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MORPHOLOGICAL AND BIOLOGICAL STUDIES OF MEDICALLY IMPORTANT HOUSE-DUST MITES

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MORPHOLOGICAL AND BIOLOGICAL STUDIES OF MEDICALLY IMPORTANT HOUSE-DUST MITES

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HOUSE-DUST
MITES
KEY
PRE-REPRODUCTIVE
PERIOD
REPRODUCTIVE
PERIOD
FECUNDITY
DEVELOPMENT TIME

ABSTRACT : The morphology of the medically important house-dust mites *Dermatophagoides pteronyssinus* (Trouessart), *D. farinae* (Hughes), *Euroglyphus maynei* (Cooreman) and *E. longior* (Trouessart) has been comprehensively reviewed. A taxonomical key for all four species was constructed and elaborated with drawings and scanning electron micrographs. Biological studies indicated significant differences occurring between these species, with respect to their pre-reproductive period, reproductive period, fecundity and development time.

ACARIENS
DES POUSSIÈRES
CLÉ
PÉRIODE
DE PRÉ-REPRODUCTION
PÉRIODE
DE REPRODUCTION
FÉCONDITÉ
DURÉE
DU DÉVELOPPEMENT

RÉSUMÉ : Nous faisons la révision de la morphologie de quatre espèces d'Acariens des poussières domestiques ayant une importance médicale, *Dermatophagoides pteronyssinus* (Trouessart), *Euroglyphus maynei* (Cooreman), *D. farinae* (Hughes) et *E. longior* (Trouessart). Nous donnons une clé pour l'identification de ces espèces, accompagnée de dessins et de photographies au Stereoscan. L'étude biologique a montré l'existence de différences significatives entre ces espèces pour la période de prè-reproduction, la période de reproduction, la fécondité et la durée du développement.

INTRODUCTION

The occurrence of allergens in house-dust, causing allergic rhinitis and asthmatic symptoms, was first suggested over 60 years ago (KERN, 1921). The allergenic factor in house-dust remains unresolved, however, important clues were found in 1964 when VOORHORST *et al.* suggested that a mite may be responsible for the house-dust allergen. In atopic patients this mite was found to produce similar skin reactions to those caused by house-dust. The mite was identified as *Dermatophagoides pteronyssinus* (Trouessart) by FAIN (1966a) and subsequent studies have shown that this is the predominant species in house-dust in many parts of the world (reviewed by FAIN, 1966b). Since then, other mite

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species have been implicated in house-dust allergies, and in particular other mites of the family Pyroglyphidae, namely *D. farinae* (Hughes), *Euroglyphus maynei* (Cooreman) and *E. longior* (Trouessart) may be important (Le Mao *et al.*, 1981 ; Charpin *et al.*, 1986).

Numerous biological and immunological studies of the two *Dermatophagoides* species have been reported, however virtually nothing is known about the biology or immunology of the *Euroglyphus* species. This is presumably due to difficulties in rearing them under laboratory conditions. We have established productive cultures of both *E. maynei* and *E. longior* in our laboratory, and herein report the first detailed study of the morphology and biology of these two species, combined with a comprehensive comparison of them with the extensively studied *D. pteronyssinus* and *D. farinae* species.

Materials and methods

Mite Cultures

Cultures of all four species were reared on a 1 : 1 : 1 mixture of fish meal, dried yeast and defatted human beard shavings at 25 ± 2°C and 75 % relative humidity.

Morphology Review and Scanning Electron Micrographs (SEMs)

A comprehensive literature search was undertaken and the salient publications on Pyroglyphid morphology reviewed (Bogdanov, 1864 ; Cooreman, 1950 ; Hughes, 1954 ; Oshima, 1964 ; Fain, 1965, 1966a, 1967 ; Oshima, 1968 ; van Bronswijk & Sinha, 1971 ; Wharton, 1976 ; Samsinak *et al.*, 1982). A key for the four major species of house-dust mites was compiled and elaborated with drawings and SEMs.

For SEMs, mites were removed from cultures and cleaned of food particles by washing for 15 min in 0.05 % HCI. They were then dehydrated by washing for 10 min in a graded series of alcohols (40 %, 50 %, 60 %, 70 %, 80 %, 90 %) and finally by 3 × 15 min washes in absolute alcohol. The mites were then critical point dried, before carefully mounting and positioning on platforms prepared with adhesive tape. Finally specimens were coated with gold and examined through a Philips 501 scanning electron microscope using accelerating voltages of 15 and 30 kV.

