THE JOURNAL OF COMPARATIVE NEUROLOGY 467:150–172 (2003)

Conserved and Convergent Organization in the Optic Lobes of Insects and Isopods, with Reference to Other Crustacean Taxa

I. SINAKEVITCH,[1] J.K. DOUGLASS,[1] G. SCHOLTZ,[2] R. LOESEL,[3] AND N.J. STRAUSFELD[1]*
[1]Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, Arizona 85721
[2]Humboldt-Universität zu Berlin, Institut für Biologie/Vergleichende Zoologie, 10115 Berlin, Germany
[3]Institut für Biologie II (Zoologie) der Rheinisch Westfaelische Technische Hochschule Aachen, 52074 Aachen, Germany

ABSTRACT

The shared organization of three optic lobe neuropils—the lamina, medulla, and lobula—linked by chiasmata has been used to support arguments that insects and malacostracans are sister groups. However, in certain insects, the lobula is accompanied by a tectum-like fourth neuropil, the lobula plate, characterized by wide-field tangential neurons and linked to the medulla by uncrossed axons. The identification of a lobula plate in an isopod crustacean raises the question of whether the lobula plate of insects and isopods evolved convergently or are derived from a common ancestor. This question is here investigated by comparisons of insect and crustacean optic lobes. The basal branchiopod crustacean *Triops* has only two visual neuropils and no optic chiasma. This finding contrasts with the phyllocarid *Nebalia pugettensis*, a basal malacostracan whose lamina is linked by a chiasma to a medulla that is linked by a second chiasma to a retinotopic outswelling of the lateral protocerebrum, called the protolobula. In *Nebalia*, uncrossed axons from the medulla supply a minute fourth optic neuropil. Eumalacostracan crustaceans also possess two deep neuropils, one receiving crossed axons, the other uncrossed axons. However, in primitive insects, there is no separate fourth optic neuropil. Malacostracans and insects also differ in that the insect medulla comprises two nested neuropils separated by a layer of axons, called the Cuccati bundle. Comparisons suggest that neuroarchitectures of the lamina and medulla distal to the Cuccati bundle are equivalent to the eumalacostracan lamina and entire medulla. The occurrence of a second optic chiasma and protolobula are suggested to be synapomorphic for a malacostracan/insect clade. J. Comp. Neurol. 467:150–172, 2003.

Indexing terms: evolution; optic lobes; immunocytology; insects; crustaceans

Early students of the arthropod nervous system, most notably Holmgren (1916) and Hanström (1926a,b), inferred evolutionary relationships on the basis of shared morphological features of the brain. It was proposed that, because their optic lobes possessed neuropils in common, malacostracan crustaceans and pterygote insects should be considered sister groups. Recent phylogenetic studies based on molecular, developmental, and anatomical characters support the view that insects and crustaceans share a common ancestor (Averof and Akam, 1995; Friedrich and Tautz, 1995; Boore et al., 1998; Strausfeld, 1998; Paulus, 2000; Dohle, 2001; Richter, 2002; Loesel et al., 2002) but leave open whether insects might have arisen from within a crustacean evolutionary trajectory or whether crustaceans as a whole are the sister group of insects. A recent molecular study even suggests that hexa-

Grant sponsor: National Institute of Health; Grant number: R01 RR08688; Grant sponsor: Office of Naval Research; Grant number: N00014-97-1-0970.

*Correspondence to: N.J. Strausfeld, ARL, Division of Neurobiology, University of Arizona, Tucson, AZ, 85721.
E-mail: flybrain@neurobio.arizona.edu

Received 5 March 2003; Revised 12 May 2003; Accepted 17 June 2003
DOI 10.1002/cne.10925
Published online the week of October 20, 2003 in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Optic Lobe Components in Crustaceans and Insects[1]

	Branchiopoda	Phyllocarida	Decapoda	Isopoda	Archaeognatha	Hymenoptera	Diptera
1st Optic neuropil[2]	+	+	+	+	+	+	+
1st Optic chiasma (o ch 1)	−	+	+	+	+	+	+
2nd Optic neuropil undivided, contiguous with brain	+	−	−	−	−	−	−
2nd Optic neuropil undivided, separated from brain[3]	−	+	+	+	−	−	−
2nd Optic neuropil divided into two nested layers by Cuccati bundle[4]	−	−	−	−	+	+	+
3rd Optic neuropil,[5] contiguous with brain	−	+	−	−	+	−	−
3rd Optic neuropil,[5] separated from brain	−	−	+	+	−	+	+
Crossed axons (o ch 2) to 3rd optic neuropil	−	+	+	+	+	+	+
4th Optic neuropil[6]	−	+	+	+	−	−	+
Uncrossed axons from medulla to 4th neuropil	−	+	+	+	−	−	+
Giant tangential layer beneath but contiguous with 3rd optic neuropil	−	−	−	−	+	+	−
Uncrossed axons into giant tangential layer	−	−	−	−	+	+	−

[1]Superscript numerals represent the following: [2]Called the "lamina" in malacostracan crustaceans and insects.[3]Classic terminology (for Crustacea): "medulla externa." Medulla is used in this account (see also Harzsch, 2002).[4]Classic terminology (for Insecta): "medulla."[5]Lenticular neuropil called "medulla interna" in crustaceans, "lobula" in insects. Lobula used in this account (see also Harzsch, 2002) or "protolobula" if contiguous with lateral protocerebrum.[6]Retinotopic neuropil (can be minute or large) separate from, but adjacent to, lobula (called "lobula plate" in Diptera, Isopods; "satellite neuropil" in *Nebalia,* Decapoda).

pods are paraphyletic and that the insects originate from within the crustaceans (Nardi et al., 2003).

If insects and crustaceans are sister groups, it might be expected that insects should not only possess the same number of optic lobe neuropils as crustaceans but should also reveal common anatomical features that are independent of constraints imposed by the organization of neuropils into retinotopic subunits. It would also be necessary to find a plausible explanation for why the optic lobes of basal crustacean taxa, such as the branchiopods, contain only two retinotopic neuropils connected by uncrossed axons. This arrangement contrasts with eumalacostracans and pterygote insects, where there are at least three nested retinotopic neuropils linked by chiasmata (Table 1). From periphery to center, these neuropils are called the lamina, medulla, and lobula, respectively. In insects, the latter is often referred to as part of the "lobula complex" because in some taxa, exemplified by the Diptera, the lenticular neuropil of the lobula is flanked by a thinner tectum-like neuropil called the lobula plate. This area typically contains systems of large tangential neurons that are tuned to the direction of motion across the retina (Hausen and Egelhaaf, 1989). In other taxa, such as the Hymenoptera, the lobula surmounts a deep level containing systems of large tangential neurons that likewise respond to the direction of motion (DeVoe et al., 1982). It has been argued that the lobula plate of Diptera and deep lobula of Hymenoptera are equivalent, if not homologous, because they are supplied by the same morphological types of small retinotopic neurons from the medulla and from an outer layer over the lobula (Cajal and Sanchez, 1915; Strausfeld, 1976).

However, not only insects possess a lobula plate. Certain isopod crustaceans, such as the littoral taxon *Ligia occidentalis*, also posses a fourth optic neuropil (Strausfeld, 1998). Like that of dipteran insects, it is tectum-like and contains systems of wide-field tangential neurons. The question, therefore, arises whether the lobula plates of isopods and insects have evolved convergently or whether they derive from a common ancestor. But because isopods are phylogenetically recent within the Malacostraca (Schram, 1970; Scholtz, 2000; Richter and Scholtz, 2001) for this to be possible the more basal malacostracan groups such as the decapods or even phyllocarids (Table 2) should also posses a fourth neuropil that is likewise supplied by uncrossed axons or have a deep level in the lobula comparable to that seen in Hymenoptera.

TABLE 2. Species Used for This Analysis and Their Classification[1]

Species	Classification
Crustaceans	
Triops longicaudatis	Branchiopoda, Notostraca
Nebalia pugettensis	Malacostraca, Phyllocarida; Leptostraca
Ligia occidentalis	Malacostraca, Peracarida, Isopoda
Pandalus platyceros[2]	Malacostraca, Decapoda, Caridea
P. dispar[2]	Malacostraca, Decapoda, Caridea
Lebbeus groenlandicus[2]	Malacostraca, Decapoda, Caridea
Palaemonetes pugio[2]	Malacostraca, Decapoda, Caridea
Hemigrapsus nudus[3]	Malacostraca, Decapoda, Brachyura
Insects	
Allomachilis froggati[3]	Ectognatha, Archaeognatha
Machilis germanicus[3]	Ectognatha, Archaeognatha
Petrobius sp.[3]	Ectognatha, Archaeognatha
Aeschna canadensis[2]	Ectognatha, Pterygota, Odonata
Periplaneta americana[4]	Ectognatha, Blattoidea
Phaenicia sericata[5]	Ectognatha, Diptera
Drosophila melanogaster[6]	Ectognatha, Diptera
Manduca sexta[7]	Ectognatha, Lepidoptera
Apis mellifera[8]	Ectognatha, Hymenoptera

[1]Superscript numerals represent the following: for crustaceans [2]"shrimps," [3]crabs; for insects [2]dragonfly; [3]bristletail; [4]cockroach; [5]blowfly; [6]fruit fly; [7]tobacco hornworm (hawk moth); [8]honey bee.

Simply put, these questions can be rephrased to ask whether the optic lobes of crustaceans and insects are fundamentally similar, as has been suggested (Hanström, 1926b; Harzsch, 2002). By identifying structures and architectures shared by modern representatives of taxa showing either ancestral or derived conditions, it should be possible to identify those neuropils and architectures that support a sister group relationship and those neuropils and architectures that suggest convergent evolution.

Comparisons between the fly *Phaenicia sericata* and the crustacean *Ligia occidentalis* serve as a starting point, because these two relatively recent taxa share many similarities (Fig. 1) but are widely separated phylogenetically and in geological time. *Ligia* is a highly mobile semiterrestrial crustacean that shares with dipteran insects large compound eyes that are contiguous with the cephalic exoskeleton (Fig. 1D) rather than being situated on eyestalks, as is the case for decapod crustaceans. As in flies, *Ligia* possesses a lenticular third optic neuropil, the lobula, lying anteroventral to a tectum-like lobula plate (Fig. 1E,F). This latter neuropil is supplied by bundles of

