ELSEVIER

Arthropod Structure & Development 33 (2004) 139–148

ARTHROPOD STRUCTURE & DEVELOPMENT

www.elsevier.com/locate/asd

Occurrence of midgut perimicrovillar membranes in paraneopteran insect orders with comments on their function and evolutionary significance

Carlos P. Silva[a], José R. Silva[a], Fábio F. Vasconcelos[a], Marílvia D.A. Petretski[a], Renato A. DaMatta[b], Alberto F. Ribeiro[c], Walter R. Terra[d,*]

[a]*Laboratório de Química e Função de Proteínas e Peptídeos, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, 28015-620 Campos dos Goytacazes, Brazil*

[b]*Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, 28015-620 Campos dos Goytacazes, Brazil*

[c]*Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, C. P. 11461, 05422-970 São Paulo, Brazil*

[d]*Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C. P. 26077, 05513-970 São Paulo, Brazil*

Received 20 August 2003; accepted 6 December 2003

Abstract

Hemipterans are characterized by the absence of the peritrophic membrane, an anatomical structure that envelopes the food bolus in the majority of insects. However, the microvillar membranes of many hemipteran midgut cells are not in direct contact with the food bolus, due to the existence of the so-called perimicrovillar membrane (PMM), which covers the microvilli extending into the gut lumen with dead ends. α-Glucosidase is a biochemical marker for PMM in the seed sucker bug *Dysdercus peruvianus* (Heteroptera: Pyrrhocoridae). In this article, we report that adults of the major hemipteran infra-orders (Sternorrhyncha, Auchenorrhyncha, and Heteroptera) have PMM and a major membrane bound α-glucosidase, which has properties similar to those of the *D. peruvianus* enzyme. A polyclonal antibody raised against the enzyme of *D. peruvianus* recognized the enzymes present in PMM from the above-mentioned hemipteran groups. The same antibody was also able of recognizing perimicrovillar α-glucosidase from thrips. No PMM nor membrane-bound α-glucosidase were found in Psocoptera and Phthiraptera midguts. This suggests that PMM and PMM-bound-α-glucosidase are widespread among insects of the order Hemiptera and of the sister order Thysanoptera. The data support the hypothesis that PMM may have originated in the Condylognatha (Paraneopteran taxon including Hemiptera and Thysanoptera) ancestral stock and are associated with plant sap feeding.

Keywords: Perimicrovillar membranes; α-Glucosidase; Sap feeding; Midgut adaptations; Hemiptera; Phthiraptera; Psocoptera; Thysanoptera

1. Introduction

The Paraneoptera group includes two superorders: Psocodea, which comprises the orders Psocoptera (booklice, barklice and psocids) plus Phthiraptera (sucking and biting lice), and the superorder Condylognatha, which includes the orders Thysanoptera (thrips) and Hemiptera (Fig. 1). The Hemiptera is by far the most successful and numerous Paraneoptera order, with many economically important species, but the other orders also have members that are considered crop pests or important vectors of plant pathogens. The ancestor of the whole order Hemiptera is supposed to have been a sap sucker insect similar to some primitive extant Auchenorrhyncha (Goodchild, 1966). Probably, associated with this fact, Hemiptera lost the peritrophic membrane, a chitin–protein anatomical structure that envelops the food bolus in the midgut of the majority of insects, leading to a compartmentalisation of the digestive process (Terra et al., 1979; Terra and Ferreira, 1994). The compartmentalisation increases the efficiency of the digestion of polymeric molecules, which were not usually present in the sap ingested by the putative Hemiptera ancestor.

The Hemiptera are unique in comprising insects that pass all their life span having solely plant saps as food. In spite of the fact that other insects have suitable mouthparts for ingesting plant liquid material, they cannot sustain all their life stages based only on this food source (Goodchild, 1966).

* Corresponding author. Fax: +55-11-3091-2186.
E-mail address: warterra@iq.usp.br (W.R. Terra).

