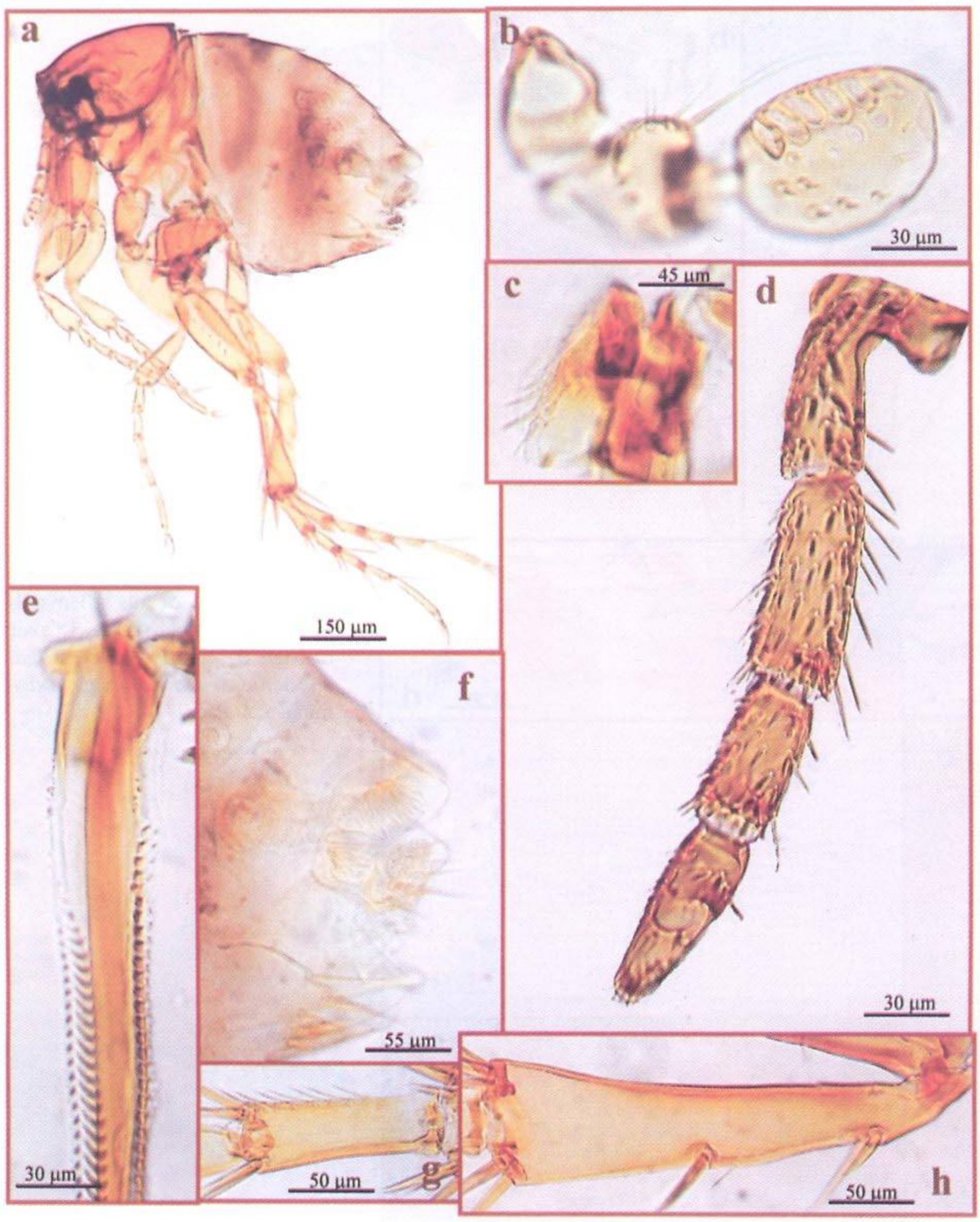

Fig. 2. – a) A non-gravid female of *Tunga penetrans*. Some details: b) antennal club; c) *maxilla*; d) maxillary palp; e) *lacinia*; f) terminal segments of the abdomen; g) first tarsal segment of the 3rd pair of legs; h) tibia of the same leg.

which are absent in *T. penetrans* (Pampiglione *et al.*, 2003). This feature is not present in non-gravid females and males and it is necessary to examine these stages with a microscope to see the most important differential characters as follows.