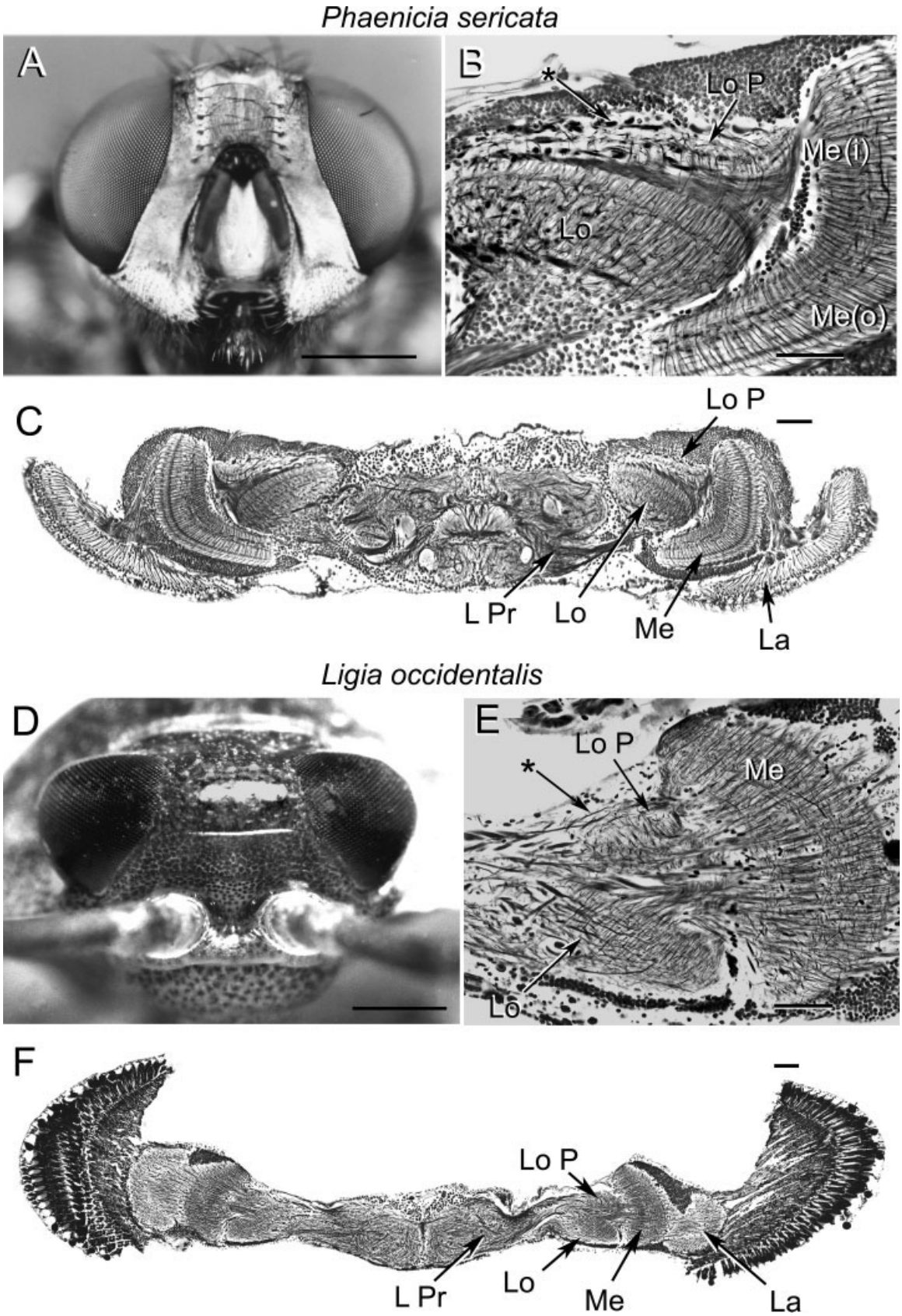

Fig. 1. Optic lobes of *Phaenicia* (A–C) and *Ligia* (D–F). **A:** Frontal view of the head and compound eyes of *Phaenicia*. **B:** The division of its deep optic lobe into four parts: the outer [Me (o)] and inner [Me (i)] medulla, the lobula plate (Lo P) and lobula (Lo). **C:** Horizontal section across the brain of *Phaenicia* showing the retinotopic optic lobes (La, lamina) distinct from the lateral protocerebrum (L Pr) but connected to it by axon bundles of the optic peduncle. **D:** Frontal view of the head and compound eyes of *Ligia occidentalis*. **E:** Divisions of its deep optic lobe neuropil into three parts: the medulla (Me), lobula plate (Lo P) with horizontally arranged giant tangential cell dendrites (asterisk), and lobula (Lo). These terms do not themselves suggest homology. **F:** Horizontal section across the brain of *Ligia* showing its retinotopic optic lobes that are distinct from the lateral protocerebrum connected to it by axon bundles. Scale bars = 1 mm in A,D; 100 μm in B,C,F; 50 μm in E.

uncrossed retinotopic axons from the second optic neuropil, the medulla, and is equipped with wide-field tangential neurons. The lobula plate of flies (Fig. 1B,C) is similarly equipped and supplied by sheets of uncrossed axons. Lobula plates have been identified in other insects groups (e.g., Lepidoptera, Coleoptera; Strausfeld, 1976) but, until the present study, not in crustaceans other than isopods.

Is the lobula plate of insects and isopods synapomorphic or homoplasic? The argument for a common origin of a lobula plate would be strengthened if isopods and insects share additional characters in the optic lobes, such as similar layer relationships and arrangements amongst comparable assemblies of neurons. The argument for a basal origin of the malacostracan lobula plate would be further strengthened if, in other crustaceans, a fourth optic lobe neuropil, not necessarily tectum-like, was identified that received uncrossed axons from the medulla.

To pursue this line of investigation, we have used antibodies against the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and standard silver staining methods to reveal neuropils and their architectures. The purpose of this study, thus, is strictly neuroanatomical and is not concerned with the putative inhibitory nature of stained structures. The optic lobes of a basal insect group, the archaeognathans, were surveyed by using Golgi impregnations and an antiserum against a tachykinin-like polypeptide.

Previous studies have used cell morphologies for discussing possible evolutionary trajectories of arthropods. The most detailed and focused approach has been by Shaw and Meinertzhagen (1986) and Shaw (1989, 1990; see also Shaw and Moore, 1989). These authors used electron microscopy and Golgi impregnation of lamina neurons in nematoceran and brachyceran Diptera to reconstruct a plausible evolutionary pathway that could account for incremental acquisition of the brachyceran neural superposition retina from an apposition eye type and, concomitantly, the incremental and adaptive remodeling of neuronal shapes and connections. Comparisons of nematoceran and brachyceran flies (Buschbeck and Strausfeld, 1996) have demonstrated that independent of their retina-to-lamina projections, a restricted subset of uniquely identifiable retinotopic neurons relaying from the lamina to the lobula plate share similar layer relationships. These conserved retinotopic neurons have since been implicated as crucial components of elementary motion detection circuits (Douglass and Strausfeld, 2003a,b). Divergent evolution of their target neurons in the lobula plate may, however, suggest differences in visual performance (Buschbeck and Strausfeld, 1997). The same subset of retinotopic neurons has also been identified in *Manduca sexta* (Wicklein and Strausfeld, 2000), and some elements of the subset have been described from crustaceans, at the level of the lamina and outer medulla (Strausfeld and Nässel, 1980). In the present account, strategies for making comparisons within a monophyletic group are extended across a broad range of arthropods.

Visual systems served by compound eyes consist of nested synaptic neuropils, each composed of columnar units that are peripherally supplied by photoreceptors from ommatidia. This common feature led Hanström (1926a) to propose that optic lobe organization unites insects and crustaceans. Renewed debate about insect–crustacean relationships has drawn heavily from neuroanatomical data. Harzsch (2002) proposed that the presence of three optic neuropils connected by two successive chiasmata in insects and malacostracans provides indisputable grounds for suggesting a common origin for these two groups, with the clear implication that the lamina, medulla, lobula, and their chiasmata are synapomorphic. Harzsch (2002) amplifies this view from developmental studies that show that the lobulas of insects and malacostracans have the common developmental property of being derived from a protocerebral cluster of proliferative cells (Wildt and Harzsch, 2002). Osorio (1991) proposed that, because the lamina and medulla have such similar construction and cell types in malacostracans and insects, these neuropils are unlikely to have evolved independently and convergently, a hypothesis reiterated by Nilsson and Osorio (1997). In support of this view, the authors drew from anatomical studies that demonstrate similarities between certain cell types in crustacean and pterygote laminae, the first optic neuropil beneath the retina. Richter and Scholtz (2001), however, suggest that the absence of a lobula separated from the protocerebrum, such as in the basal taxa Branchiopoda and Phyllocarida (Table 1), argues for its being a convergent apomorphic character of eumalacostracans and of dicondylians (Zygentoma and Pterygota) within insects. At a superficial level (Table 1), there appear to be only minor differences between the optic lobes of eumalacostracans and pterygote insects. However, a previous report showing differences between the medulla's organization in decapod eumalacostracans and pterygote insects suggests that deeper levels of their optic lobes may have evolved convergently (Strausfeld, 1998).

Agreeing in principle that similarities between insect and crustacean optic lobes offer a compelling case for homology, Meinertzhagen (1991) cautions that, if retinotopic organization is imposed by the developing compound eye, this itself must constrain the growth and, hence, shape of optic lobe neurons. Such constraints are evidenced by olfactory neuropils where odortypic receptors cluster into glomeruli, which in widely disparate taxa are developmentally equipped with analogous cell types and connections (Strausfeld and Hildebrand, 1999).

In the present account, we show that similarities between pterygote insects and eumalacostracan crustaceans cannot be ascertained throughout the optic lobes. The identification of a discrete neuropil in a basal malacostracan receiving uncrossed axons suggests a precursor to the isopod lobula plate. But a comparable neuropil is not seen in the Archaeognatha, in many respects the most primitive group of extant insects. Specific differences in the organization of the crustacean and insect medulla as well as differences of retinotopic organization in the lobulas of these groups all suggest caution when proposing homologies between pterygotes and eumalacostracans. A preliminary report of this work has appeared in abstract form (Sinakevitch et al., 2000).

MATERIALS AND METHODS

Specimens of sexually mature *Ligia occidentalis* were collected from tidal zones in Southern California. Flies (*Phaenicia sericata*) were raised to adulthood in breeding facilities of the laboratory, as were hawk moths (*Manduca sexta*; Lepidoptera: Sphingidae), cockroaches (*Periplaneta americana*), and fruit flies (*Drosophila melanogaster* Oregon R). Foraging *Apis mellifera* workers were collected locally. Dragonflies (*Aeschna canadensis*), decapod crusta-

ceans (*Hemigrapsus nudus* [crab], *Pandalus platyceros*, *P. dispar*, and *Lebbeus groenlandicus* [shrimps]), and the phyllocarid *Nebalia pugettensis* were collected at University of Washington-designated sites on or in waters around San Juan Island, Washington, and processed at the University of Washington Friday Harbor Laboratories. The grass shrimp *Palaemonetes pugio* was obtained from the Gulf of Mexico. Specimens of *Triops longicaudatus* were raised in the laboratory. Adult machilids (*Allomachilis froggati*) were obtained from Pebble Beach, New South Wales, Australia. Specimens of *Machilis germanicus* were obtained from the vicinity of Würzburg, Germany, and a third machilid, *Petrobius sp.*, was obtained from the Olympic Peninsula, Washington.

Reduced-silver staining

Reduced-silver preparations were used to illustrate general morphology. Brains were dissected and fixed in acetic acid–alcohol–formalin, washed in 70% ethanol, dehydrated in ascending ethanols, cleared in terpineol followed by xylene, and embedded in Paraplast Plus (Corning, Corning, NY). Hardened blocks were serially sectioned at 10 μm. Sections mounted on slides were dewaxed and incubated for 24 hours at 60°C in 1% silver proteinate (Roques, Paris, France) with 3 g of pure copper wire fragments per 100 ml of solution. Afterward, tissue was treated according to Bodian's (1937) original method.

Immunohistochemistry

GABA antiserum (raised in rabbits using GABA conjugated with glutaraldehyde [a] to bovine serum albumin [BSA], bovine hemoglobin, or poly-L-lysine) was obtained from GEMAC (Talence, France). Antiserum specificity has been described elsewhere (Seguela et al., 1984; Sinakevitch et al., 1996). Brains were fixed at 4°C overnight in 0.8–1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0) containing 1% sodium metabisulfite ($Na_2S_2O_5$, SMB, Sigma). After fixation, whole brains were incubated for 15 minutes in a solution of 0.05 M Tris-HCl buffer with 0.5% SMB, pH 7.5, containing 0.13 M $NaBH_4$ to saturate double bonds. After washing in 0.05 M Tris-HCl-SMB buffer (4 × 30 minutes), the preparations were embedded in 8% agarose and, after hardening, were sectioned at 80–100 μm at frontal and horizontal orientations. Sections were preincubated with 10% normal swine serum (Dako, Carpenteria, CA) in 0.05 M Tris-HCl-SMB with 0.5% Triton X-100. Conjugated GABA antiserum, diluted 1:1,000, was added to the sections of each brain for overnight incubation at room temperature. After washing in Tris-HCl-Triton X-100, the sections were incubated overnight with goat anti-rabbit immunoglobulin (Jackson Laboratories, West Grove, PA) conjugated to Texas red or Alexa 488 (Molecular Probes, Eugene, OR) diluted 1:250 in the Tris-HCl-Triton X-100. After a final wash in Tris-HCl, the sections were embedded in the mounting medium. To test the specificity of immunostaining, the working dilutions of GABA antibodies were preincubated overnight with conjugate containing 10^{-5} or 10^{-4} M hapten-conjugated GABA-G-BSA. Preadsorption of the primary antiserum with GABA-G-BSA completely abolished staining in *Phaenicia* and *Ligia* (Fig. 2).