1467-8039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.asd.2003.12.002

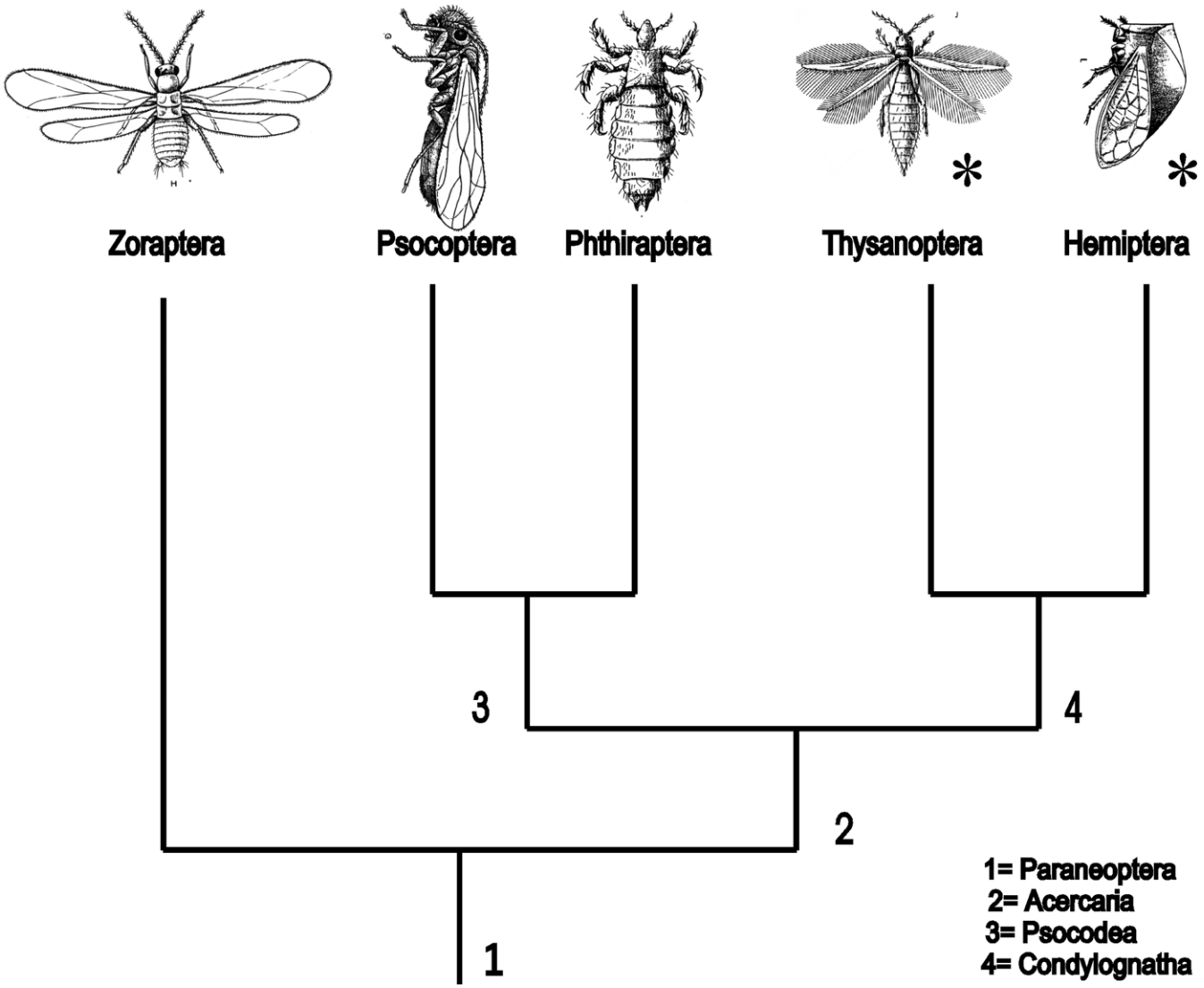

Fig. 1. Phylogeny of extant Paraneoptera as proposed originally by Kristensen (1981). At present, the order Zoraptera is no longer considered as true paraneopteran insects (Kukalova-Peck and Peck, 1993; Rasnitsyn, 1998). The asterisks show the orders which have perimicrovillar membranes.

This observation suggests that some structure, other than the appropriate mouthparts, might be responsible for Hemiptera being capable of exploiting this extremely restricted ecological niche. In fact, besides being an exception because of the lack of a true peritrophic membrane, hemipteran insects have an unusual extra-cellular lipoprotein membrane ensheathing the microvilli of midgut cells. This membrane was named perimicrovillar membrane (PMM) by Terra (1988). The PMM and the microvillar membranes limit a closed space, the perimicrovillar space (Terra, 1988).

The PMM was not reported in Cicadoidea and Cercopoidea (Marshall and Cheung, 1974), Cicadelloidea (Lindsay and Marshall, 1980) and Aphididae (O'Loughlin and Chambers, 1972). Nevertheless, an inspection in the micrographeis and published descriptions suggests that this kind of membrane is also present in those insects, in spite of the fact that aphids have modified perimicrovillar membranes (Cristofoletti et al., 2003). There are also published micrographeis showing similar membranes in the midgut of the Hemiptera sister order Thysanoptera (Kitajima, 1975; Del Bene et al., 1991), but as far as we are aware, there is no demonstration of the presence or absence of PMM in the other two related orders Psocoptera and Phthiraptera.

It has been shown that an α-glucosidase is a biochemical marker for PMM in the seed sucker bug *Dysdercus peruvianus* (Heteroptera: Pyrrhocoridae) (Silva et al., 1995, 1996). Based on the results from immunolocalisation of the PMM-bound α-glucosidase, it has been proposed that the membranes of the perimicrovillar system are originated from internal membranes of double membrane vesicle (Silva et al., 1995), which were found budding from the trans area of the Golgi complex (Andries and Torpier, 1982; Werner et al., 1991; Silva et al., 1995). The model states that the inner membranes in the double-membrane vesicles form the perimicrovillar membranes following two membrane fusion events. The first event consists of the fusion of the

outer membrane of the double vesicles with the microvillar membrane, and the second one is the fusion of the inner membrane with the PMM (Silva et al., 1995).

The present investigation was undertaken with two aims. First, evaluation of the presence or absence of PMM in species representative of all orders classified within Paraneoptera. Second, verification if the biochemical marker for PMM described for a relatively evolved species of Heteroptera is also valid for PMM from more primitive groups. It was demonstrated in this article that PMM and the PMM-bound α-glucosidase are conserved in the condylognathan orders Hemiptera and Thysanoptera, and are absent in the psocodean orders Psocoptera and Phthiraptera, suggesting that the perimicrovillar system of membranes evolved from a condylognathan ancestor.

2. Material and methods

2.1. Rearing and collecting insects

Colonies of *Dysdercus peruvianus* (Pentatomomorpha: Pyrrhocoridae) and *Rhodnius prolixus* (Cimicomorpha: Reduviidae) were maintained in our laboratory since 1994. *D. peruvianus* was reared inside plastic bottles, covered with a piece of cloth, under natural photoperiod conditions at a relative humidity of 60–70% at 26 ± 1 °C. The insects had free access to water and to cotton (*Gossypium hirsutum*) seeds. *R. prolixus* was reared in the dark inside an incubation chamber at 26 ± 1 °C and fed on rabbit blood.

Insects collected in the field were placed on plastic containers and kept on ice until the dissection of the animals. The following paraneopteran representatives were collected for this study: adult Psocoptera (Liposcelidae) from rice grains; adult lice *Pediculus humanus* (Phthiraptera: Pediculidae) from school children living in Campos dos Goytacazes; adult thrips *Frankliniella* sp. (Thysanoptera: Thripidae) from flowers of *Canavalia ensiformis*; aphids *Myzus persicae* (Sternorrhyncha: Aphididae) from leaves of lettuce; adult cercopid *Mahanarva posticata* (Auchenorrhyncha: Cercopidae) from sugar cane.

2.2. Preparation of samples from insects

The animals were immobilized by cold and dissected to remove the whole midgut in cold 250 mM NaCl. Only insects with food-filled gut tracts were chosen for experiments. Pooled midgut tissues were homogenized in cold distilled water in a hand-held Potter–Elvehjem homogenizer immersed in ice. Midgut tissue homogenates were centrifuged at 20,000*g* for 30 min at 4 °C and the supernatant (soluble fraction) was collected, and the sediment (membrane fraction) was homogenized in distilled water with the aid of the Potter–Elvehjem homogenizer. When necessary, membrane-bound proteins were extracted with the detergent Triton X-100 at the ratio of 1 mg protein/mg Triton X-100 and incubation time of 15 h at 4 °C. Following this incubation, the suspension was centrifuged at 100,000*g* for 60 min at 4 °C, and the extracted proteins were recovered in the supernatant.