FEMALES

30 specimens measured for each species.

Size: the average total length of *T. trimamillata* is slightly greater (798-1,210 µm) than that of *T. penetrans* (650-1,050 µm). When compared the shape of the body of the former is more elongate ("torpedo-like") than the latter (Figs 1a, 2a).

Maxillary palps: whereas in *T. penetrans*, the second segment is generally longer than the others (Westwood, 1840; Hopkins & Rothschild, 1953) and numerous bristles are implanted on the entire surface of the palps, in *T. trimamillata* the first segment is always the longest and, in addition to bristles, bears very characteristic, short, thick, slightly claw-like spines (Figs 1d, 2d); in general the palps of the latter species are a little thinner and longer than those of the former.

Maxillae: six-seven claw-like teeth are also present on the anterior surface of the maxilla in *T. trimamillata*, while in *T. penetrans* the teeth are thinner, less numerous and less curved (Figs 1c, 2c).

Antennae: on the surface of the club in *T. penetrans* the bristles are always more numerous (from eight to 16) than in *T. trimamillata* (from four to seven) (Figs 1b, 2b).

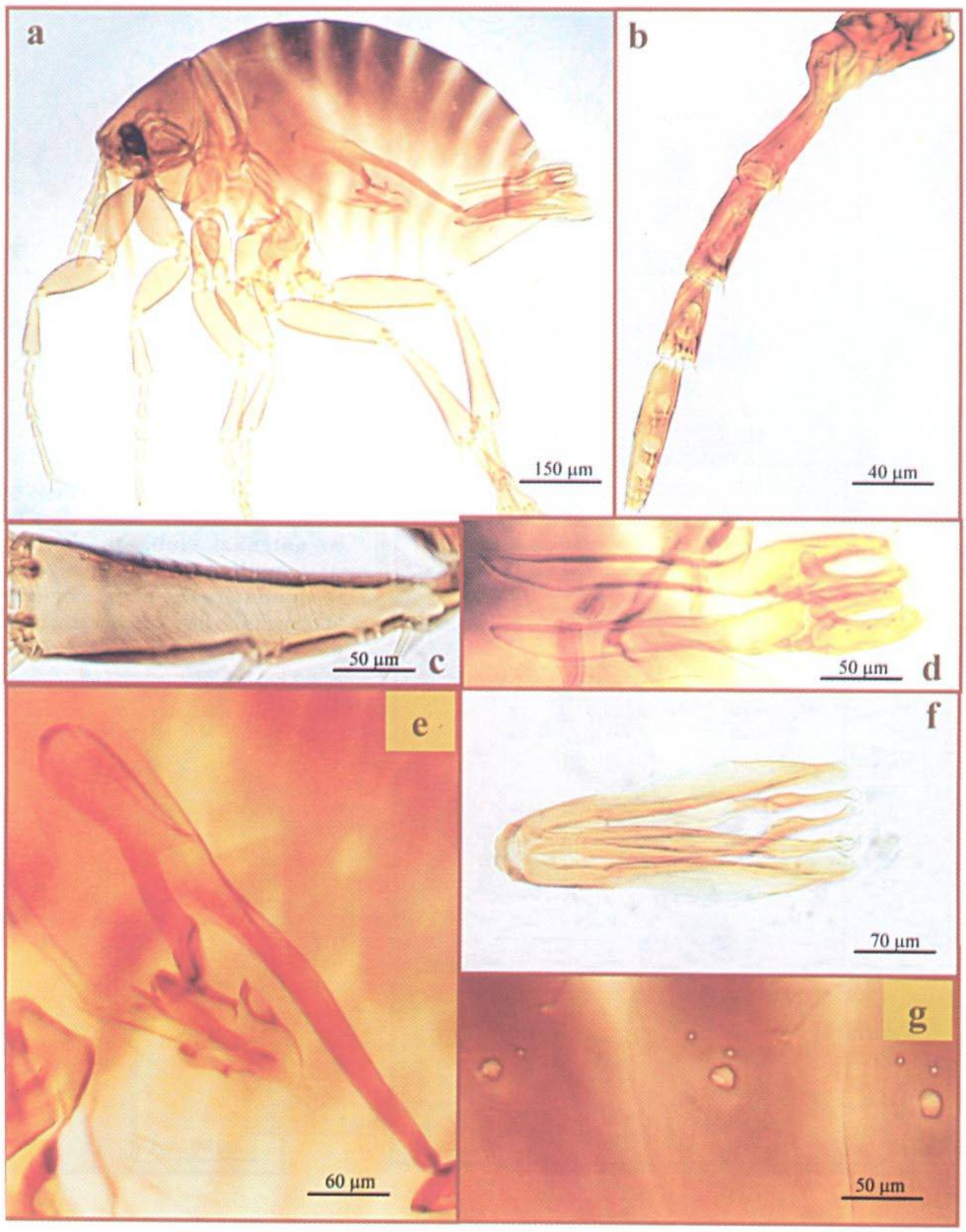

Fig. 3. – a) A male of *T. trimamillata*. Some details: b) maxillary palp; c) tibia of the 3rd pair of legs; d) claspers; e) aedeagal apodeme; f) *aedeagus*; g) abdominal spiracles.