The polyclonal antiserum to tachykinin-related peptide (TRP), which recognizes the C-terminus of known insect and crustacean TRPs, was raised in rabbits inoculated with locust TRP conjugated to BSA (Winther and Nässel, 2001). Anti-TRP was used to stain general architectural features of the archaeognathan insect *Machilis*. Animals were anesthetized by chilling on ice. Dissections were made under fixative consisting of 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS), pH 7.4. After fixing for approximately 18 hours at room temperature, brains were dehydrated, incubated in propylene oxide for 10 minutes for membrane permeabilization, rehydrated, embedded in gelatin/albumin, and sectioned at 85 μm with a Vibratome (VT1000S, Leica, Nussloch, Germany). Sections were rinsed in 0.1% Triton X-100 in PBS and incubated overnight in 5% normal swine serum (Dako) in PBS containing 0.5% Triton X-100. Next, sections were

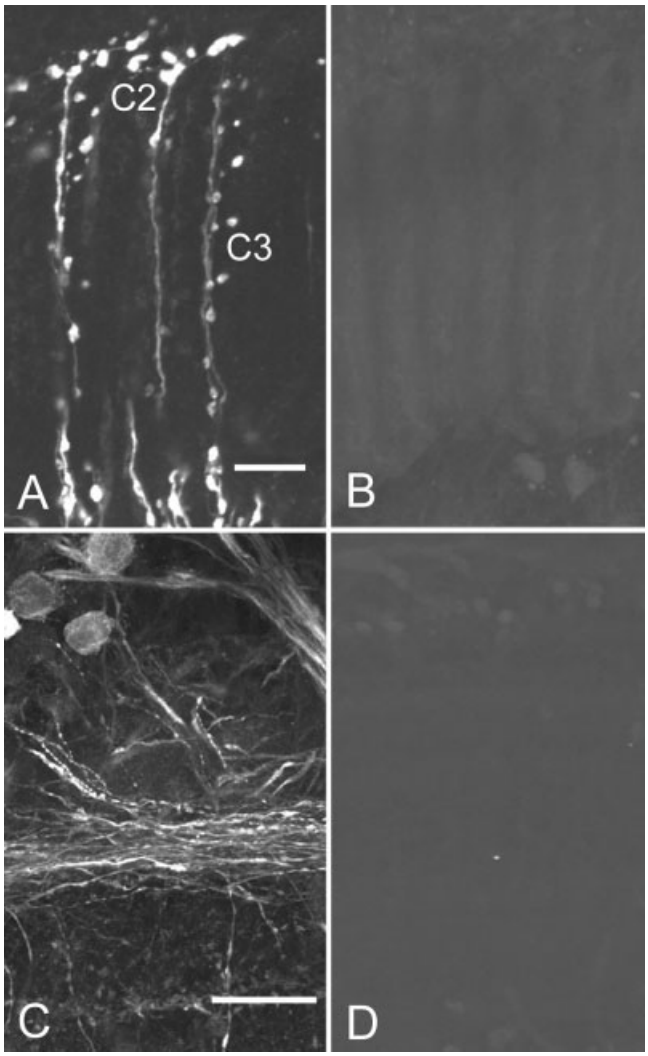

Fig. 2. Adsorption controls for anti–γ-aminobutyric acid (GABA) specificity. **A:** C2 and C3 terminals in the lamina of *Phaenicia* revealed by treatment with anti-GABA followed by treatment with the Texas red–conjugated secondary antibody (see Materials and Methods section) **B:** *Phaenicia* lamina-treated with anti-GABA preadsorbed with GABA-G-BSA followed by the Texas red–conjugated antibody. **C:** Cell bodies and neurites revealed above the medulla of *Ligia*, using anti-GABA and Texas red–conjugated secondary antibody. **D:** Control as in B. Scale bars = 10 μm in A (applies to A,B), C (applies to C,D).

incubated in primary antiserum for approximately 26 hours at room temperature. After rinsing in 0.1% Triton X-100 in PBS, sections were incubated overnight in Texas red–conjugated goat anti-rabbit immunoglobulin (Jackson Laboratories) used at a dilution of 1:500 in PBS containing 0.5% Triton X-100 and 1% normal swine serum. After rinsing in PBS, sections were mounted in Elvanol (Rodriguez and Deinhardt, 1960) on chromalum/gelatin-covered slides.

Confocal microscopes (Nikon PCM 2000 or Zeiss LSM 5 Pascal) were used for data acquisition. Series of optical sections, equivalent in depth to a thick reduced-silver section (15–25 μm) comprising images of 512 × 512 or 1,024 × 1,024 pixels at 8-bit or 12-bit color depth, were scanned through 20×/0.5 Neofluar, 40×/1.0 oil iris or 63×/1.40 oil iris plan-apochromat objectives. Figures were assembled and labeled in Adobe Photoshop version 6.0.

RESULTS

Use of GABA antisera as a neuroanatomical tool

Historically, early studies on the vertebrate brain and central nervous system relied on reduced-silver methods to characterize architectural features of specific brain regions (Cajal and De Castro, 1933). Likewise, the classic studies on crustacean and insect optic lobes (Cajal and Sanchez, 1915; Hanström, 1926b) relied on the same methods to identify the arrangements of retinotopic columns, chiasmata, stratifications, and tracts leading from optic neuropils to the brain. However, when neurons are small or do not provide a reducing substrate for silver impregnation, neural tissues are refractory to reduced-silver analysis and other strategies are required. Neuropils that comprise dense arrangements of neurons are also difficult because they may be so heavily stained that their architectures are largely obscured. These problems were recognized by some of the earliest anatomists working on invertebrates who then used methylene blue on living tissue to provide, within a species, consistently selective staining of subsets of neurons that then allowed comparisons across species (Retzius, 1891, 1891, b; Zawarzin, 1913). In contrast, stochastic methods such as the Golgi procedures, although essential for revealing the morphologies of single neurons and relating these to known architectures, do not alone provide information about overall architecture.

The present account uses an antiserum raised against the common inhibitory transmitter GABA to provide a useful stain for revealing populations of neurons in neuropils, including many that are refractory or too small to be investigated by reduced silver. Anti-GABA has the advantage that it generally reveals fewer neurons than would be stained by reduced silver. Its reliability is supported by studies that have used anti-GABA sera from different sources, used on appropriately fixed and embedded materials, which show the same specificity of staining, such as uniquely identifiable neurons in the optic lobes of cyclorrhaphan flies (Meyer et al., 1986; Strausfeld et al., 1995). Preadsorbtion tests using fly and isopod tissue (see Materials and Methods section) further demonstrate that neurons are revealed by the antibody raised against GABA and not by the secondary antibody alone. Antibodies raised against tachykinin are used to show general features of the machilid brain and optic lobes.

The following sections compare the optic lobes of the fly *Phaenicia* with that of the isopod *Ligia* and then compare the range of morphological variations in their laminas with those of other pterygote insects and eumalacostracan crustaceans. This comparison is followed by comparisons of medulla architectures and organization in the lobula complex. Comparisons with basal taxa and observations on retinotopic organization in the lobula conclude the Results section.

Similarities of optic lobe organization in the fly *Phaenicia* and the isopod *Ligia*

Brachyceran flies such as *Phaenicia sericata* (Fig. 1A) have "neural superposition" eyes, a phylogenetically recent modification of the apposition type compound retina (Land and Nilsson, 2002). In flies, deep optic lobe neuropils (Fig. 1B) consist of two synaptic regions: a lenticular lobula and, opposing its outer surface, a tectum-like lobula plate. Together, these regions comprise the lobula complex. The more peripheral regions of the optic lobe consist of a lamina lying immediately beneath the retina that is linked by means of a chiasma to the medulla (Fig. 1C). The medulla is divided into two obvious levels, the outer and inner medulla, by the Cuccati bundle. This bundle provides axons to and receives axons from the posterior optic tract (Strausfeld, 1976). The medulla is connected to the lobula by a second chiasma and to the lobula plate by sheets of uncrossed axons (Braitenberg, 1970). In flies, as in most pterygote insects, the optic lobes are separated from the lateral protocerebrum of the midbrain, connected to it by bundles of efferent and afferent axons.

Oniscid isopods such as *Ligia occidentalis* (Fig. 1D) are thought to have evolved relatively recently (Brusca and Wilson, 1991), the first isopod fossils being recorded from Upper Carboniferous deposits (Schram, 1970). The visual system of *Ligia* is served by an apposition-type eye (Hariyama et al., 2001). Silver-stained horizontal sections reveal that its deep optic lobe neuropils are similarly divided into a lobula and lobula plate (Fig. 1E) and are also separated from the lateral protocerebrum (Fig. 1F). Without prejudice to phylogenetic considerations, we here use the terms lamina, medulla, lobula, and lobula plate for this taxon as well.

The optic lobes of *Phaenicia* and *Ligia* are retinotopically organized into columnar subunits that are intersected by tangential strata. These occur at characteristic depths through each of the neuropils. Strata comprise the dendrites and terminals of tangential cells, as well as the processes of amacrine cells and the lateral processes (dendrites and collaterals) of retinotopic neurons that contribute to the retinotopic columns.

Morphological range: comparisons of lamina architectures provided by centrifugal cells

The lamina of *Phaenicia sericata* is composed of columns, each of which is defined by the six axon terminals of photoreceptors that share the same optical alignment (Kirschfeld, 1967), thus constituting a visual sampling unit (Franceschini, 1975). Pairs of axons, from a seventh and eighth photoreceptor in each ommatidium, project across the first optic chiasma to end in the outer medulla. During development, the first axons to differentiate are the R8 "long visual fibers" that project through the presumptive lamina to the medulla, where each defines a retinotopic column (Meyerowitz and Kankel, 1978). In

flies, associated with each set of terminals in the lamina are six efferent neurons (Strausfeld, 1971). Five (the monopolar cells) originate from a stratum of cell bodies above the synaptic external plexiform layer (Fig. 3A). This layer contains amacrine processes, tangential endings of wide-field centrifugal cells, and the retinotopically organized terminals of two types of narrow-field feedback neurons from the medulla called C2 and C3 centrifugal cells (Strausfeld, 1971). It is these neurons that are selectively revealed by anti-GABA (Fig. 3B,C; see also Meyer et al., 1986). In flies, centrifugal neurons to the lamina originate from cell bodies in rind beneath and lateral to the posterior margin of the medulla (Strausfeld, 1971). Their axons project across the first optic chiasma (Fig. 3B) as do centrifugal cells supplying the lamina of *Ligia* (Fig. 3E). C2 and C3 neurons have entirely different arborizations in the medulla (Strausfeld, 1976), and their terminals in the lamina are also distinct, those of C3 neurons providing rows of knob-like specializations that extend along one side of the dendritic branches of the L1 and L2 monopolar cells (Strausfeld, 1971). Each C2 neuron terminal is capped by a characteristic flattened specialization (Figs. 3C, 4A), as originally described from Golgi impregnations (Strausfeld, 1971), where the cap was shown to reside at the outermost dendrites of the two largest efferent neurons in the lamina, the L1 and L2 monopolar cells. C2 and C3 centrifugal endings are restricted to single cartridges and are often so closely apposed to each other that they can appear to belong to a single element (Fig. 3C,4A).