2.3. Hydrolase assays and protein determination

α-Glucosidase activity was determined using *p*-nitrophenyl α-D-glucopyranoside (NPαGlu) (5 mM) as substrate and following the appearance of *p*-nitrophenolate according to the method of Terra et al. (1979). All assays were performed at 30 °C. Incubations were carried out for at least four different periods of time, unless otherwise stated, and initial rates of hydrolysis were calculated. One unit of enzyme is defined as the amount that catalyses the cleavage of 1 μmol of substrate/min.

In samples without Triton X-100, proteins were determined following the dye-binding method of Bradford (1976) and, in the presence of the detergent, the bicinchonic acid-based method of Smith et al. (1985) was used, both utilizing ovalbumin as a standard.

2.4. Transmission electron microscopy

The adult insects were dissected in their own hemolymph and their midguts were fixed in 2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2. Following this, the material was rinsed three times in 0.1 M cacodylate, pH 7.2. Post fixation was carried out in 1% OsO_4 and 0.8% $K_4(Fe(CN)_6)$. Following fixation, the material was rinsed three times in 0.1 M cacodylate, pH 7.2 and dehydrated through a graded acetone series. The specimens were embedded in epon. Ultrathin sections (60–70 nm) were cut with an ultramicrotome and mounted on copper grids. The samples were stained in uranyl acetate and lead citrate. The materials were analyzed in a Zeiss EM 900 electron microscope.

2.5. Immunogold labeling

Midguts were fixed in 0.1% glutaraldehyde, 4% paraformaldehyde in 0.1 M cacodylate, pH 7.2. After that, the materials were rinsed three times in 0.1 M cacodylate pH 7.2, dehydrated through a graded ethanol series and further embedded in LR gold. Ultrathin sections were cut with an ultramicrotome and mounted on nickel grids. Tissue sections were treated with 10 mM phosphate buffer pH 7, containing 100 mM NaCl and 1% albumin. The tissues were further incubated (2 h) with the antibody raised in rabbits against *D. peruvianus* α-glucosidase (Silva et al., 1995) in the dilution of 1:100. After incubation, grids were washed in the above-mentioned buffer, followed by anti-rabbit IgG conjugated to 10 nm gold particles for 1 h. Sections were then stained with uranyl acetate and observed in a Zeiss EM 900 electronmicroscope.

3. Results

3.1. Occurrence of midgut perimicrovillar membranes among Paraneoptera

As previously described, *D. peruvianus* (Silva et al., 1995) and *R. prolixus* (Lane and Harrison, 1979; Ferreira et al., 1988) display in their midgut cell apices microvilli showing two membranes: the inner (true) microvillar membrane and the outer microvillar (perimicrovillar) membrane (PMM). PMMs ensheath the microvillar membranes and extend deep into the midgut lumen as narrow tubes (Fig. 2A and B). PMMs apparently form a continuous domain from one cell to another. In longitudinal sections, PMM may present arrangements with circular profiles, and are composed of multiple layers of membranes, while in transversal sections, the microvilli appear with two membranes (Fig. 2A and B).

M. posticata (cercopid) PMM may extend far into midgut lumen (not shown), as described above for *D. peruvianus* and *R. prolixus* although frequently they are not highly developed (Fig. 2C). PMMs have not been described before in Auchenorrhyncha insects, despite the fact that inspection in published micrographies (Marshall and Cheung, 1974) suggests their occurrence.

The apex of the midgut cells of the aphid *M. persicae* is modified into apical lamellae that are associated with one another through trabeculae, resembling septate junctions (Fig. 2D and inset). Associated with the lamellae there are amorphous membranes masses (Fig. 2D, arrows). The midgut cell apex of *M. persicae* is similar to that of *A. pisum* (Cristofoletti et al., 2003), suggesting that this peculiar arrangement is characteristic of aphids. The amorphous membranes associated with lamellae were named modified perimicrovillar membranes and were thought to be originated from Golgi and rough endoplasmic reticulum membranes, then moving through the apical lamellae to become associated with the luminal surface of the lamellae, thus projecting into the midgut lumen (Cristofoletti et al., 2003).

P. humanus (Phthiraptera) and a species of book louse (Psocoptera) do not have a PMM (Fig. 3A and B) nor a peritrophic membrane. It is probable that the dark material seen among the book louse microvilli corresponds to the remains of a peritrophic gel separating cells and midgut contents. The peritrophic gel is an anatomical structure occurring instead of a peritrophic membrane in some insects (Terra, 2001). The peritrophic gel may be removed during the fixation of the samples for electron microscopy (Terra, 2001) and perhaps this explains the lack of material associated with *P. humanus* midgut microvilli (Fig. 3B).

Frankliniella sp have PMM in their midgut cells (Fig. 3C) as previously described (Kitajima, 1975; Del Bene et al., 1991). PMM in these insects and in the above-mentioned Hemiptera are present in all midgut sections in fed and in fasting animals (data not show). These observations agree with the pioneering work done by Lane and Harrison (1979) with *R. prolixus*.

3.2. Occurrence of midgut membrane-bound α-glucosidase among Paraneoptera

Hemipterans and thysanopterans, but not psocopterans and phthirapterans, have a major midgut membrane-bound α-glucosidase (Tables 1 and 2). The enzyme is efficiently extracted by the detergent Triton X-100, with typical yields of 150% in relation to the initial activity assayed in the crude membrane preparation (not shown). These unexpected yields may be explained if the α-glucosidase is localized in the outside surface of the PMM of the midgut cells of those insects, as discussed by Silva and Terra (1995). During homogenisation, PMM are thought to form right-side-out and inside-out vesicles. α-Glucosidase in inside-out vesicles must be almost inactive, because the substrate does not easily permeate membranes. Thus, about 50% of the enzyme in homogenates is expected to be inactive unless the membranes are solubilized in detergent. If α-glucosidase were bound to microvillar membranes, no detergent effect in activity would be expected, because microvillar membranes (due to the cytoskeleton) only form right-side-out vesicles (Proux, 1991).

In *D. peruvianus*, the midgut membrane-bound α-glucosidase was proposed to be PMM-bound based on density-gradient centrifugation and differential centrifugation in isotonic and hypotonic conditions (Silva et al., 1996). This proposal was later confirmed by immunocytolocalization with a polyclonal antibody raised against the purified *D. peruvianus* α-glucosidase (Silva et al., 1995; this paper, Fig. 4A). This antibody also immunolocalizes an α-glucosidase on *R. prolixus* PMM (Fig. 4B), confirming previous proposal based on centrifugation data (Ferreira et al., 1988) and on the PMM of *M. posticata* (Fig. 4C) and *Frankliniella* (Fig. 4D). The data suggest that the α-glucosidases and PMM of hemipterans and thysanopterans are homologous.