Laciniae: 10-12 sclerotised teeth of graduated size are present on the base of the *laciniae* in *T. trimamillata*; in *T. penetrans* they are much smaller and less sclerotised (Figs 1e, 2e).

3rd pair of legs: there is a row of 9-12 spines on the antero-medial surface of the tibia of *T. trimamillata* which are absent on the tibia of *T. penetrans* (Figs 1h, 2h). This is one of the most important differential characteristics because it is constant and easily seen. Also, 9-13 spines are present on the edge of the 1st tarsal segment of the former species while, in the latter species, the number is always less (Figs 1g, 2g).

Abdominal spiracles: in *T. trimamillata*, the first abdominal spiracle is smaller (8-10 µm) than that of *T. penetrans* (12-14 µm). The last pair of abdominal spiracles protrudes outwards in the former species but not in the latter (Figs 1f, 2f), forming a sort of small "tail".

Sex organs: the *spermathecae* of non-gravid females of the two species show only few notable differences. They appear to be formed by a large *area cribriformis* (of the *bulga*) connected on one side to the fecundation chamber by a long and twisted canal and, on the other side, to a tail (*hilla*) terminating in a hood with a slightly striated wall. Almost always the *area* and the hood are easily seen with a microscope in specimens cleared in Hoyer's medium (Figs 5a, 5b), but are less apparent after treatment with 10 % NaOH solution. The connection between the *bulga* and the *hilla* seems to