Biological studies

Adult males and female tritonymphs were used as parents to ensure that no eggs were laid before observations commenced. Each couple was placed in small glass dish (13 mm diaemter × 10 mm high) with " tanglefoot " applied to the rim to prevent escape of the mites. The food mixture described for mite cultures was then added and the dishes placed in an unlit controlled temperature cabinet at 25 ± 2°C with a 75 % relative humidity. Observations for any eggs laid, were made three times weekly. Ten couples from each species were used for these studies and ten eggs were also observed for determination of the time taken to develop from birth to adult.

Statistical Analyses

All statistical analyses were found to have a 5 % probability from analysis of variance and multiple paired comparisons using an Apple Macintosh " Statview " computer programme.

Results

Systematic Position and Key of the Pyroglyphidae

The Pyroglyphidae belong to the order Astigmata of the Acari. This order is distinguished from the other Acari orders by the absence of stigmata on the idiosoma. The order Astigmata is further divide into two sub-orders, the Acaridia which are free-living and the Psoroptidia which are parasitic. The former sub-order is divided into many families, including the Pyroglyphidae, to which house-dust mites belong. Distinguishing morphological characteristics, detailed drawings and SEMs were used to

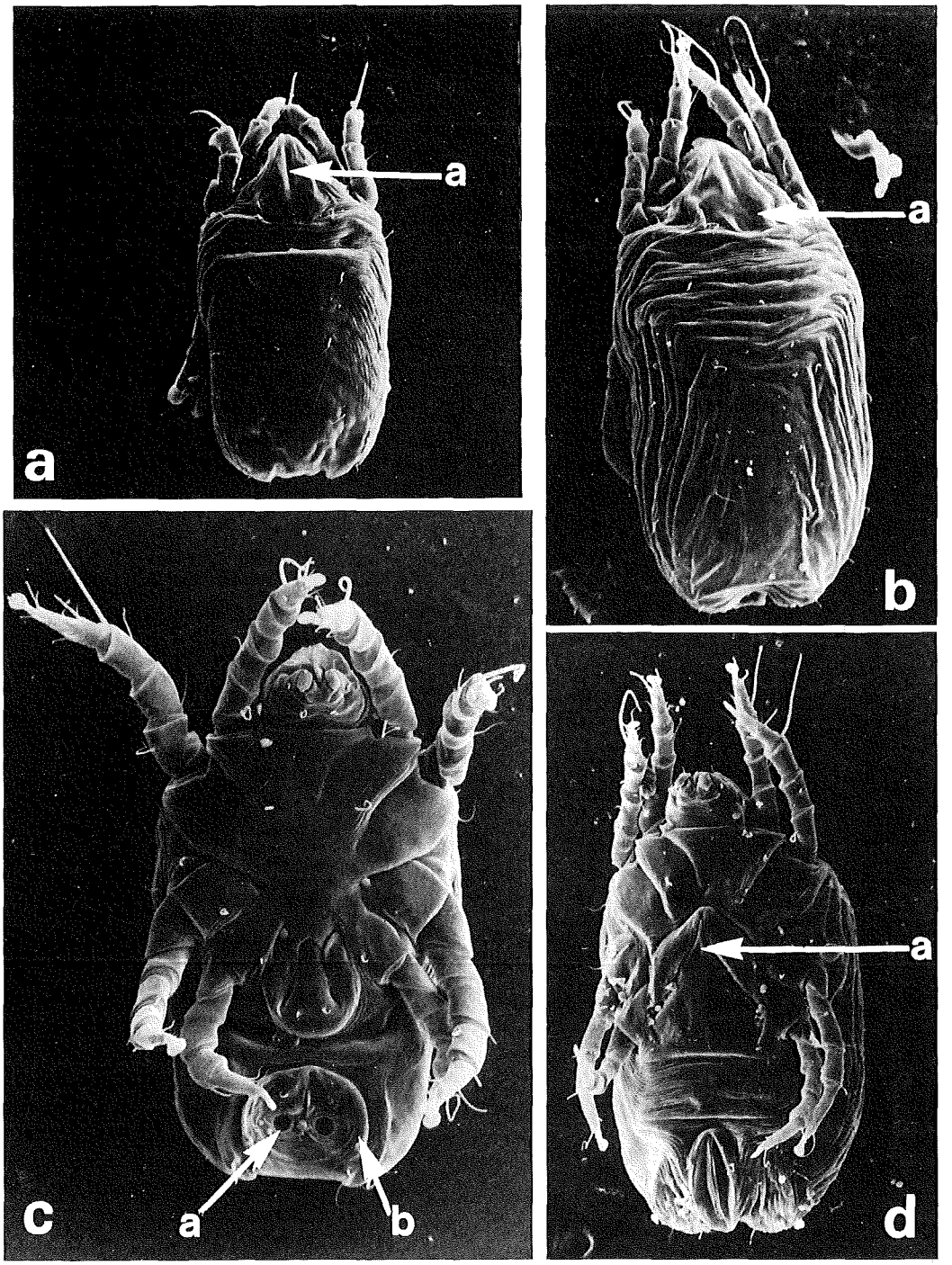

FIG. 1 : Scanning electron micrographs of *Euroglyphus maynei* (Cooreman). A. Dorsal male × 320 showing tegmen (a) ; B. Dorsal female × 320 showing tegmen (a) ; C. Ventral male × 480 showing anal suckers (a) and anal plate (b) ; D. Ventral female × 480 showing vulval lip (a).