Hariyama et al. (2001) demonstrated seven photoreceptors in each ommatidium of *Ligia*, of which only one ends in the medulla and six in the lamina. As in flies, the lamina is divided into a cell body layer and an external plexiform layer (Fig. 3D). Receptor terminals confer retinotopy into the lamina, the columns of which are visited by retinotopically organized GABAergic centrifugal cells (Fig. 3E,F). These have bifurcating endings that overlap each other so as to provide a characteristic architecture that is apparent both in reduced-silver stained (Fig. 3D) and immunostained material (Fig. 3E,F). Overlapping GABAergic processes (Fig. 3F) give rise to branches that ascend distally through the external plexiform layer toward bundles of incoming photoreceptor axons, amongst which they provide beaded specializations (Fig. 3E,F). Beads, as opposed to spine-like structures, are indicative of presynaptic specializations in arthropods (Strausfeld and Meinertzhagen, 1996).

Unless considered in the context of variations within groups, these differences in centrifugal organization could be interpreted as distinguishing insect and crustacean laminas. However, observations of other insects and crustaceans demonstrate that such differences may be adaptations that reflect retinal organization or habitat rather than phylogeny. Comparisons of laminas serving apposition retinas in other taxa show that the GABAergic supply to the lamina of *Ligia* is within the range of eumalacostracan and pterygote morphologies and is almost indistinguishable from some. This range includes unistratified systems of GABAergic terminals, as in the honey bee lamina (Fig. 4B,C), and a unistratified organization seen in the lamina of the decapod crustacean *Pandalus platyceros*, a shallow water shrimp (Fig. 4D). Comparisons between anti-GABA–labeled elements (Fig. 4C) and Golgi-impregnated lamina neurons suggest that GABA-immunoreactive neurons in the honey bee may correspond to the type C1 centrifugal cells described by Ribi (1975), whose overlapping processes extend laterally across several neighboring optic cartridges at this level. Columnar and stratified arrangements of centrifugal terminals are seen in dragonflies, such as *Aeschna canadensis* (Fig. 4E,F), the lamina of which comprises discrete rows of optic cartridges each supplying a sheet of axons into the first optic chiasma (see, Meinertzhagen, 1976). In *Aeschna* (Fig. 4E), each centrifugal GABAergic ending bifurcates to provide a system of tangential processes and systems of climbing fibers to three or four optic cartridges. These fibers end as beaded tufts at the level of the incoming receptor axons (Fig. 4E). A second system of GABAergic terminals provides tangential processes, the collaterals of which approximately coincide with optic cartridges (Fig. 4F). The moth *Manduca sexta* is a crepuscular and nocturnal species equipped with superposition eyes (Fig. 4G,H), but its centrifugal terminals are also similar to those of *Ligia* (Fig. 3F). In *Manduca*, there are pairs of GABAergic axons, some of which bifurcate beneath the lamina to provide branches into adjacent columns. Centrifugal terminals in *Manduca* ascend through the whole depth of the external plexiform layer where they are decorated with either varicosities or bead-like swellings, possibly suggestive of two cell types. The widths of these terminals may correspond to the lateral spread of dendrites of monopolar cells described by Wicklein and Strausfeld (2000). The cockroach *Periplaneta americana* is also a crepuscular and nocturnal species but has apposition eyes. In this species, anti-GABA–labeled centrifugal endings (Fig. 4I,J) divide beneath the lamina to provide a dense plexus of overlapping branches that ascend through the external plexiform layer before extending varicose processes toward bundles of incoming receptor axons (Fig. 4J).

Medulla neuroarchitectures in pterygote insects and eumalacostracan

Reduced-silver stained medullas of *Phaenicia* and *Ligia* show them to receive bundles of axons that confer the peripheral retinotopic map of the lamina into this neuropil (Fig. 1B,C,E,F). In *Phaenicia*, as in other insects, the medulla is divided into a thick outer layer (the outer medulla; Fig. 5A) and a thinner inner layer (the inner medulla; Fig. 5A) by a prominent stratum of tangentially directed axons. This is the Cuccati bundle (Fig. 5A), which carries axons of tangential neurons to and from the posterior optic tract (Cajal and Sanchez, 1915; Strausfeld, 1976). Columnar arrangements of small-field retinotopic neurons extend the retinotopic map from the medulla's surface through all of its strata (Fig. 5A,B). Above the medulla, GABAergic cell bodies contribute to GABAergic columnar neurons. Some of these appear to be contained only within the medulla, connecting deep strata of the outer medulla to its outermost stratum (Fig. 5C,D) and corresponding to local feedback neurons identified by Golgi impregnations (Douglass and Strausfeld, 2003a). In addition, through-projecting GABAergic axons contribute to immunoreactive boutons beneath and at two levels above the Cuccati bundle. The boutons above the bundle (lower arrow in Fig. 5E) are similar to those seen in a system of GABAergic retinotopic neurons in *Ligia* (lower arrow in Fig. 5F). However, in the isopod, these boutons do not lie above a Cuccati-like bundle because no such bundle can be identified. Thus, the levels of and relationships

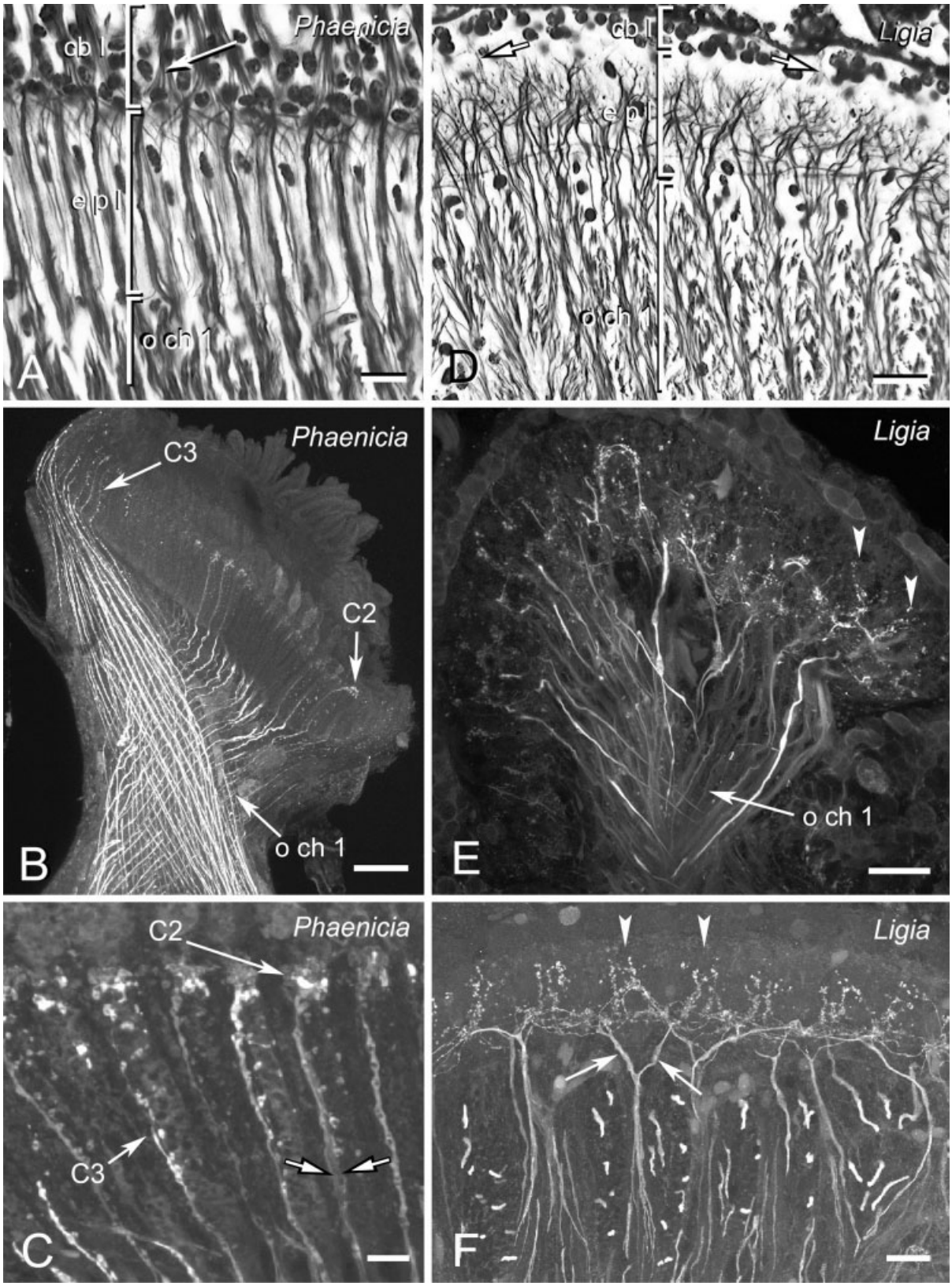

Fig. 3. Comparison of γ-aminobutyric acid (GABA)ergic organization in the laminas of *Phaenicia* (A–C) and *Ligia* (D–F). **A,D:** Reduced-silver stained laminas of both taxa showing the cell body layer (cb l) and the external plexiform layer (e p l), the latter supplied by photoreceptor endings (arrows in A,D). Axons from the lamina extend centrally into the first optic chiasma (o ch 1). **B,E:** Anti-GABA–stained centrifugal neurons from the medulla ending in the lamina: a pair of dissimilar neurons (C2 and C3) enters each column of the lamina in *Phaenicia*, whereas in *Ligia* a population of similar cell types ends as tufts (arrowheads in E), the locations of which correspond to bundles of incoming receptor axons. **C:** Detail showing the capped structure of C2 endings and the unilateral blebs from C3 terminals. Axons providing these terminals lie adjacent to each other (paired outlined arrows) and often cannot be resolved separately. **F:** In *Ligia*, the terminals of centrifugal endings bifurcate into two tributaries (arrows), which then provide a system of tangentially oriented processes and ascending tufts (arrowheads). Scale bars = 10 μm in A,C,F; 25 μm in B,E; 20 μm in D.