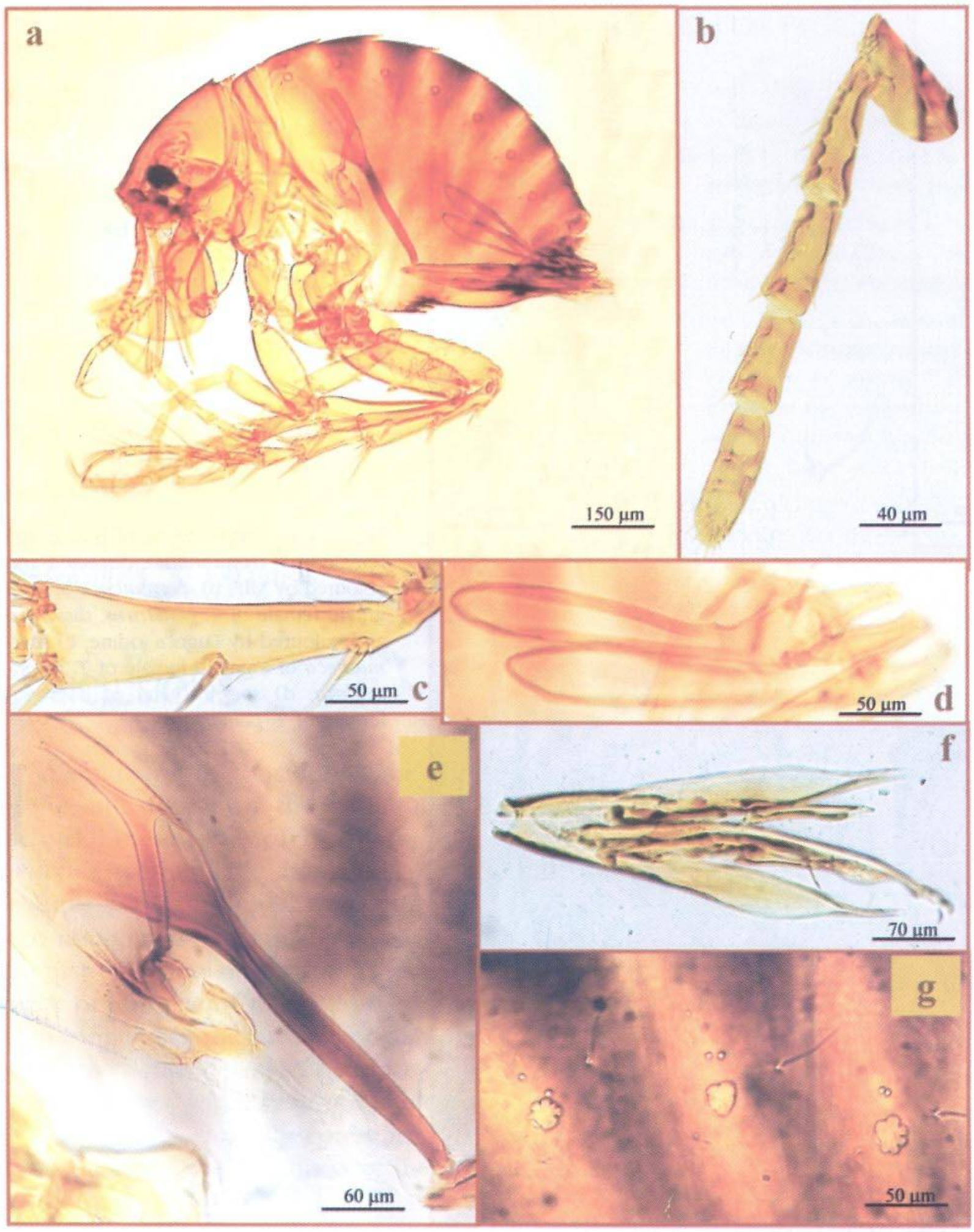

Fig. 4. – a) A male of *T. penetrans*. Some details: b) maxillary palp; c) tibia of the 3rd pair of legs; d) claspers; e) aedeagal apodeme; f) *aedeagus*; g) abdominal spiracles.

be made of a sleeve of very loose transparent tissue; however, we have never been able to demonstrate this clearly in any of our preparations. Both *bulga* and *hilla* appear to be a little smaller in *T. trimamillata* than in *T. penetrans*. In the latter species the hood of the *hilla* is always surmounted by a papilla which is absent in the former. This structure is present in both gravid and non-gravid specimens of *T. penetrans*. The aspect of the *spermatheca* of non-gravid females of both species is notably different from that of gravid females in which this organ assumes a completely different shape with strongly sclerotic walls (Hopkins & Rothschild, 1953; Beaucournu, 2003, com. pers.) (Figs 5c, 5d). Unlike the *spermatheca* of non-gravid females, the *spermatheca* in gravid specimens appears notably larger in *T. trimamillata* than in *T. penetrans* (280-340 µm × 160-250 *vs.* 205-260 × 90-140 µm, N = 10 specimens of both species) (Figs 5c, 5d).