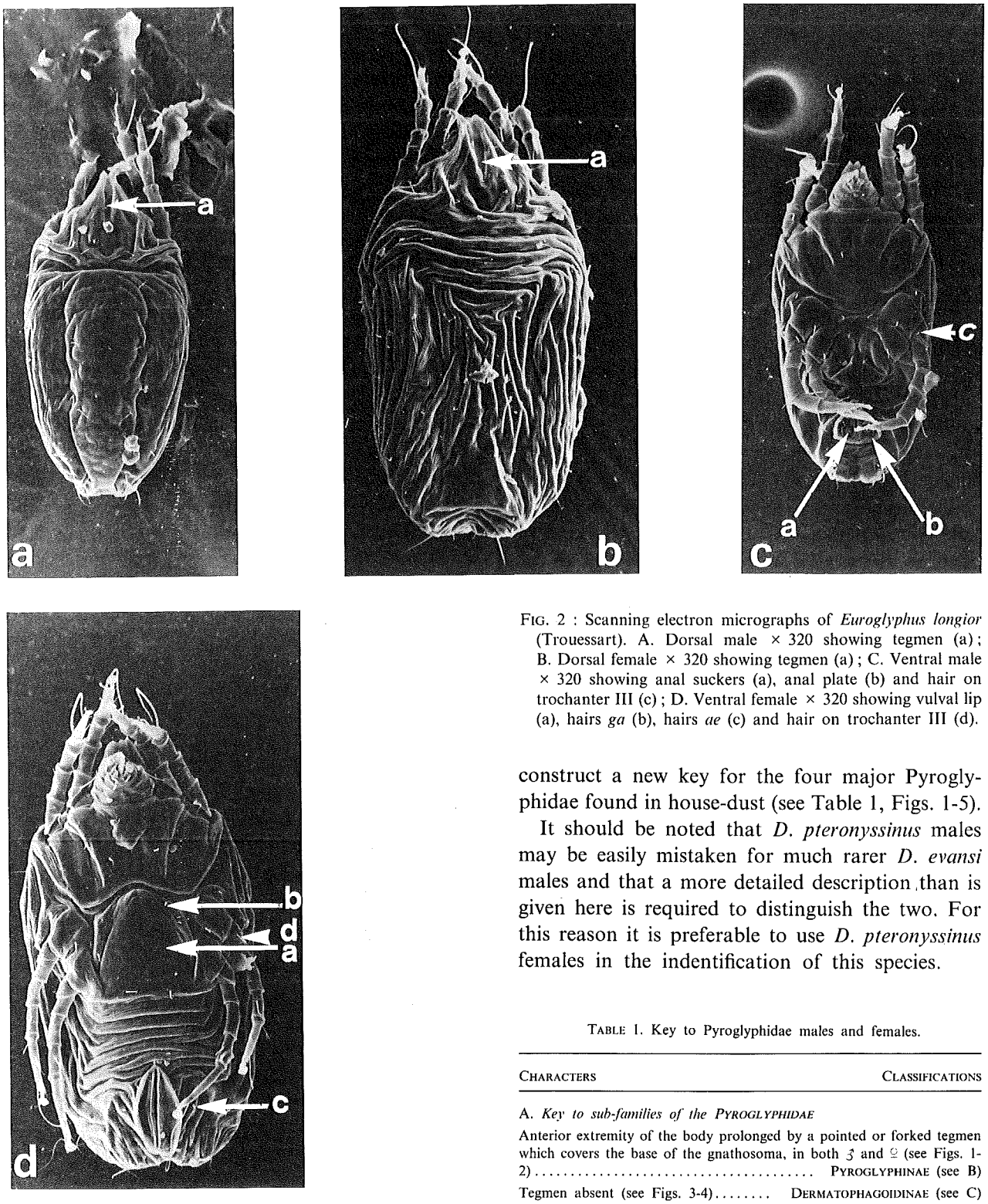

FIG. 2 : Scanning electron micrographs of *Euroglyphus longior* (Trouessart). A. Dorsal male × 320 showing tegmen (a); B. Dorsal female × 320 showing tegmen (a); C. Ventral male × 320 showing anal suckers (a), anal plate (b) and hair on trochanter III (c); D. Ventral female × 320 showing vulval lip (a), hairs *ga* (b), hairs *ae* (c) and hair on trochanter III (d).

construct a new key for the four major Pyroglyphidae found in house-dust (see Table 1, Figs. 1-5).