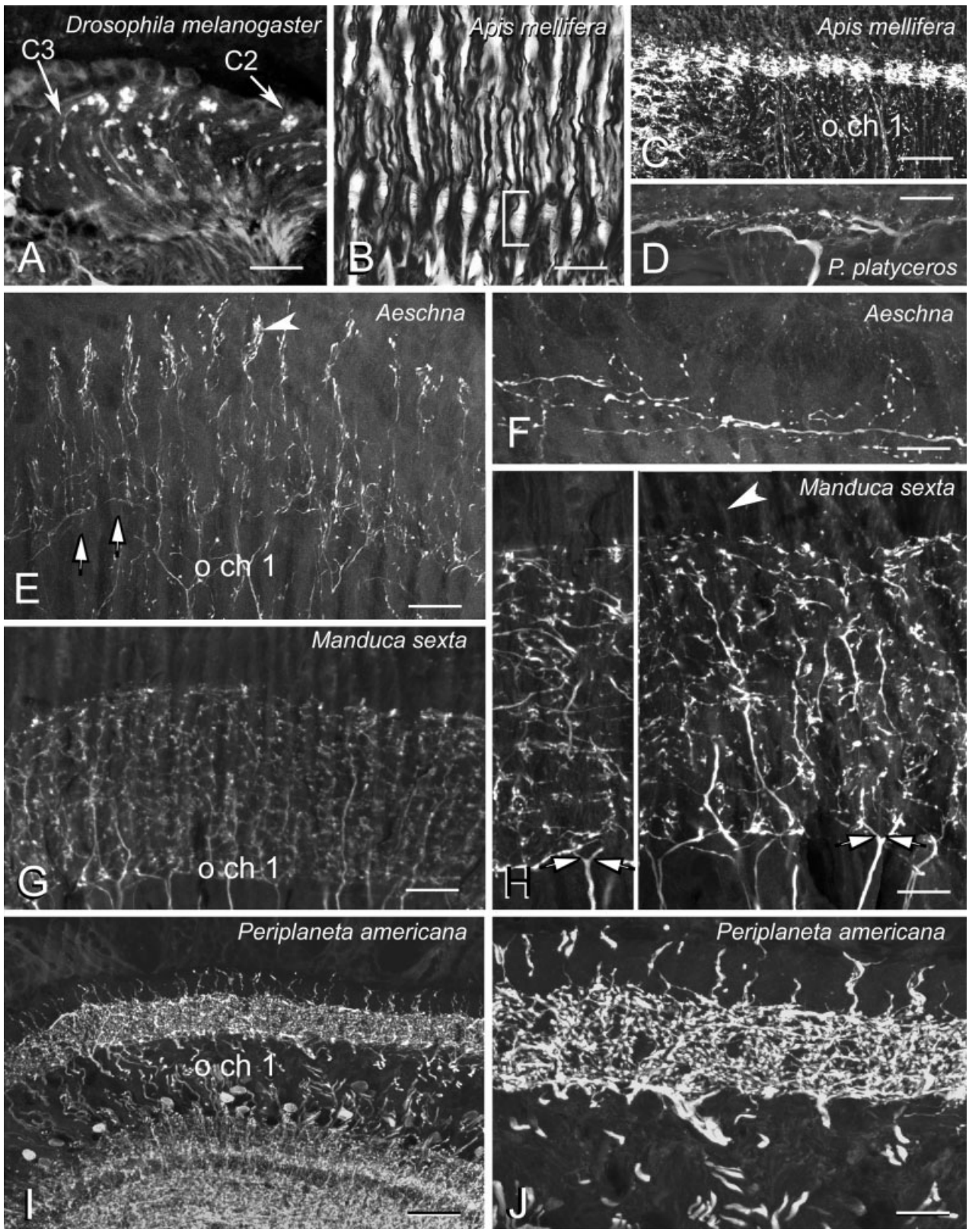

Fig. 4. Centrifugal terminals. **A:** Characteristic terminals of C2 (capped ending) and C3 (unilateral beaded ending) in *Drosophila melanogaster* are restricted to single lamina columns (optic cartridges). **B,C:** Honey bee laminas are also organized as discrete columns, shown stained with reduced silver in B, showing a layer of tangential processes (bracket) that is at the same level as γ-aminobutyric acid (GABA)ergic centrifugal cells in **C**. **D:** Tangential centrifugal endings in the lamina of the decapod crustacean *Pandalus platyceros*. **E:** In the dragonfly *Aeschna canadensis*, each centrifugal cell extends to adjacent cartridges and, as in *Ligia*, provides distal tufts (arrowhead) to incoming receptor axons. Each cartridge is indicated by its unstained axon bundle (arrows) into the first optic chiasma (o ch 1). **F:** Tangential centrifugal endings in *Aeschna*. **G,H:** The lamina of *Manduca sexta* receives pairs of GABAergic terminals (some bifurcating shown by paired arrows in two panels of H) that extend across groups of incoming receptor axons (one indicated by the arrowhead). **I,J:** Likewise, in *Periplaneta americana*, the GABAergic centrifugals provide a dense system of overlapping terminals. Note the distal processes ascending toward the retina. Scale bar = 10 μm in A; 25 μm in B–E; 20 μm in F,H,J; 30 μm in G; 15 μm in I.

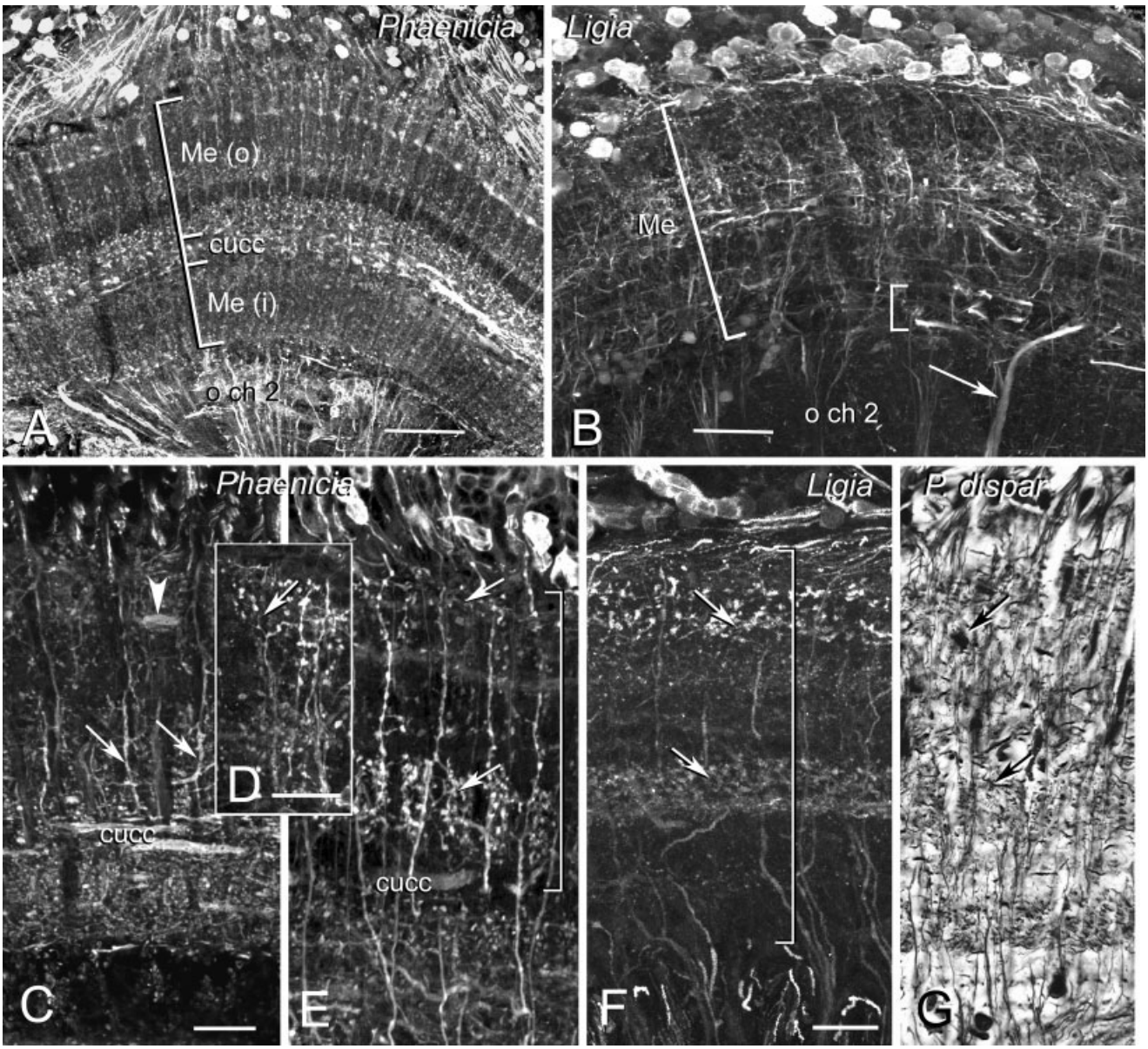

Fig. 5. γ-Aminobutyric acid (GABA) -like immunoreactivity reveals principal differences in medulla organization between *Phaenicia* (A,C–E), *Ligia* (B,F), and the decapod *Pandalus dispar* (G), here scaled to match the depth of the *Ligia* medulla in F. **A:** A layer of tangential axons [Cuccati's bundle (cucc)] divides the medulla of *Phaenicia* into an outer two thirds [Me(o)] and inner one third [Me(i)]. o ch 2, second optic chiasm. **B:** This organization is not seen in *Ligia*. Instead, tangentials (arrow) enter the proximal level of the medulla where they branch (small bracket). The entire medulla (large bracket) is equivalent to the outer medulla of *Phaenicia*. **C,D:** In *Phaenicia*, although some GABAergic tangentials (arrowhead) occur within its outer stratum, most GABAergic elements reveal the medulla's retinotopic organization. A discrete system of dendrites (arrow pair in C) and terminals (arrow in D) of local centrifugal cells links the inner and outer strata of the outer medulla. **E,F:** Comparable organization of retinotopic GABAergic neurons in *Phaenicia* and *Ligia* (compare arrows in E and F). Equivalent levels in *Phaenicia* and *Ligia* are bracketed. Stratification revealed by anti-GABA corresponds to strata typical of other eumalacostracans, such as the decapod *Pandalus dispar* (**G**), here shown stained with reduced silver (compare arrows in G, with arrows in F). G is scaled so that the upper and lower margins of the medulla align with F. Scale bars = 20 μm in A; 15 μm in B–D; 10 μm in F (applies to E–G).

between GABAergic specializations provided by what appear to be equivalent systems of GABAergic intrinsic neurons suggest an important difference between the dipteran and isopod medullas. In isopods, there is an absence of two nested and well-defined components of the medulla. That this absence is not merely due to a lack of GABA-immunoreactive tangential axons is shown by reduced-silver preparations of *Ligia* and other eumalacostracans, such as *Pandalus dispar* (Fig. 5G).

The presence of a Cuccati bundle in insects (Fig. 6A) but not in crustaceans was suggested to be an important difference between these two groups (Strausfeld, 1998). How-

ever, this difference could be explained as an acquired change of position such that tangentials deep in the crustacean medulla have shifted to a more peripheral position in the insect medulla. Here, we ask whether tangential neurons provide a deep bundle in malacostracan medulla. And, are cell bodies of tangential cells in crustaceans located as they are in insects, at the anterior edge of the medulla neuropil?

Observations of reduced-silver preparations reveal specific differences in the organization of wide-field neurons in the medulla of *Phaenicia* and *Ligia*. Tangential neurons serving the isopod medulla extend their axons centrally from the anterior and dorsal edge of the medulla (Fig. 6B,C). Their cell bodies are located distant from the neuropil, however. Dendrites or terminals belonging to these neurons penetrate into medulla neuropil from the side and from its lower surface without dividing this region into an obvious inner and outer component. By comparison, GABA immunohistology shows that, in the honey bee (Fig. 6D) and dragonfly (Fig. 6E), numerous bundled GABAergic tangential axons divide the medulla into a discrete outer and inner component. These two levels have distinctive cytoarchitectures, with the inner medulla appearing denser than the outer. This organization again contrasts with that of the medulla of the decapod *Hemigrapsus* (Fig. 6F) and the isopod *Ligia* (Fig. 5B) where, in both taxa, there appear to be no discrete bundles of tangential neurons. The deep medulla level in which tangential processes can be shown by reduced silver (*Ligia*; Fig. 6C) lacks GABA-immunoreactive tangential axons.

Comparisons of fly, moth, and isopod lobula complexes

The term "lobula complex" includes one or more neuropils that receive retinotopic axons from the medulla (Table 1). In flies, the lobula complex consists of a bean-shaped lobula flanked by a tectum-like neuropil, the lobula plate. The linear order of axon bundles entering the lobula is reversed from that in the medulla by the second optic chiasma, whereas the linear order of axons entering the lobula plate is not (Braitenberg, 1970). This latter projection (called "homotopic") typifies projections into a comparable tectum-like neuropil in *Ligia* (see Table 1). In this section, we first compare the architectonics of the lobula and then discuss its accompanying neuropil.

GABA immunohistology reveals several discrete strata in the dipteran lobula (Fig. 7A). An outer stratum, called the T5 layer, contains relatively few GABAergic neurons except for extremely slender retinotopic terminals from the medulla (not shown) and a wide-field tangential cell, the processes of which cover the whole lobula surface (Fig. 7B) and send bouton-like specializations among the dendrites of immunonegative T5 (bushy T-cells) neurons. The organization of these small neurons, which supply information about the direction of motion to the lobula plate from the lobula (Douglass and Strausfeld, 1995), has been described from Golgi impregnations of Diptera and Lepidoptera (Strausfeld, 1976; Fischbach and Dittrich, 1989; Strausfeld and Lee, 1991; Buschbeck and Strausfeld, 1996; Strausfeld and Blest, 1970; Wicklein and Strausfeld, 2000). As shown by Cajal and Sanchez (1915), there is an equivalent level of T5 neurons in the undivided lobulas of Hymenoptera, the axons of which terminate on large tangential neurons deep in the lobula.