Males

15 specimens measured for each species: as with non-gravid females, males of the two species can be identified by morphological features, some of which are the same as the differential characters of non-gravid females whereas others, relating to the reproductive and respiratory systems, are seen only in males.

Size: the total length is almost the same in the two species (780-1,040 µm for *T. trimamillata*, 650-1,110 µm for *T. penetrans*) (Figs 3a, 4a).

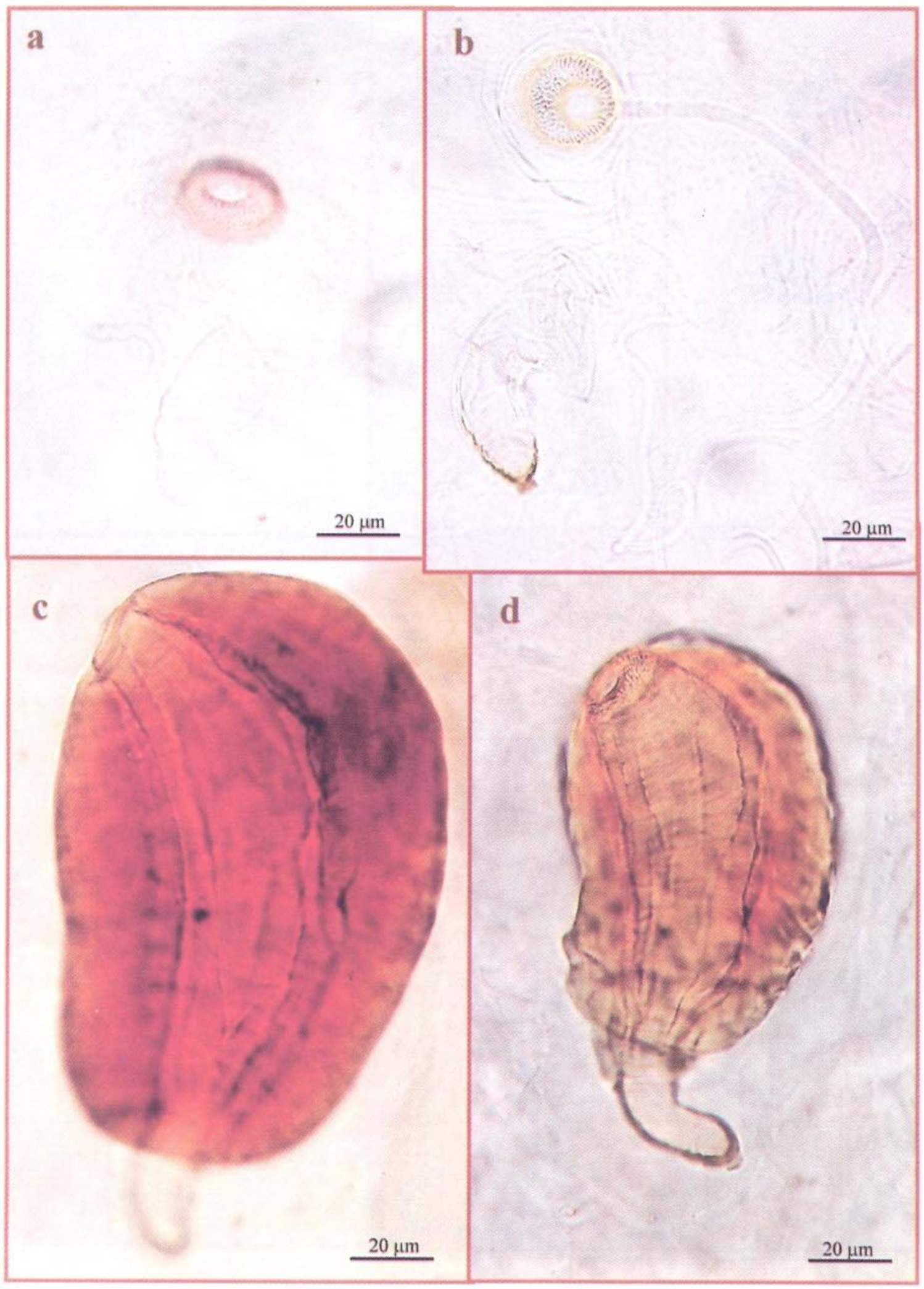

Fig. 5. – a) *Spermatheca* of non-gravid female of *T. trimamillata*, dissected and coloured by MIF; b) *spermatheca* of non-gravid female of *T. penetrans*, dissected and coloured by Lugol's jodine; c) *spermatheca* of a gravid female of *T. trimamillata*; d) *spermatheca* of a gravid female of *T. penetrans*.

3rd pair of legs: as in the females, the presence of a row of spines on the antero-medial surface of the tibia is a constant finding in the male of *T. trimamillata*, while it is absent in *T. penetrans* (Figs 3c, 4c); and, again, as in the females, there are 9-13 spines on the edge of the 1st tarsal segment of males of *T. trimamillata* while, in *T. penetrans*, the number is always less.

Abdominal spiracles: in *T. penetrans*, they are in the form of a flower, having a diameter of 18-22 µm, with six more or less irregular "petals"; in *T. trimamillata*, they are less irregular, appearing more rounded and having a smaller diameter (10-12 µm) (Figs 3g, 4g).

Sex organs: both the claspers and the *aedeagus* are shorter in *T. trimamillata* (250-288 and 310-330 µm, respectively) than in *T. penetrans* (280-360 and 370-450 µm, respectively) (Figs 3e, 4e). The profile of the claspers is also slightly different, especially in the handle, which is thinner and more pointed in the former than in the latter. The profile of the aedeagal apodeme (*lamina media*) is also different: the upper part is thicker and larger in *T. penetrans* than in *T. trimamillata* (Figs 3f, 4f).