4. Discussion

A screening amongst some representative groups of paraneopteran insects revealed that the insects from the orders Thysanoptera and Hemiptera have PMM, as shown by transmission electron microscopy and α-glucosidase immunocytolocalisation (Figs. 2–4). In contrast, Psocoptera and Phthiraptera lack PMM and a midgut membrane-bound α-glucosidase. These results suggest that the PMM appeared in a putative condylognathan ancestor (Fig. 1).

Before hypothesizing on the origin and evolution of PMM, it is necessary to review the present knowledge on the evolution and feeding habits of Condylognatha.

Based on the fossil record and on comparative biology (Gillot, 1995), the condylognathan ancestor is supposed to

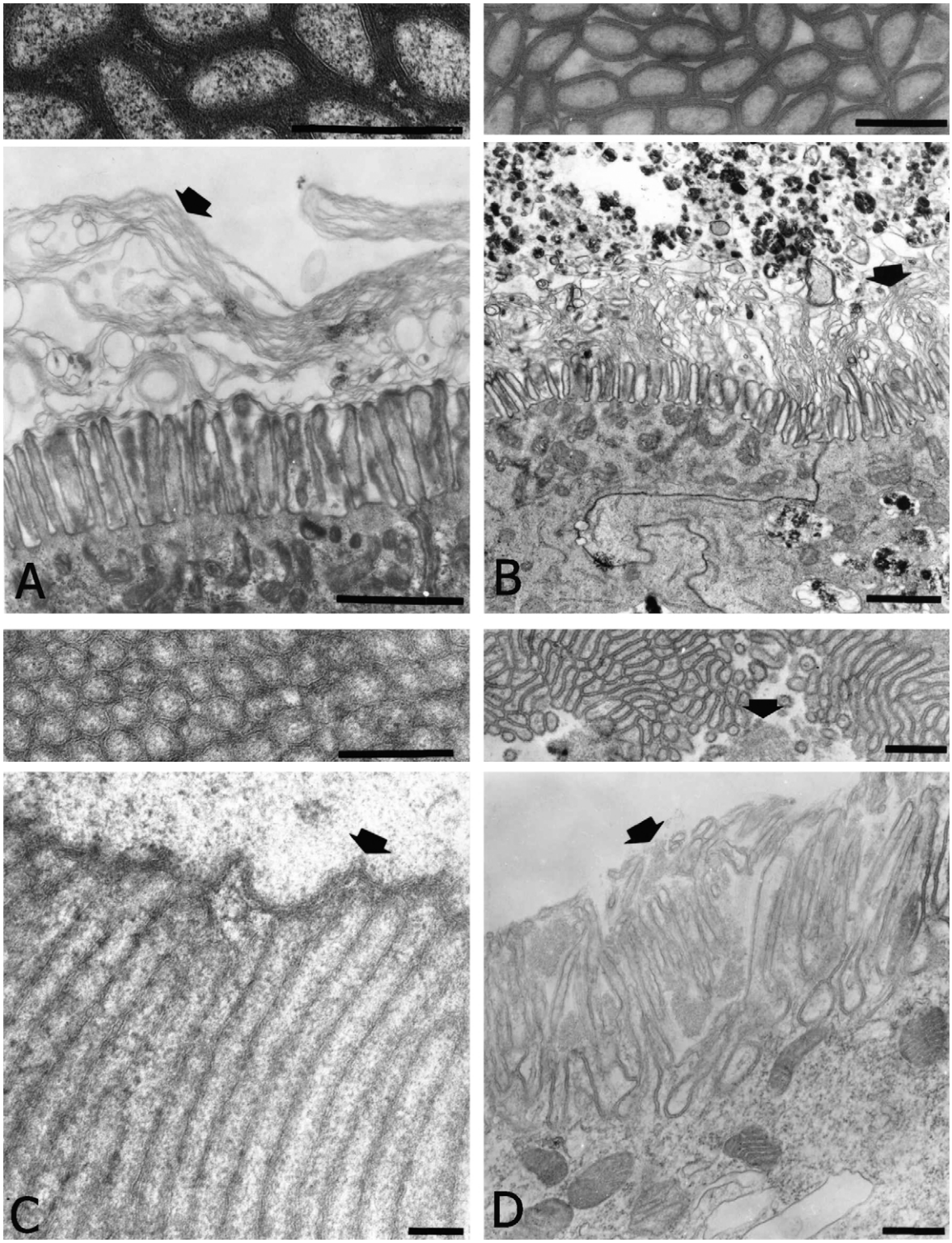

Fig. 2. Transmission electron microscopy of midgut cell apices of different hemipteran insects in longitudinal sections. A, anterior midgut of the cotton stainer bug *Dysdercus peruvianus* (Pentatomomorpha: Pyrrhocoridae); B, posterior midgut of the kissing bug *Rhodnius prolixus* (Cimicomorpha: Reduviidae); C, posterior midgut of the cercopid *Mahanarva posticata* (Auchenorrhyncha: Cercopidae); D, anterior midgut of the aphid *Myzus persicae* (Sternorrhyncha: Aphididae). Note perimicrovillar membranes in all species (arrows). The inset in each figure shows a transversal section through microvilli, where it is possible to see perimicrovillar membranes ensheathing them, except in the case of the aphid (see inset in panel D). In the aphid, the microvilli are tightly arranged side by side and the perimicrovillar membranes are absent between them, being found only in their apices as amorphous membrane masses (arrows in D). Bars: in A, 2 μm (inset 0.6 μm); in B, 2 μm (inset 0.4 μm); in C, 0.1 μm (inset 0.2 μm); in D, 0.5 μm (inset 0.5 μm).