It should be noted that *D. pteronyssinus* males may be easily mistaken for much rarer *D. evansi* males and that a more detailed description than is given here is required to distinguish the two. For this reason it is preferable to use *D. pteronyssinus* females in the indentification of this species.

TABLE I. Key to Pyroglyphidae males and females.

CHARACTERS	CLASSIFICATIONS
A. *Key to sub-families of the* PYROGLYPHIDAE	
Anterior extremity of the body prolonged by a pointed or forked tegmen which covers the base of the gnathosoma, in both ♂ and ♀ (see Figs. 1-2)	PYROGLYPHINAE (see B)
Tegmen absent (see Figs. 3-4)	DERMATOPHAGOIDINAE (see C)

TABLE 1. Key to Pyroglyphidae males and females.

CHARACTERS	CLASSIFICATIONS

B. *Key to the* PYROGLYPHINAE

1. ♀ with the distal part of the bursa copulatrix in the form of a small,oval, strongly sclerified pocket (Fig. 5). ♂ with anal suckers (Figs. 1-2) . ..... *Euroglyphus* (see 2)

♂ and ♀ without this combination of cahracters (no data shown).. ..... *Pyroglyphus*

2. ♂ with a large, oval anal plate reaching close to the posterior edge of the body. Trochanters I to III without hairs.

♀ with a short posterior vulval lip which does not cover the anterior of the vulva. Hairs *ga, ae* and those trochanters I to III lacking..... ..... *E. maynei* (Fig. 1)

♂ with a small, hexagonal anal plate, distant from the posterior edge of the body. Trochanters I to III with one hair.

♀ posterior vulval lip long, almost entirely covering the vulva. Hairs *ga, ae* and those on trochanters I to III present .... *E. longior* (Fig. 2)

C. *key to the* DERMATOPHAGOIDINAE

1. ♂ with hairs *sc e* much longer and thicker than *sc i*. Tarsus III without spines. Simple perianal ring (not denticulate). With a hysteronotal shield (Figs. 3-4).

♀ with hairs *sc e* much longer and thicker *sc i*. Legs III and IV equal or subequal in length. Hysteronotal shield absent. (Figs. 3-4)..... ..... *Dermatophagoides* (see 2)

Without this combination of characters (no data shown) .... *Malayoglyphus, Hirstia, Sturnophagoides*

2. ♂ hysteronotal shield long (longer than broad) and extending further forward than hairs *d2*. Epimeres I parallel or diverging. Legs I normal.

♀ with middle region of dorsum between hairs *d2-d2-d3-d3* only with longitudinal striations. Proximal part of bursa copulatrix with a sclerite in the form of a daisy ; distal part very slightly dilated (Fig. 5)... ..... *D. pteronyssinus* (Fig. 3)

♂ hysteronotal shield short (broader than long) and not reaching the base of hairs *d2*. Epimeres I either free or fused to form a sternum. Legs I generally swollen.

♀ with middle region of dorsum between hairs *d2-d2-d3-d3* with transverse striations in the anterior half and with convex or oblique striations in the posterior half. Proximal part of bursa copulatrix without sclerifications ; distal part dilated into a small,sclerified, triangular pouch (Fig. 5) ..... *D. farinae* (Fig. 4)

TABLE 2. Adult reproduction and development of immatures of *Dermatophagoides pteronyssinus, D. farinae, Euroglyphus maynei* and *E. longior* at 25°C ± 2°C, 75 % RH.

Species	Pre-reproductive[a] period	Reproductive[a] period	Fecundity[b]	Rate of[c] reproduction	Development[a] egg to adult
D. pterony.	9.00 ± 0.95	33.89 ± 3.72	58.22 ± 4.66	1.79 ± 0.15	14.30 ± 0.52
D. farinae	10.70 ± 1.30	47.00 ± 5.67	84.10 ± 10.61	1.80 ± 0.13	34.63 ± 1.32
E. maynai	13.80 ± 0.20	60.20 ± 6.53	84.20 ± 10.32	1.47 ± 0.18	33.00 ± 4.00
E. longior	12.78 ± 1.06	39.78 ± 4.99	48.00 ± 3.89	1.33 ± 0.18	30.14 ± 3.49

Legend to Table 2 :

Figures given are the means ± standard error calculated from 10 mites of each species.