Lobula architecture beneath the T5 layer is defined by layered ensembles of columnar neurons that are retinotopically organized, but which coarsen the retinotopic mosaic (Braitenberg, 1970; Strausfeld and Hausen, 1977; Strausfeld and Gilbert, 1992). Although none of these efferent neurons is itself GABAergic, many fibers that ascend amongst their dendrites are. These elements provide alternating layers defined by the density of their GABAergic profiles (Fig. 7A). Large immunoreactive cell bodies reside beneath the lobula. In contrast, the lobula of the crab *Hemigrapsus* is densely immunoreactive, including a superficial stratum (see Fig. 7A, inset) and shows clear evidence of a retinotopic organization that is not coarsened (Fig. 7A, inset). This architectural feature is further considered in the last section of the Results and in the Discussion section.

The lobula plate of *Phaenicia* is richly supplied with immunoreactive processes (Figs. 7B,C, 8A), of which the most prominent (Fig. 7B) are the paired centrifugal horizontal cells (CH neurons; see Hausen and Egelhaaf, 1989) that originate from the posterior–medial protocerebrum and which have been identified previously by using two other anti-GABA sera (Meyer et al., 1986; Strausfeld et al., 1995). The terminal arbors of CH neurons stretch across the whole outer surface of the lobula plate into which they send many thousands of immunopositive swellings that lie at the level of the dendrites of centripetal horizontal motion sensitive neurons (HS cells; see Hausen and Egelhaaf, 1989). Deeper in the lobula plate (Fig. 7C), immunonegative profiles of vertical motion-sensitive neurons (VS; see Hausen and Egelhaaf, 1989) are accompanied by a variety of immunopositive tangential cells, whose origin from the contralateral lobula plate has been identified previously using a different GABA antiserum (Strausfeld et al., 1995). Together, the CH neurons and these deeper tangential elements provide the lobula plate with two GABA-immunoreactive levels (Fig. 8B), one at the level of VS neurons (the inner level) and the other at the level of HS neurons (outer level). A comparable layering in the lobula is seen in *Manduca sexta* (Wicklein and Strausfeld, 2000) as are two levels of immunoreactive processes in its lobula plate (Fig. 8C,D). However, in *Manduca*, the slender but intensely immunoreactive GABAergic tangentials extending across the outer and inner surfaces of the lobula plate appear to be more numerous than those in flies.

The lobula complex of *Ligia* contrasts with those of these two species of insects. GABAergic retinotopic profiles extend distally to the lobula's outer surface (Fig. 8E), a feature that is not seen in *Phaenicia* or *Manduca* but is seen in the decapod *Hemigrapsus* (Fig. 7A, inset). In *Ligia*, the lobula plate lacks wide-field GABAergic tangential processes. Instead, GABAergic specializations are diffusely arranged through its depth (Fig. 8G). Axons within the chiasma leading to the lobula plate appear bundled in *Ligia* (Fig. 8E,F) as opposed to the sheet-like organization of uncrossed axons that connect the medulla and lobula plate in insects (Fig. 8A,C).

Comparison of archaeognathan, branchiopod, and phyllocarid optic lobes

As reported by Harzsch and Walossek (2001), *Triops* (Fig. 9A) has two optic neuropils, which are connected to each other by uncrossed axons (Fig. 10A), as is typical for the Branchiopoda (Elofsson and Dahl, 1970). The second

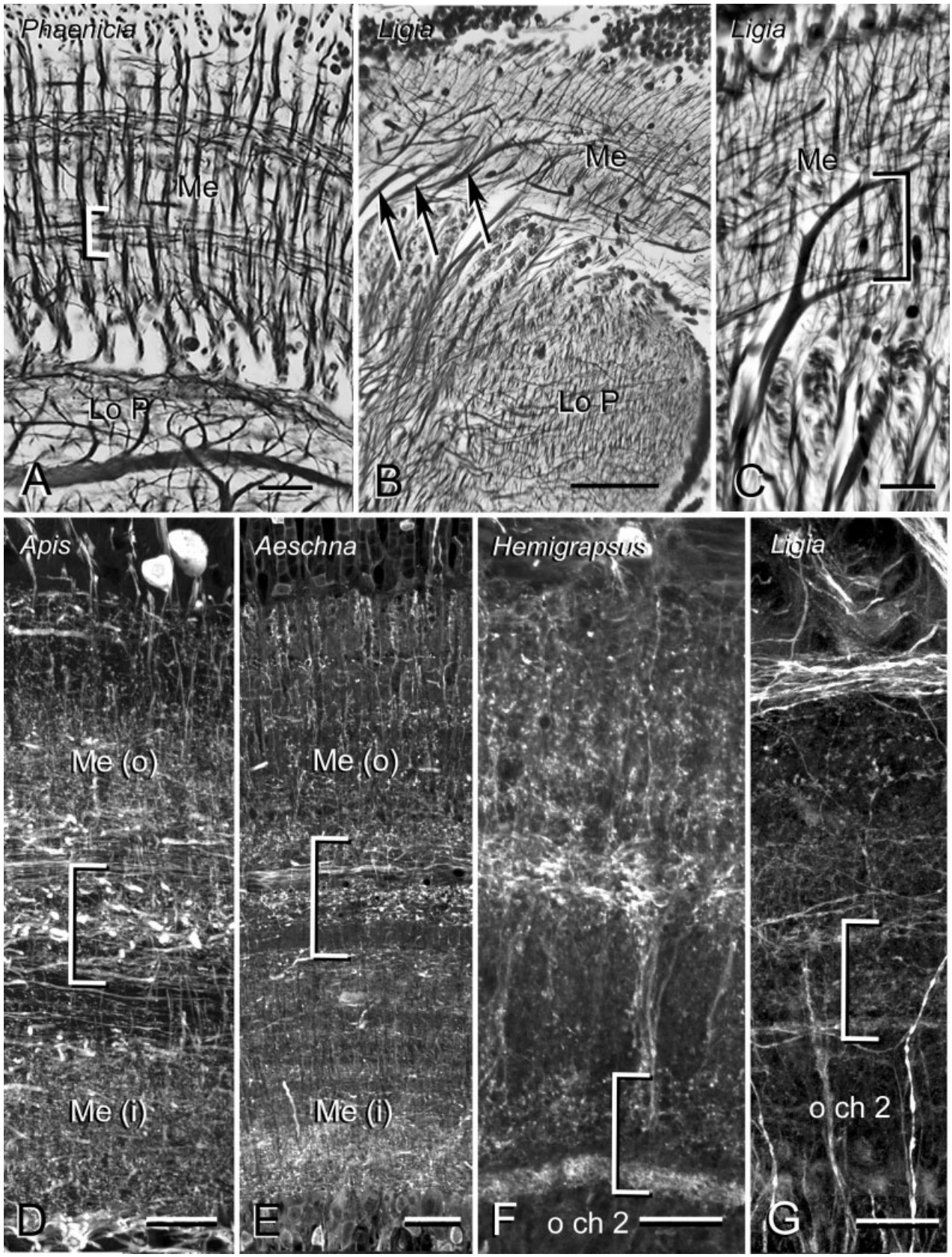

Fig. 6. Medulla organization in insects and eumalacostracans. **A:** In insects, the medulla (Me) is divided into two parts by axons of many different types of tangential neurons making up the Cuccati bundle (bracketed). **B:** In *Ligia*, tangential neurons enter or leave the proximal strata of the medulla, extending their axons over the lobula plate (Lo P) into the midbrain. **C:** Detail of tangential cell axon extending from the proximal strata (bracketed). **D–F:** γ-aminobutyric acid (GABA) immunoreactivity reveals clustered axon bundles of tangential neurons (bracketed in D,E) but not in eumalacostracans (F; the crab *Hemigrapsus*). In *Hemigrapsus* (F) as in the medulla of *Ligia* (**G**), although the proximal strata of the medulla (bracketed) possess tangential processes, most of these are not GABAergic. o ch 2, second optic chiasma; Me (o), outer medulla; Me (i), inner medulla. Scale bars = 25 μm in A,B,D–G; 10 μm in C.

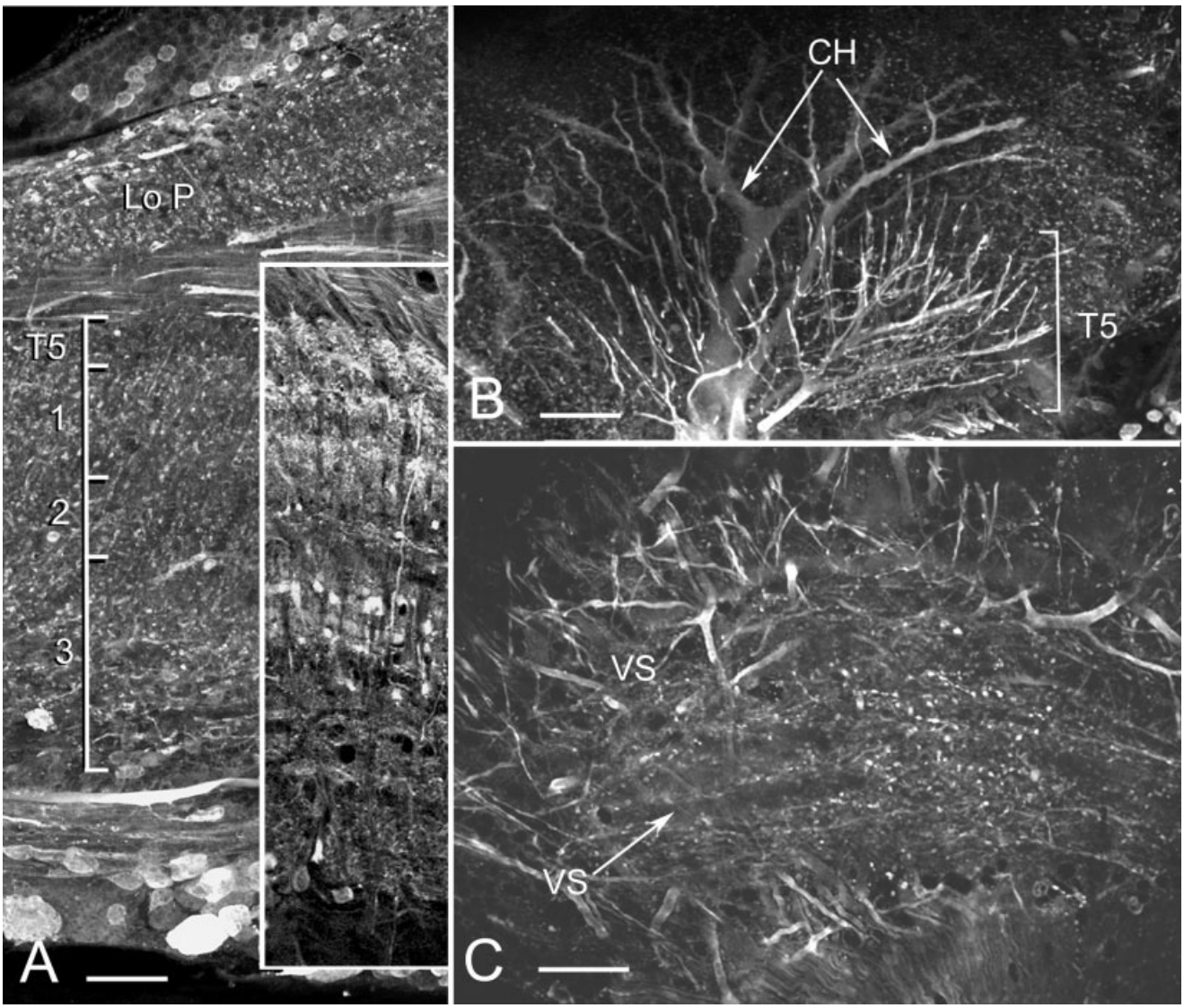

Fig. 7. γ-Aminobutyric acid (GABA) -like immunoreactivity in the lobula and lobula plate of *Phaenicia sericata*. **A:** Horizontal section showing the relative depths of stratification in the lobula, which is divided into four major levels: T5, 1, 2, and 3. The T5 layer receives terminals of small-field retinotopic neurons from the medulla and contains the immunonegative dendrites of T5 bushy cells (not visible here) that terminate in the lobula plate (Lo P). Inset shows comparable depth of the lobula of *Hemigrapsus* showing three dense immunoreactive layers in the outer one third of the lobula, including an outermost stratum, and the retinotopic organization of columns. **B:** Top-down view of the lobula plate of *Phaenicia*, showing the main terminal branches of the two centrifugal motion-sensitive neurons (CH). The many small spot-like profiles at the same level belong to these neurons. The intensely stained processes (bracketed) belong to a GABAergic local neuron in the lobula, extending over the T5 layer. **C:** Bottom up view of the lobula plate, showing immunonegative profiles of vertical motion-sensitive (VS) tangential neurons accompanied by many smaller GABAergic tangential processes. Scale bars = 20 μm in A; 25 μm in B,C.