Finally, we think it useful to point out some of the morphological differences we observed between the males and non-gravid females in each of the two species stu-

died. These data have not been clearly explained by previous observers.

Maxillary palps: in the males of both species, the bristles which are present on the various segments are much fewer and more slender than in the females and in *T. penetrans* the first segment is often longer than the second (Figs 3b, 4b).

Antennae: unlike the females, the surface of the club of the males of both species is completely devoid of bristles; the six oval *fossae* and three other roughly circular sensory organs are always present.

Proventriculus: as in other Siphonaptera, the *proventriculus* of females of both species is almost double that of males.

Laciniae: this is slightly shorter in the males than in the females (270-330 µm *vs.* 360-380 µm, respectively).

CONCLUSIONS

In summary, in considering the non-gravid females and the males, the differential diagnosis between these forms of *T. trimamillata* and *T. penetrans* can essentially be based on the following features:

– in non-gravid-females: differences in the lengths of the first two segments of the maxillary palps; the presence or absence of thick short spines on the surface of the segments; the presence or absence of a row of spine on the antero-medial surface of the tibia of the 3rd pair of legs; whether or not the last abdominal spiracle protrudes; and the presence or absence of a papilla surrounding the hood of the *billa* of the *spermatheca*;

– in males, the presence or absence of a row of spines on the antero-medial surface of the tibia of the 3rd legs; the size and regularity of the edge of the abdominal spiracles; the lengths of the claspers and the *aedeagus;* and differences in the profile of the aedeagal apodemes.

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