[a] Figures in days.
[b] Figures in eggs per female.
[c] Figures in eggs laid per female per day of the reproductive period.

Biological Studies

The determination of the life cycles of these four mite species has provided invaluable comparative information on their biology (Table 2). Significant interspecific differences in reproduction and development were found and are summarised in Table 3. The pre-reproductive period, defined as the period from mating to the birth of the first eggs, was significantly longer in *E. maynei* and *E. longior* than in *D. pteronyssinus*. The pre-reproductive period of *E. maynei* was also significantly longer than that of *D. farinae*. The reproductive period, the period between the production of the first and last eggs, was significantly longer in *E. maynei* than in *D. pteronyssinus* and *E. longior*. Fecundity, the total number of eggs laid per female, was significantly smaller in *D. pteronyssinus* and *E. longior* than in *D. farinae* and *E. maynei*, however, rate of reproduction, calculated as the number of eggs laid per

TABLE 3. Summary of statistically significant differences found between *Dermatophagoides pteronyssinus, D. farinae, Euroglyphus maynei* and *E. longior* at 25°C ± 2°C, 75 % RH.

Parameter	Significant interspecific differences
Pre-reproductive period	*E. maynei, E. longior* > *D. pteronyssinus* *E. maynei* > *D. farinae*
Reproductive period	*E. maynei* > *D. pteronyssinus*, E. longior
Fecundity	*E. maynei, D. farinae* > *D. pteronyssinus, E. longior*
Ratte of reproduction	No significant differences found
Development from egg to adult	*D. farinae, E. maynei, E. longior* > *D. pteronyssinus* *D. farinae* > *E. longior*