neuropil is a lateral extension of and is contiguous with the lateral protocerebrum (Fig. 10A). There is no evidence for wide-field tangential cells within this second optic neuropil of *Triops*, but GABA antisera reveal extensive connections from this inner optic neuropil to the outer one. In contrast, in basal archaeognathan insects (Fig. 9B) the optic lobe consists of three discrete neuropils, a lamina, a medulla that is divided into an outer and inner layer, and a columnar protolobula consisting of a lateral extension of the protocerebrum (Fig. 9C,D). In machilids, the lamina is linked by an optic chiasma to the medulla (Fig. 9C). The inner medulla provides crossing axons to the protolobula, which is retinotopically organized and which contains a deep layer of wide-field "giant" tangential neurons (Fig. 10C). Thus, the optic lobes of archaeognathans are essentially like those of pterygote insects, such as hymenopterans, that do not possess a lobula plate, but in which the lobula complex consists of two distinct levels: an outer component that receives retinotopic inputs from the medulla and is itself retinotopically organized, and a deeper component that contains wide-field tangential neurons and is supplied by retinotopic neurons from the outer

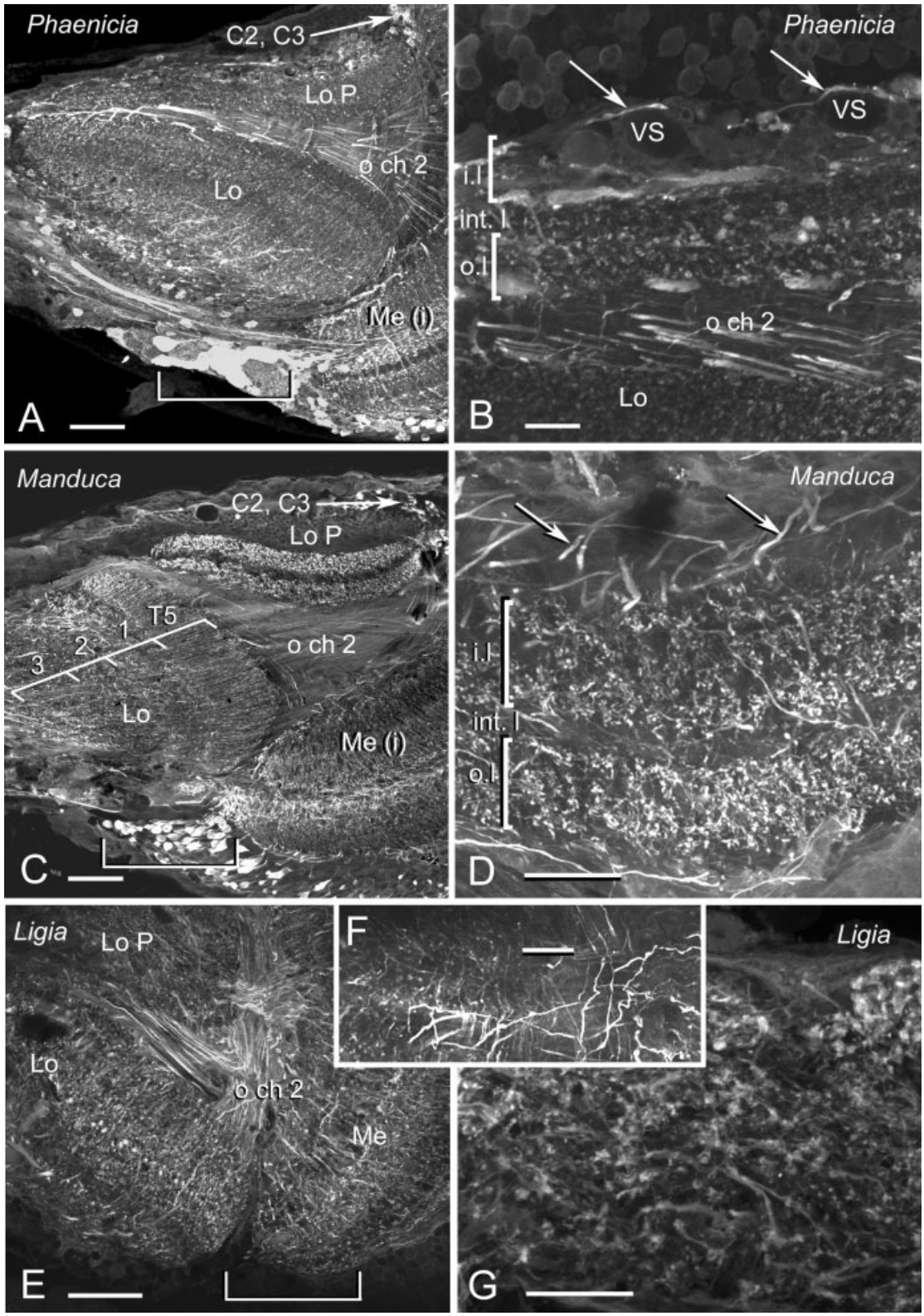

Fig. 8. Lobula (Lo) complexes compared. *Phaenicia sericata* (**A,B**) and the hawk moth *Manduca sexta* (**C,D**) both possess prominent lobula plates and similarly layered lobulas (compare layers T5, 1–3 in C with layering in Fig. 7A). In both insect species, the cell bodies of C2 and C3 centrifugal cells (arrowed in A,C) are clustered between the posterior edge of the inner medulla [Me(i)] and tip of the lobula plate (Lo P). Prominent γ-aminobutyric acid (GABA)ergic cell bodies of tangential neurons (lower bracket in A,C) have axons leaving the Cuccati bundle at the anterior edge of the medulla. B,D: In both insect species, the lobula plate is divided into an inner (i.l) and outer (o.l) level separated by an immunoreactivity-poor intermediate level (int. l). In *Phaenicia*, unstained profiles of vertical motion-sensitive neurons (VS) are shown in B, accompanied by small-diameter GABAergic profiles (arrows). Similar but more numerous elements are seen at the same level in *Manduca* (arrows, D). **E,F:** The lobula complex of *Ligia* differs from that of insects. Its second optic chiasma (o ch 2) is not orderly and contains bundles rather than sheets of uncrossed axons to the Lo P, which enter the plate in a rather haphazard manner (F). **G:** As in insects, the lobula plate of *Ligia* is densely supplied by GABAergic profiles, but these are uniformly distributed rather than organized in two levels. Scale bars = 40 μm in A; 10 μm in B; 60 μm in C; 25 μm in D,F,G; 50 μm in E.

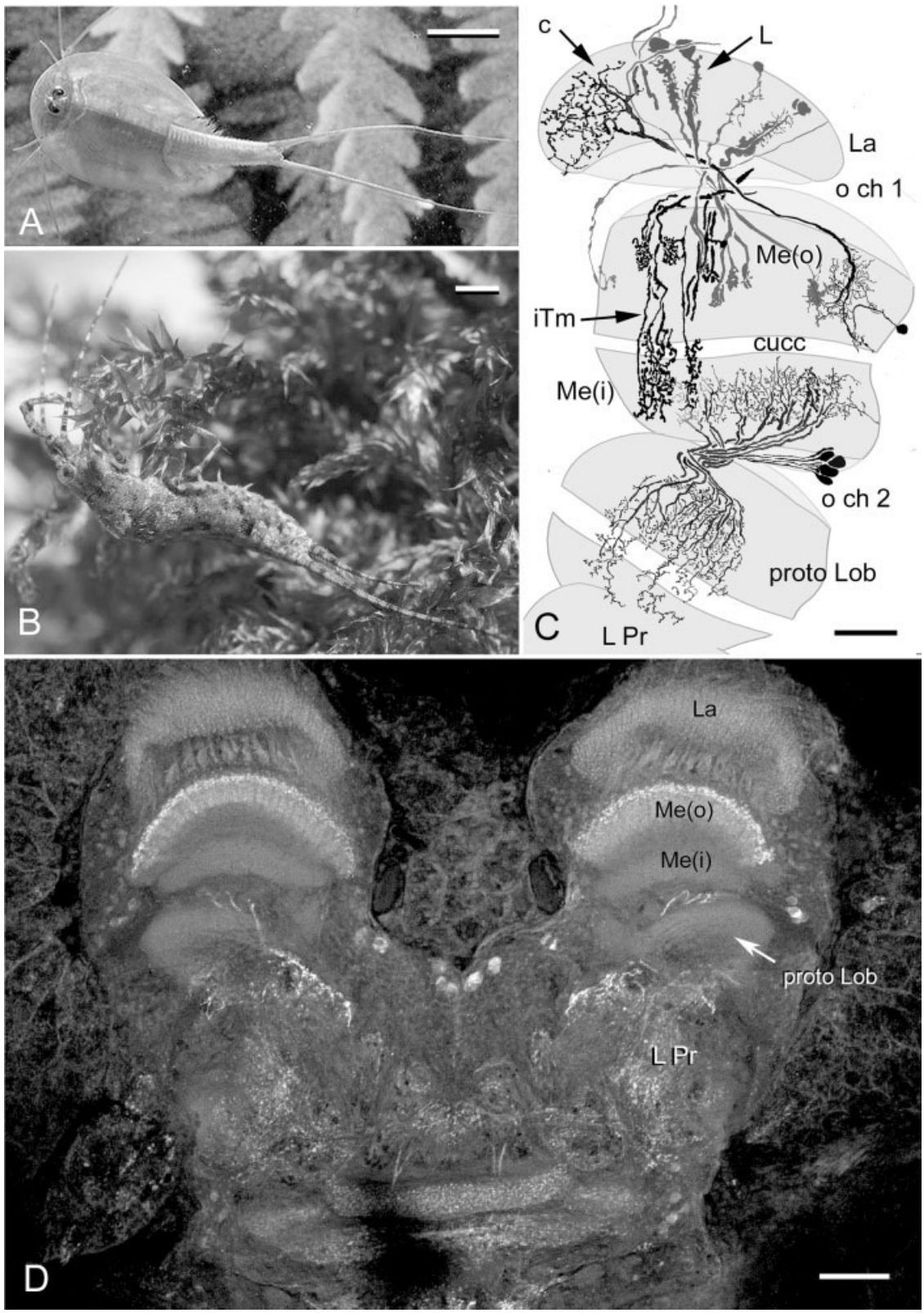

Fig. 9. **A,B:** Basal branchiopod crustacean (*Triops longicaudatus*, A) and archaeognathan (machilid) insect (*Machilis germanicus*, B). **C:** Camera lucida composite from four Golgi preparations of *M. germanicus*, showing retinotopic neurons linking an inner medulla [Me (i)] by means of an optic chiasma (o ch 2) with a lobular extension (proto Lob) of the lateral protocerebrum (L Pr). The deepest endings reach layers of large field tangential cells illustrated in Figure 10C (Tan) from the machilid *Petrobius sp.*. The inner medulla is supplied by retinotopic interneurons (iTm) with dendritic trees in the outer medulla [Me (o)]. A Cuccati bundle (cucc) separates the inner from the outer medulla. The lamina (La) possesses at least three morphological types of monopolar cells (L) that terminate at three levels in the outer medulla. Centrifugal neurons (c) link the outer medulla back to the lamina. **D:** Neuropil staining using anti-tachykinin demonstrates that the optic lobes of the Australian machilid *Allomachilis froggatti* comprise three retinotopic neuropils: a lamina (La), a medulla divided into an outer and inner layer [Me (o) and Me (i)], and a protolobula (proto Lob), which receives retinotopic projections from the medulla and extends from the lateral protocerebrum (L Pr). Scale bars = 500 μm in A,B; 50 μm in C; 150 μm in D.