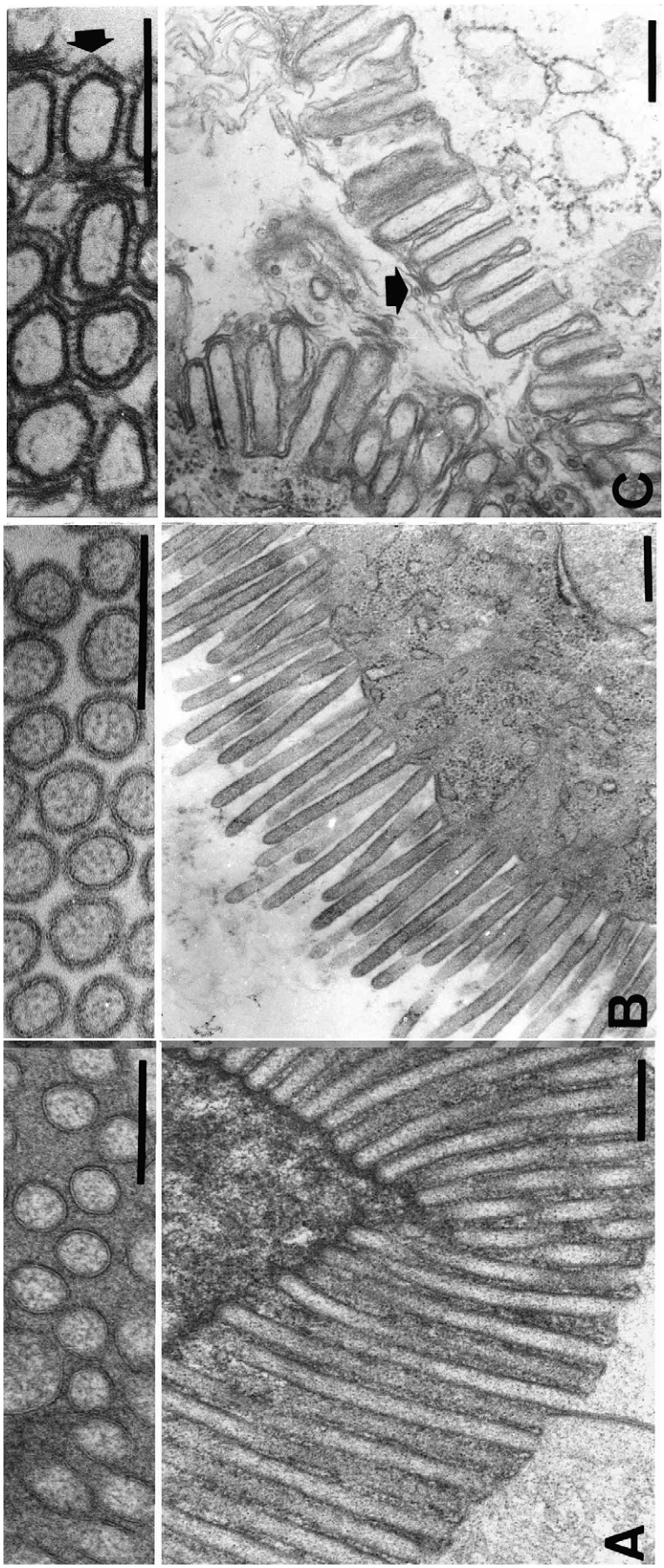
C
B
A

Table 1
α-Glucosidase activity along the midgut tissue in species pertaining to several paraneopteran taxa

Animal	V_1 Tissue		V_2 Tissue	
	mU/animal	mU/mg	mU/animal	mU/mg
R. prolixus (Heteroptera)[a]	2.3 ± 0.3	3.5 ± 0.3	7 ± 1	90 ± 20
D. peruvianus (Heteroptera)[b]	53 ± 8	160 ± 30	19 ± 3	110 ± 20
M. posticata (Auchenorrhyncha)	0.5 ± 0.2	7.4 ± 1.5	5.2 ± 0.6	50 ± 6
M. persicae (Sternorrhyncha)	1.05 ± 0.06	35 ± 2	0.3 ± 0.1	15 ± 3
Frankliniella sp. (Thysanoptera)[c]	1.5 ± 0.6	7 ± 2	–	–

Figures are means and SEM calculated from four assays performed in each of three different preparations obtained from 10 animals. V_1, a dilated anterior midgut region frequently named the stomach for *R. prolixus* and aphids and corresponding to the filter chamber in *M. postica*; V_2, slender and longer midgut region frequently named the intestine for *R. prolixus* and aphids. No α-glucosidase activity was found in midgut homogenates of a species of Pscoptera from rice grains and the Phthiraptera *Pediculus humanus*.

[a] Taken from Terra et al. (1988).

[b] Taken from Silva and Terra (1994).

[c] Whole midguts were assayed.

Table 2
Membrane-bound and soluble α-glucosidase activities in midgut sections of species pertaining to several paraneopteran taxa

Animal	Membrane-bound		Soluble	
	mU/animal	mU/mg	mU/animal	mU/mg
R. prolixus (Heteroptera)	7 ± 1	160 ± 30	0.8 ± 0.2	20 ± 5
D. peruvianus (Heteroptera)	70 ± 7	400 ± 70	2.4 ± 0.4	13 ± 3
M. posticata (Auchenorrhyncha)	5.0 ± 0.6	100 ± 10	0.20 ± 0.04	3.6 ± 0.3
M. persicae (Sternorrhyncha)	0.3 ± 0.1	30 ± 5	<0.03	–
Frankliniella sp. (Thysanoptera)	1.0 ± 0.3	20 ± 5	0.5 ± 0.1	3 ± 1

Midgut sections were homogenized in water and, after centrifuging the homogenates at 20,000*g* for 30 min at 4 °C, the resulting supernatant (soluble fraction) and pellets (membrane-bound fractions) were assayed for α-glucosidase activity. Figures are means and SEM calculated from assays performed in each of three different preparations obtained from 10 animals. V_1 were used for *D. peruvianus* and *M. persicae*; V_2, for *R. prolixus* and *M. posticata*; whole midgut, for *Frankliniella*.

have given origin to the hemipteran and thysanopteran ancestors in the Carboniferous. The hemipteran ancestor originated Auchenorrhyncha (fulgorids, cicadas, cercopids) and Sternorrhyncha (aphids, scale insects), known from lower and upper Permian, respectively. Heteroptera (pentatomids, cimicids) irradiated from the Jurassic and is the sister group of Auchenorrhyncha (Fig. 5). Present-day cicadas and cercopids feed on xylem sap; fulgorids, aphids, and scale insects feed on phloem sap; heteropteran insects feed on parenchyma and plant seeds as well as invertebrate and vertebrate tissue and blood (Carver et al., 1991).