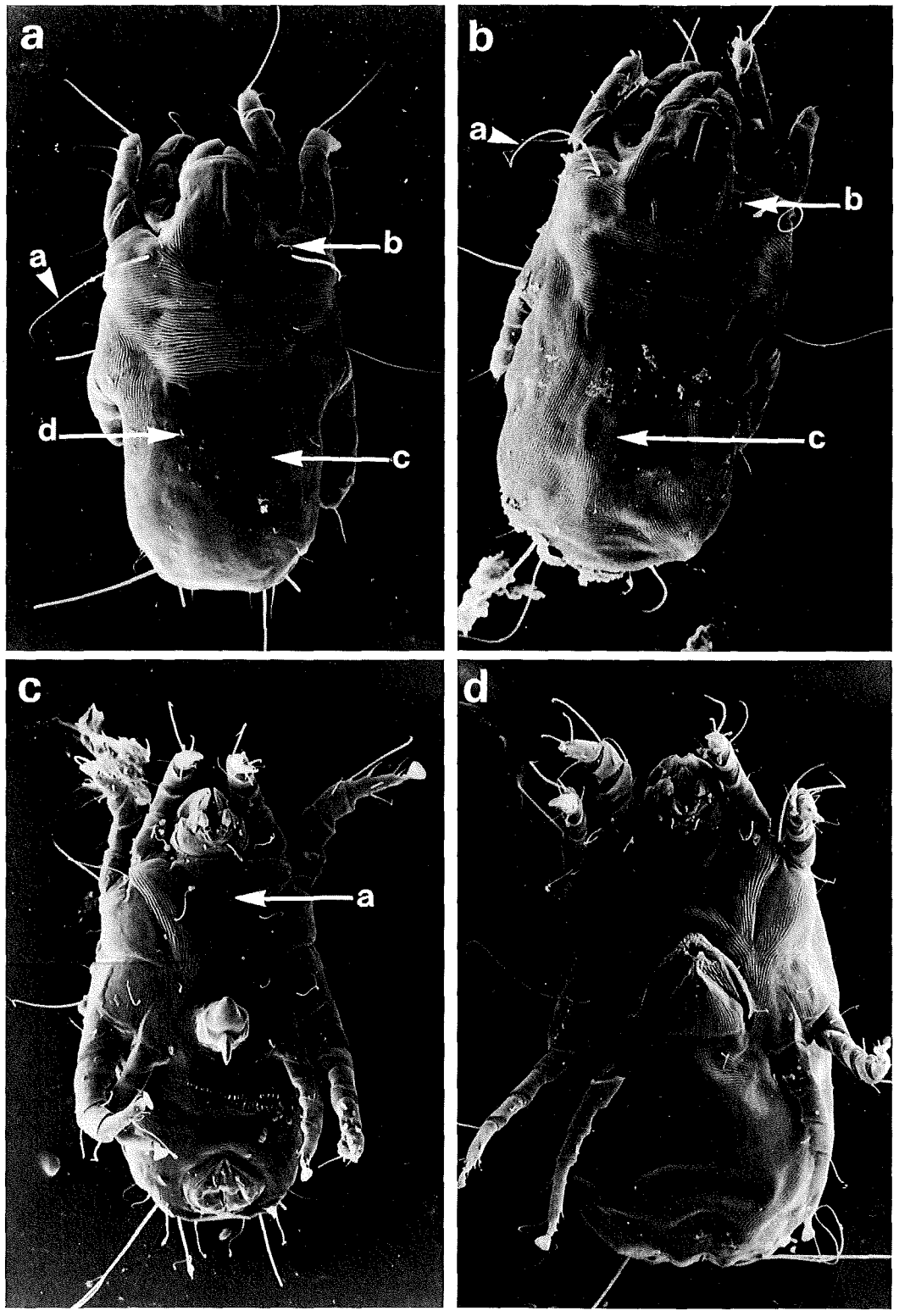

FIG. 3 : Scanning electron micrographs of *Dermatophagoides pteronyssinus* (Trouessart). A. Dorsal male × 320 showing hairs *sc e* (a), hairs *sc i* (b), hysteronotal shield (c) and hairs *d2* (d) ; B. Dorsal female × 320 showing hairs *sc e* (a), hairs *sc i* (b) and longitudinal striations (c) ; C. Ventral male × 320 showing epimeres (a) ; D. Ventral female × 320.

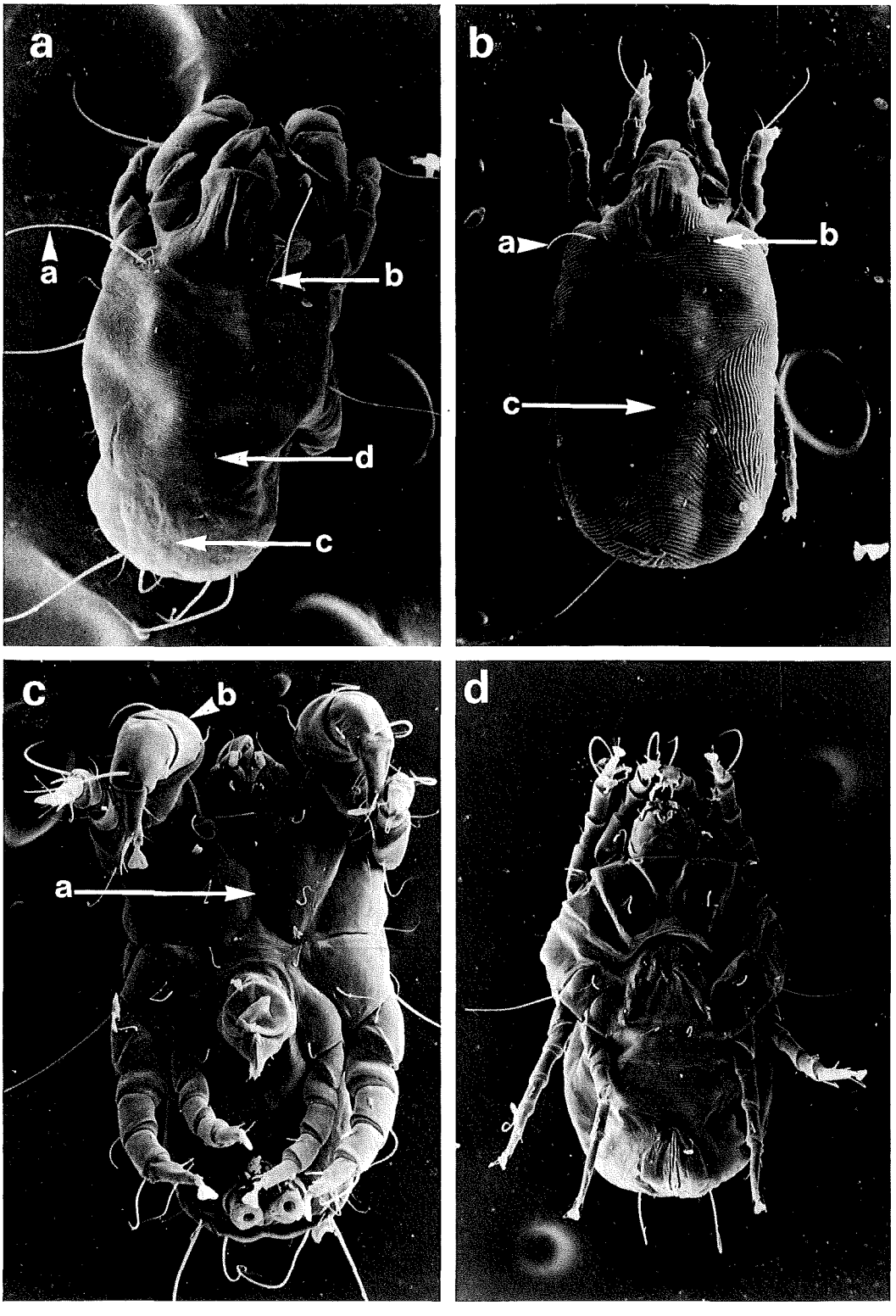

FIG. 4 : Scanning electron micrographs of *Dermatophagoides farinae* (Hughes). A. Dorsal male × 320 showing hairs *sc e* (a), hairs *sc i* (b), hysteronotal shield (c) and hairs *d2* (d) ; B. Dorsal female tritonymph × 320 showing hairs *sc e* (a), hairs *sc i* (b) and transverse striations (c) ; C. Ventral male × 320 showing epimeres (a) and enlarged legs one (b) ; D. Ventral female × 240.

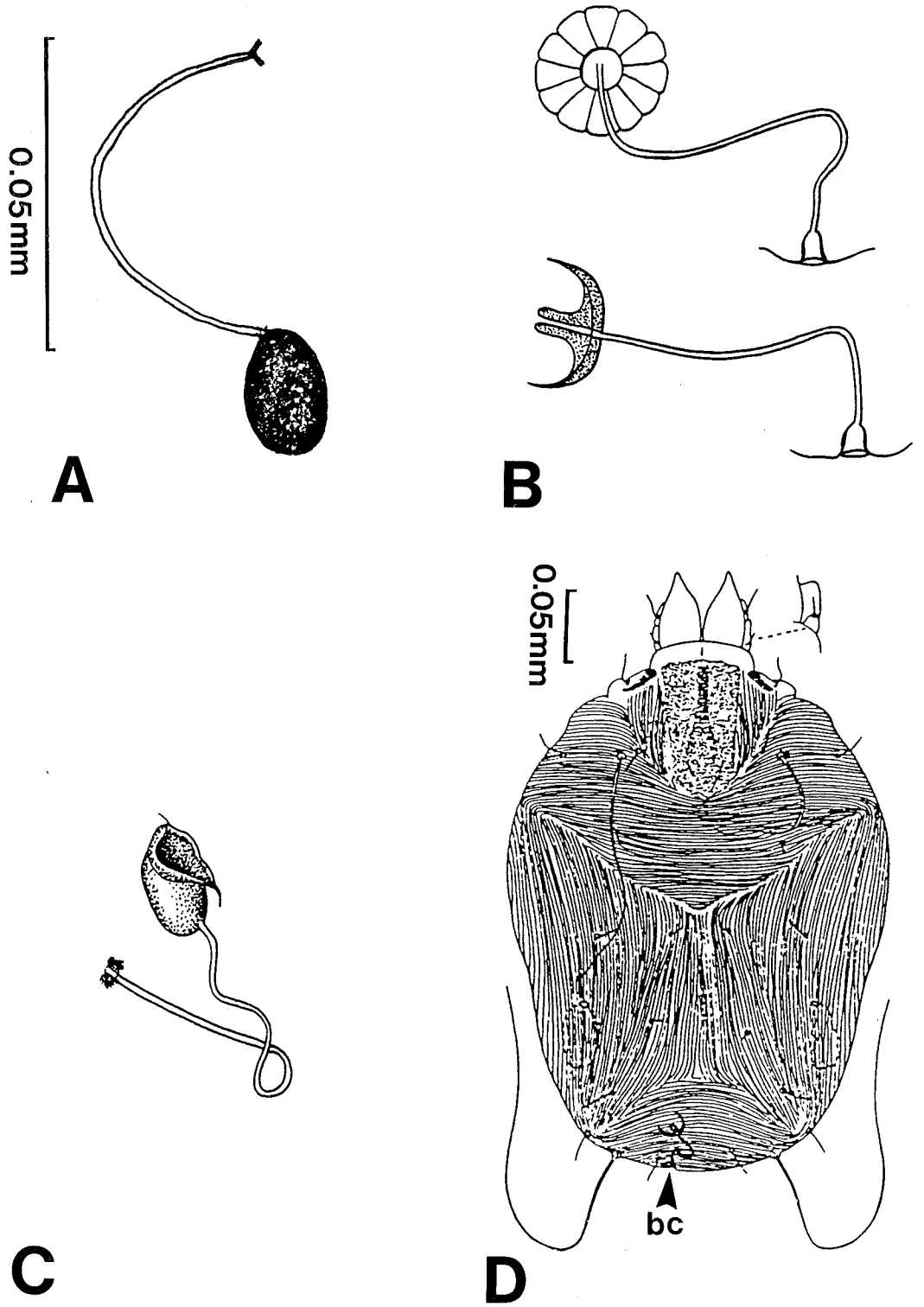

Fig. 5 : Drawings showing details of bursa copulatrix from four species of Pyroglyphidae. (A) *Euroglyphus maynei* and *E. longior*; (B) *Dermatophagoides pteronyssinus*; (C) *D. farinae*; (D) Drawing from slide preparation of *D. pteronyssinus* showing position of bursa copulatrix (bc) (from Fain, 1966a).