The condylognathan ancestors should feed as present-day thrips on phloem sap obtained from plant tissues pierced by the oral stylets, the so-called 'punch and suck' mechanism. Phloem has very low contents of protein (with the exception of few phloem saps, see below) and carbohydrate polymers and is rich in sucrose and relatively poor in the free amino acids (Terra, 1990). Thus, except for dimer (sucrose) hydrolysis, no food digestion is usually necessary in sap-suckers. Upon adapting to this food, condylognathan ancestors would lose the enzymes involved in initial and intermediate digestion and associated with the lack of luminal digestion also lose the peritrophic membrane. The major problem facing a sap-sucking insect is to absorb nutrients, such as essential amino acids, that are present in very low concentrations in sap. Amino acids may be absorbed according to a hypothesized mechanism that depends on PMM as follows (Terra, 1988; Terra and Ferreira, 1994): the microvillar membrane would transport actively potassium ions (the most important ion of the plant sap) from the perimicrovillar space into the midgut cells, generating a concentration gradient between the sap in the lumen and that in the perimicrovillar space. This concentration gradient would be used as a driving force for the absorption of organic substances, like amino acids, through carriers in the PMM. The organic substances, once in the perimicrovillar space, would be absorbed through carriers at the surface of the microvillar membrane. The α-glucosidase

Fig. 3. Transmission electron microscopy of midgut cell apices of psocodean and thysanopteran insects in longitudinal sections. A, a book louse (Psocoptera: Liposcelidae); B, the louse *Pediculus humanus* (Phthiraptera: Pediculidae); C, the thrip *Frankliniella* sp. (Thysanoptera: Thripidae). The insets are transversal sections through microvilli. Note the absence of perimicrovillar membranes in A and B and their presence (C, arrows) in the thrip midgut. Bars, in A, 0.3 μm (inset 0.2 μm); in B, 0.5 μm (inset 0.2 μm); in C, 0.3 μm (inset 0.5 μm).

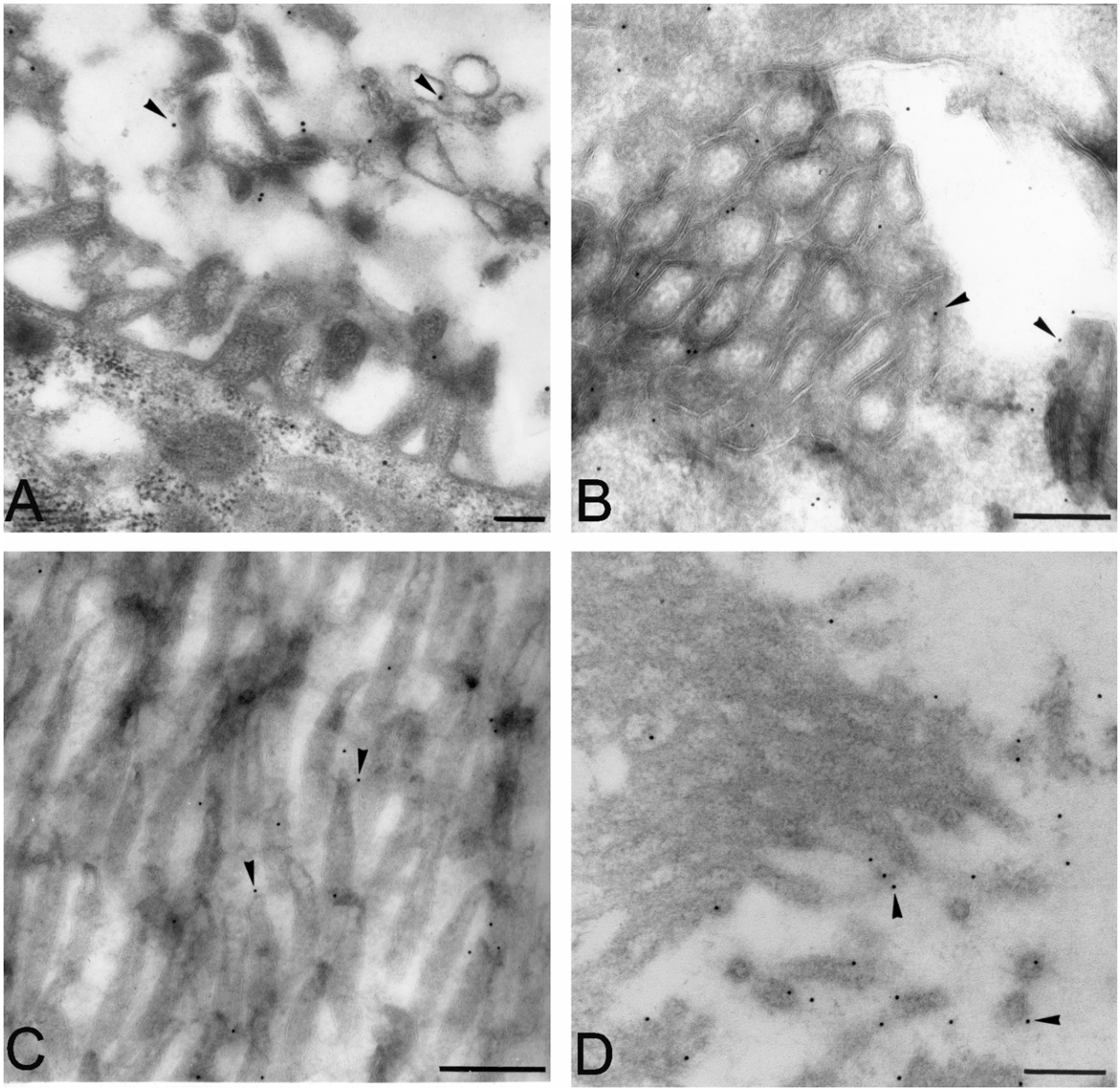

Fig. 4. Immunocytochemical localisation of membrane-bound α-glucosidase in midgut cells of different insects. A, *Dysdercus peruvianus* (Pentatomomorpha: Pyrrhocoridae); B, *Rhodnius prolixus* (Cimicomorpha: Reduviidae); C, *Mahanarva posticata* (Auchenorrhyncha: Cercopidae); D, *Frankliniella* sp. (Thysanoptera: Thripidae). Note labelling (arrows) in perimicrovillar membranes of the four insects. No reaction was seen in controls with non-immune serum. Bars 0.2 μm.

being bound to PMM efficiently cleaves sap sucrose without being excreted.

The hemipteran ancestor evolved from the condylognathan ancestor in acquiring piercing–sucking mouthparts (Gillot, 1995), thus becoming able to suck plant sap directly from the plant vascular system. These hemipteran ancestors were probably similar to present-day Auchenorrhyncha and were adapted to suck xylem and dilute phloem sap. As will be discussed below, high-sucrose phloem-sap feeding implies in several midgut derived characters found in aphids (Sternorrhyncha).

Organic compounds in xylem sap and in dilute phloem sap need to be concentrated before they can be absorbed by the perimicrovillar system. This occurs in the filter chamber of cicadoidea and cercopoidea, which concentrates xylem-sap 10-fold, or in the filter chamber of cicadelloidea which is able to concentrate dilute phloem about 2.5-fold (Terra, 1990).

The evolution of Heteroptera was associated with regaining the ability to digest polymers. Because the appropriate digestive enzymes were lost, these insects instead used enzymes derived from lysosomes, that is cysteine and aspartic proteinases characterized by being active in acid conditions. Compartmentalization of digestion was maintained by PMM as a substitute for the lacking peritrophic membrane (Terra, 1990).

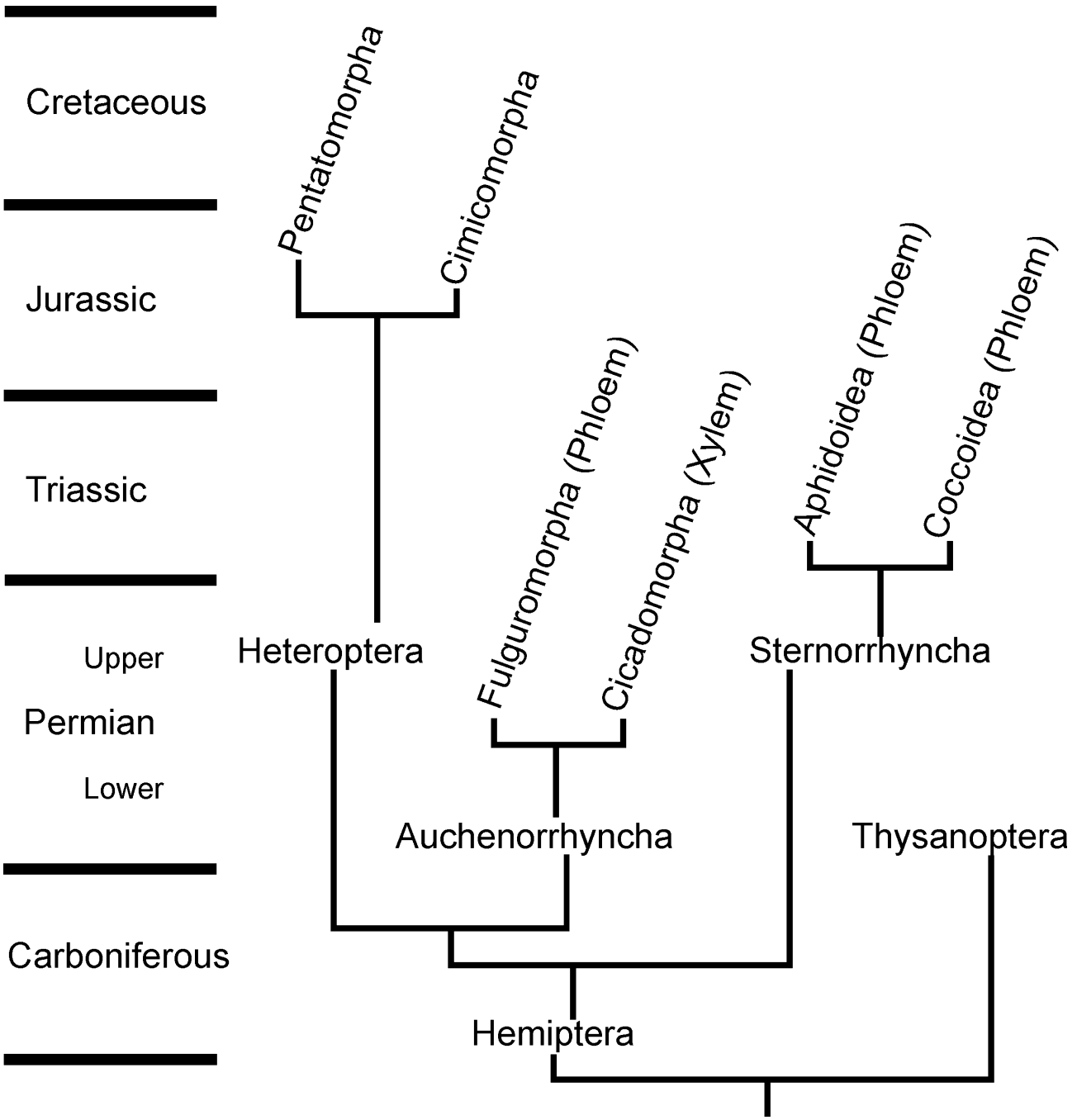

Fig. 5. Proposed evolution of Condylognatha, showing relations among major hemipteran infraorders and the order Thysanoptera. See Gillot (1995) for details. Sap fed by the major infraorders of Auchenorrhyncha and Sternorrhyncha is indicated in parentheses.

Aphids may suck more-or-less continuously phloem sap of sucrose concentration up to 1.0 M and osmolarity up to three times that of the insect hemolymph (Ashford et al., 2000). As the ingested phloem passes through the aphid midgut, its osmolarity decreases, resulting in a honeydew isoosmotic with hemolymph (Fisher et al., 1984). As a consequence, the anterior part of the midgut must withstand higher hydrostatic pressures than the posterior ones. Midgut stretching resistance is apparently helped by the existence of links (trabeculae) between apical lamellae that became less conspicuous along the midgut (Ponsen, 1991; Cristofoletti et al., 2003). According to Cristofoletti et al. (2003), following the appearance during evolution of apical lamellae links, PMM were replaced by the membranes seen associated with the tips of the lamellae, the modified perimicrovillar membranes.

The physiological role of the modified perimicrovillar membranes are similar to PMM and may include (Cristofoletti et al., 2003): (a) making amino acid absorption easier

by increasing the concentration of amino acids by binding them in a reversible way; (b) immobilizing α-glucosidase in a large area, so that this enzyme is not lost in the honeydew and is able to efficiently release fructose from sucrose without increasing the osmolarity of midgut contents; (c) immobilizing a cathepsin-L-like cysteine proteinase in a large area to avoid excretion. This proteinase may process toxins or be important in digesting proteins occurring in high concentrations in some phloem saps (Cristofoletti et al., 2003).