day of the female's reproductive period, did not differ significantly between the four species studied. Finally, the development of immatures was significantly faster in *D. pteronyssinus* than in the other three species, and immatures of *E. longior* also developed significantly more rapidly than those of *D. farinae*.

Discussion

The taxonomy of *D. pteronyssinus* and *D. farinae* has been studied in detail using both drawings and SEMs (e.g. Fain 1966a, van Bronswijk, 1973 ; Mumcuoglu, 1976). The present review of their taxonomy complements these studies and provides one of the first detailed comparisons of these species with *E. maynei* and *E. longior*, using a key fully illustrated with drawings and SEMs. Apart from the original descriptions however, *E. maynei* and *E. longior* have been studied only very rarely (Fain, 1965 ; Mumcuoglu, 1976). The present study therefore, provides an invaluable insight into the morphology of these two species in comparison with the extensively studied *D. pteronyssinus* and *D. farinae*. Further comparative studies of the isoenzymes and antigens of these four mite species have been reported (Hart *et al.*, 1988 ; Le Merdy *et al.*, 1988).

Interest in house-dust mites and house-dust allergies is rapidly growing due to an alarming increase in allergies over the last ten years (e.g. Fleming & Crombie, 1987). The key presented herein therefore provides an invaluable tool, not only for acarologists, but also for clinicians, immunologists and commercial companies with an interest in house-dust allergies and the causative mite species. The key should facilitate the unequivocal identification of these important mite species.

Scanning electron micrographs have proved to be extremely useful in the study of mite morphology. A limitation of SEMs is, however, the inability to view certain internal organs which may help in the identification of a species. For example, the bursa copulatrix is usually very distinct in female Pyroglyphidae, and therefore is a very important character for separating both genera and species of this family. This is very easily seen, by light microscopy, on slide preparations, but is impossible to see on SEM preparations. We believe therefore, that slide preparations and SEMs both have a place in mite taxonomy, and that they indeed complement one another extremely well in this area of acarology.

The results obtained for the reproduction and developmental capacities of *D. pteronyssinus* and *D. farinae* correlate well with previous reports of these species at 25°C and 75-80 % RH (Oshima & Sugita, 1966 ; Spieksma, 1967 ; Larson *et al.*, 1969 ; Furumizo, 1973). In addition, the developmental time calculated by Nannelli *et al.* (1983) for *E. maynei* compares favourably with the value obtained in the present study. The biology of *E. longior* has not been studied previously.

E. maynei and *E. longior* are more difficult to rear under laboratory conditions than *D. pteronyssinus* and *D. farinae*, suggesting that the former two species may have a lower reproductive potential with respect to their adult reproductive and immature development parameters. Suprisingly, we did not find any general trend towards poorer reproduction and development in *E. maynei* and *E. longior* compared to the two *Dermatophagoides* species. The pre-reproductive period was the only parameter to suggest poorer reproductive potential in both *Euroglyphus* species, and it is possible that this could account for the difficulties in laboratory rearing of *E. maynei* and *E. longior*. A behavioural difference between the two groups of mites, however, could also provide part of the explanation. *E. maynei* and *E. longior* are much smaller in size and are slower moving than *D. pteronyssinus* and *D. farinae*. Under laboratory conditions, these males and females may actually take longer to find one another, which could reduce mating frequency. Thus, despite having similar reproduction to the two *Dermatophagoides* species, the suggested difficulty in males and females making contact and mating, together with the longer pre-reproductive period, may limit reproduction and population development in laboratory cultures of the two *Euroglyphus* species. Nevertheless, other features of mite biology, such as mortality of eggs and lifespan, may influence population development in laboratory cultures. These aspects are under further

investigation, as are the influences of temperature, humidity and food.

In conclusion, the observations reported herein have considerably increased our understanding of the morphology and biology of house-dust mites, and in particular *E. maynei* and *E. longior* which were previously poorly understood. These species are of potential importance as causative agents of house-dust allergies, and the construction of this morphological key, as well as the new information on their biology, could prove to be of vital importance in understanding the aetiology of house-dust allergy.

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