Acknowledgements

This work was supported by the Brazilian research agencies CNPq, FAPERJ, PRONEX, FINEP and FAPESP. We thank Dr C. Ferreira for helpful discussions. C.P. Silva, A.F. Ribeiro, R.A. DaMatta and W.R. Terra are staff members of their respective departments and research fellows of CNPq.

References

Andries, J., Torpier, G., 1982. An extracellular brush border coat of lipid membranes in the midgut of *Nepa cinerea* (Insecta Heteroptera): ultrastructure and genesis. Biology of the Cell 46, 195–202.

Ashford, D.A., Smith, W.A., Douglas, A.E., 2000. Living on a high sugar diet: the fate of sucrose ingested by a phloem-feeding insect, the pea aphid *Acyrthosiphon pisum*. Journal of Insect Physiology 46, 335–341.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry 72, 248–254.

Carver, M., Gross, G.F., Woodward, T.E., 1991. In: CSIRO, (Ed.), Hemiptera, Second ed., The Insects of Australia, vol. 1. Melbourne University Press, Melbourne, pp. 429–509.

Cristofoletti, P.T., Ribeiro, A.F., Deraison, C., Rahbé, Y., Terra, W.R., 2003. Midgut adaptation and digestive enzyme distribution in a phloem feeding insect, the pea aphid *Acyrtosiphon pisum*. Journal of Insect Physiology 49, 11–24.

Del Bene, G., Dallai, R., Marchini, D., 1991. Ultrastructure of the midgut and the adhering tubular salivary glands of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). International Journal of Insect Morphology and Embryology 20, 15–24.

Ferreira, C., Ribeiro, A.F., Garcia, E.S., Terra, W.R., 1988. Digestive enzymes trapped between and associated with the double plasma membranes of *Rhodnius prolixus* posterior midgut cells. Insect Biochemistry 18, 521–530.

Fisher, D.B., Wright, J.P., Mittler, T.E., 1984. Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharides. Journal of Insect Physiology 30, 387–393.

Gillot, C., 1995. Entomology, Second ed., Plenum Press, New York.

Goodchild, A.J.P., 1966. Evolution of the alimentary canal in the Hemiptera. Biological Reviews 41, 97–140.

Kitajima, E.W., 1975. A peculiar type of glycocalyx on the microvilli of the midgut epithelial cells of the thrips *Frankliniella* sp. (Thysanoptera: Thripidae). Cytobiologie 11, 299–303.

Kristensen, N.P., 1981. Phylogeny of insect orders. Annual Review of Entomology 26, 135–157.

Kukalova-Peck, J., Peck, S., 1993. Zoraptera wing structures: evidence for new genera and relationship with the blattoid orders (Insecta: Blattoneoptera). Systematic Entomology 18, 333–350.

Lane, N.J., Harrison, J.B., 1979. An unusual cell surface modification: a double plasma membrane. Journal of Cell Science 39, 355–372.

Lindsay, K.C., Marshall, A.T., 1980. Ultrastructure of the filter chamber complex in the alimentary canal of *Eurymela distincta* Signoret (Homoptera: Eurymelydae). International Journal of Insect Morphology and Embryology 15, 211–224.

Marshall, A.T., Cheung, W.W.K., 1974. Studies on water and ion transport in homopteran insects: ultrastructure and cytochemistry of the cicadoid and cercopid Malpighian tubules and filter chamber. Tissue and Cell 6, 153–171.

O'Loughlin, G.T., Chambers, T.C., 1972. Extracellular microtubules in the aphid gut. Journal of Cell Biology 53, 575–578.

Ponsen, M.B., 1991. Structure of the digestive system of aphids, in particular *Hyalopterus* and *Coloradoa*, and its bearing on the evolution of filterchambers in the Aphidoidea. Wageningen Agricultural University Papers 91-5, 3–61.

Proux, P., 1991. Structure–function relationship in intestinal brush border membranes. Biochimica et Biophysica Acta 1071, 255–271.

Rasnitsyn, A., 1998. On the taxonomic position of the insect order Zorotypida = Zoraptera. Zoologischer Anzeiger 237, 185–194.

Silva, C.P., Terra, W.R., 1994. Digestive and absorptive sites along the midgut of the cotton seed sucker bug *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae). Insect Biochemistry and Molecular Biology 24, 493–505.

Silva, C.P., Terra, W.R., 1995. An α-glucosidase from perimicrovillar membranes of *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) midgut cells. Purification and properties. Insect Biochemistry and Molecular Biology 25, 487–494.

Silva, C.P., Ribeiro, A.F., Gulbenkian, S., Terra, W.R., 1995. Organization, origin and function of the outer microvillar (perimicrovillar) membranes of *Dysdercus peruvianus* (Hemiptera) midgut cells. Journal of Insect Physiology 41, 1093–1103.

Silva, C.P., Ribeiro, A.F., Terra, W.R., 1996. Enzyme markers and isolation of the microvillar and perimicrovillar membranes of *Dysdercus peruvianus* (Hemiptera: Pyrrhocoridae) midgut cells. Insect Biochemistry and Molecular Biology 26, 1011–1018.

Smith, P.R., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provezano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurements of protein using bicinchoninic acid. Analytical Biochemistry 150, 76–85.

Terra, W.R., 1988. Physiology and biochemistry of insect digestion: an evolutionary perspective. Brazilian Journal of Medical and Biological Research 21, 675–734.

Terra, W.R., 1990. Evolution of digestive systems of insects. Annual Review of Entomology 35, 181–200.

Terra, W.R., 2001. The origin and functions of the insect peritrophic membrane and peritrophic gel. Archives of Insect Biochemistry and Physiology 47, 47–61.

Terra, W.R., Ferreira, C., 1994. Insect digestive enzymes: properties, compartmentalization and function. Comparative Biochemistry and Physiology 109B, 1–62.

Terra, W.R., Ferreira, C., De Bianchi, A.G., 1979. Distribution of digestive enzymes among the endo- and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physiological significance. Journal of Insect Physiology 25, 487–494.

Terra, W.R., Ferreira, C., Garcia, E.S., 1988. Origin, distribution, properties and functions of the major *Rhodnius prolixus* midgut hydrolases. Insect Biochemistry 18, 423–434.

Werner, K., Moutairou, K., Werner, G., 1991. Formation and structure of the surface coat in the midgut of a waterstrider, *Gerris najas* Deg. (Heteroptera: Gerridae). International Journal of Insect Morphology and Embryology 20, 69–77.