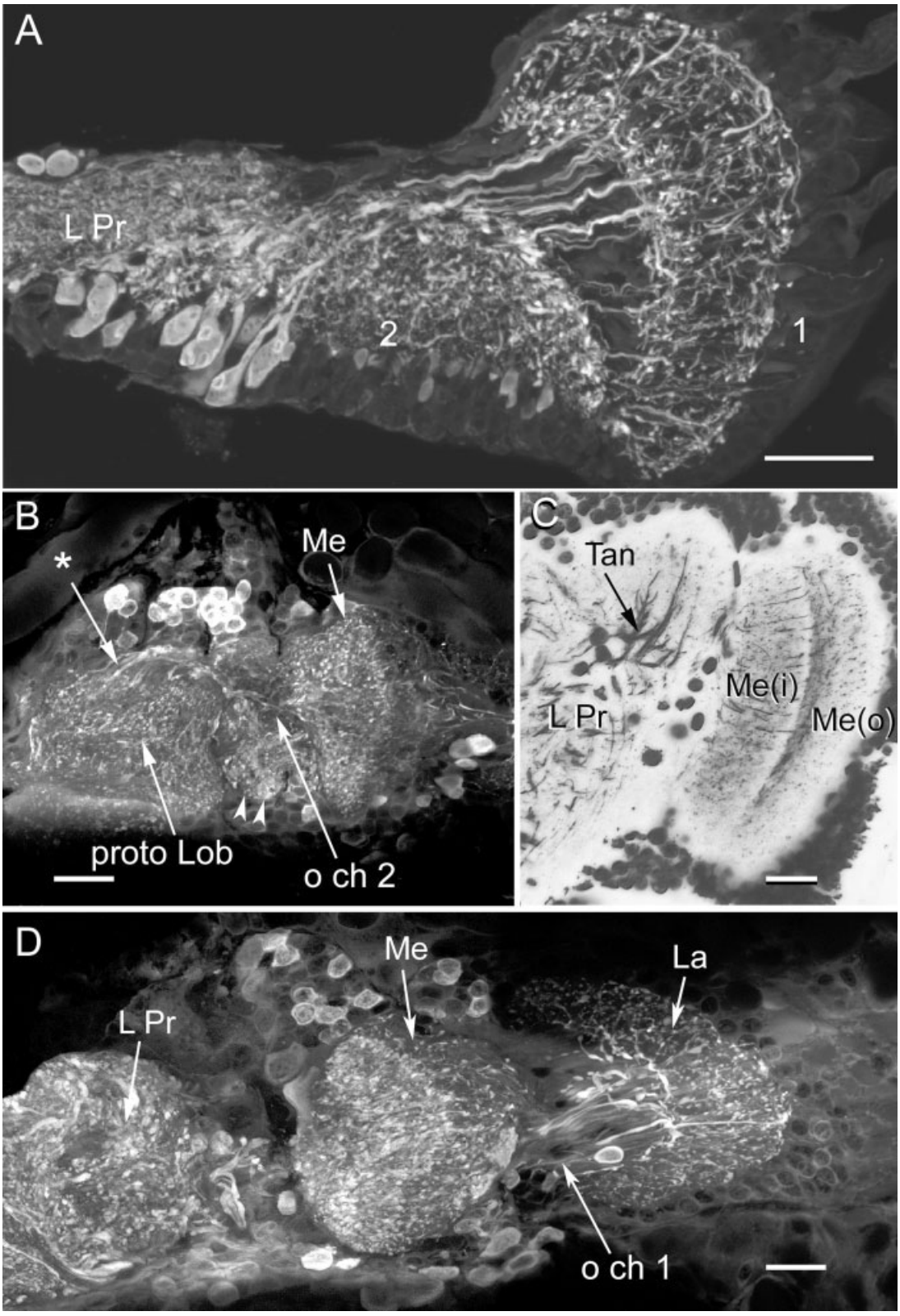

Fig. 10. Basal insect and malacostracan compared. **A:** γ-Aminobutyric acid (GABA) immunohistology of an optic lobe of *Triops* reveals two retinotopic synaptic neuropils (1, 2) connected by uncrossed axons. The second of the two neuropils is an extension of the lateral protocerebrum (L Pr). **B:** GABA immunohistology of the protolobula (proto Lob) of the lateral protocerebrum of the phyllocarid malacostracan *Nebalia pugettensis* supplied by retinotopic projections (asterisk arrow) from the medulla (Me), by means of a chiasma (o ch 2). Arrowheads indicate a small and discrete neuropil supplied by uncrossed fibers from the medulla. The medulla shows no evidence for a division into two parts by a Cuccati bundle. This finding contrasts with the outer and inner layers [Me (o), Me(i)] of the archaeognathan medulla (here *Petrobius sp.*) shown in **C**, stained by reduced silver. The lateral protocerebrum (L Pr) and its protolobula are revealed with a system of wide-field tangential neurons (Tan). **D:** GABA immunohistology of *Nebalia pugettensis*, showing the lateral protocerebrum (L Pr) at the level beneath the protolobula. Axons extend centrifugally from the medulla (Me) by means of the first optic chiasma (o ch 1) out to the lamina (La) where they end in a manner similar to that of the centrifugal neuron observed in *Machilis* (c in Fig. 9C). Scale bars = 25 μm in A–D.

component, as has been described for hymenopterans (Cajal and Sanchez, 1915; Strausfeld, 1976).

In the basal malacostracan Crustacea, here exemplified by the phyllocarid *Nebalia pugettensis* (Fig, 10B,D), GABA immunohistology shows that the lamina is connected to the medulla by means of a system of crossed axons (a first optic chiasma; Fig. 10D). The medulla (Fig. 10B,D), however, shows no evidence of being divided into two layers by a Cuccati bundle. However, as in the Archaeognatha, axons from the medulla form a second optic chiasma. They project into a lateral extension of the protocerebrum, here also termed the protolobula (Fig. 10B), where they confer an approximate retinotopic map of the medulla. There is also a diminutive fourth optic neuropil, which is detached from the protolobula and is supplied by uncrossed axons from the medulla (Fig. 10B, arrowheads). The significance of this satellite neuropil is discussed below.

Retinotopic organization in pterygotes and eumalacostracans

There exist specific differences between insects and crustaceans with respect to the retinotopic organization of neurons in their medullas and lobula complexes.

As described previously (Strausfeld and Nässel, 1980), in malacostracans and pterygotes, the retinal mosaic of visual sampling units is faithfully represented by discrete retinotopic ensembles of neurons in the lamina (the optic cartridges) and medulla (columns). Even in nocturnal insects such as *Sphinx ligustri*, the laminas of which lack clearly defined cartridges (Strausfeld and Blest, 1970), R7 and R8 photoreceptor axons from the retina and monopolar cell axons from the lamina nevertheless project point for point into the medulla, so that there are as many columns in the medulla as there are ommatidia in the retina.

In insects and eumalacostracans, a bundle of axons from each medulla column confers the retinotopic mosaic one-to-one into the lobula, by means of the second optic chiasma. In the lobula, inputs from the medulla end amongst palisades of efferent neurons, the axons of which extend into lateral protocerebrum, where they terminate in islets of neuropil termed optic foci (Strausfeld and Nässel, 1980). In most species, the dendritic trees of neurons in the lobula are arranged as columnar arrays and have bushy or inverted pyramid-like arborizations (Strausfeld and Nässel, 1980). However, the spacing of these lobula neurons can be radically different in insects and eumalacostracans (Strausfeld and Nässel, 1980; Strausfeld, 1998). Reduced-silver preparations of the eumalacostracan optic lobe (Fig. 11A–C) show that the spacing of retinotopic columns across the lobula (Fig. 11B) is the same as that of columns across the medulla (Fig. 11C). This organization is reflected by the dendritic domains of lobula neurons, which Golgi impregnations show are narrow, overlapping the dendrites of the immediately neighboring neurons (Strausfeld and Nässel, 1980). This one-to-one relationship between the retinotopic mosaic of the medulla and that of neurons originating in the lobula, however, is not characteristic of insects. In insects, such as the fly *Musca domestica* (Fig. 11D), incoming bundles to the lobula are retinotopically organized (Braitenberg, 1970), but the dendritic fields of neurons in the lobula are widely spaced, each dendritic tree representing between six (for example, in odonates and cyclorrhaphan Diptera) or multiples of six retinotopic columns (Braitenberg, 1970; Strausfeld and Hausen, 1977). Such differences in architecture can be seen by comparing the lobulas in Figure 11A and D.

DISCUSSION

GABA immunoreactivity

To claim that similar organizations occur in divergent taxa requires a method or methods that reliably select for certain types of neurons. This requirement was the singular advantage of the vital dye methylene blue, which in the early 1920s, was used to reveal comparable thoracic interneurons in different insects (Zawarzin, 1924). In this account, we have relied mainly on antibodies against GABA, with supporting reduced-silver stains, to reveal comparable architectures across taxa.

Sera that recognize GABA have been used to study the distribution of this transmitter in a variety of species and systems. Such studies include observations of the optic lobes of flies (Meyer et al., 1986; Strausfeld et al., 1995), the brains of honey bees (Shäfer and Bicker, 1986, Meyer et al., 1986), the optic lobes of cockroaches (Fuller et al., 1989), and the brain of the moth *Manduca sexta* (Homberg et al., 1987). Of significance is that, in those cases where different anti-GABA sera were used on the same system, the sera recognized the same species of neurons (Meyer et al., 1986; Strausfeld et al., 1995; Shäfer and Bicker, 1986). The present study likewise reveals the same neurons that were resolved by different GABA antisera (Meyer et al., 1986; Strausfeld et al., 1995). For example, GABAergic profiles previously identified in the lobula and lobula plate of *Calliphora* and *Sarcophaga* (Meyer et al., 1986: Buchner et al., 1988; Strausfeld et al., 1995) do not differ from those described here from *Phaenicia*. The present identification of stratified GABAergic centrifugal endings in the honey bee lamina confirms observations of the same GABAergic system, using a different antibody against GABA, by Schäfer and Bicker (1986). GABAergic profiles in *Manduca sexta* revealed by the present antibody show the same types of GABA-immunoreactive olfactory lobe neurons described previously by Homberg et al. (1987), also using a different anti-GABA serum. However, as also reported by Meyer et al. (1986), we were unable to identify any GABA-like immunoreactivity in photoreceptor axons (Datum et al., 1986), possibly because those authors used hot paraffin embedding, which can result in false positives. Used with appropriate embedding methods, antibodies against GABA are reliable: they do not reveal centrifugal cells to the lamina in some taxa and centripetal cells from the lamina in others. Rather, across taxa, GABA immunostaining consistently reveals comparable classes of neurons.

Differences between eumalacostracan and pterygote optic lobes

A hiatus of almost 75 years separates the early use of optic lobe anatomy for inferring arthropod phylogeny (Holmgren, 1916; Hanström, 1926a) from recent molecular and developmental studies that support the insects as close relatives of the crustaceans (Averof and Akam, 1995; Friedrich and Tautz, 1995; Giribet et al., 2001) as do modern neuroanatomic studies (Strausfeld, 1998; Loesel et al., 2002). Yet, still undetermined is to which crustacean lineage or lineages insects are sisters of. The selec-

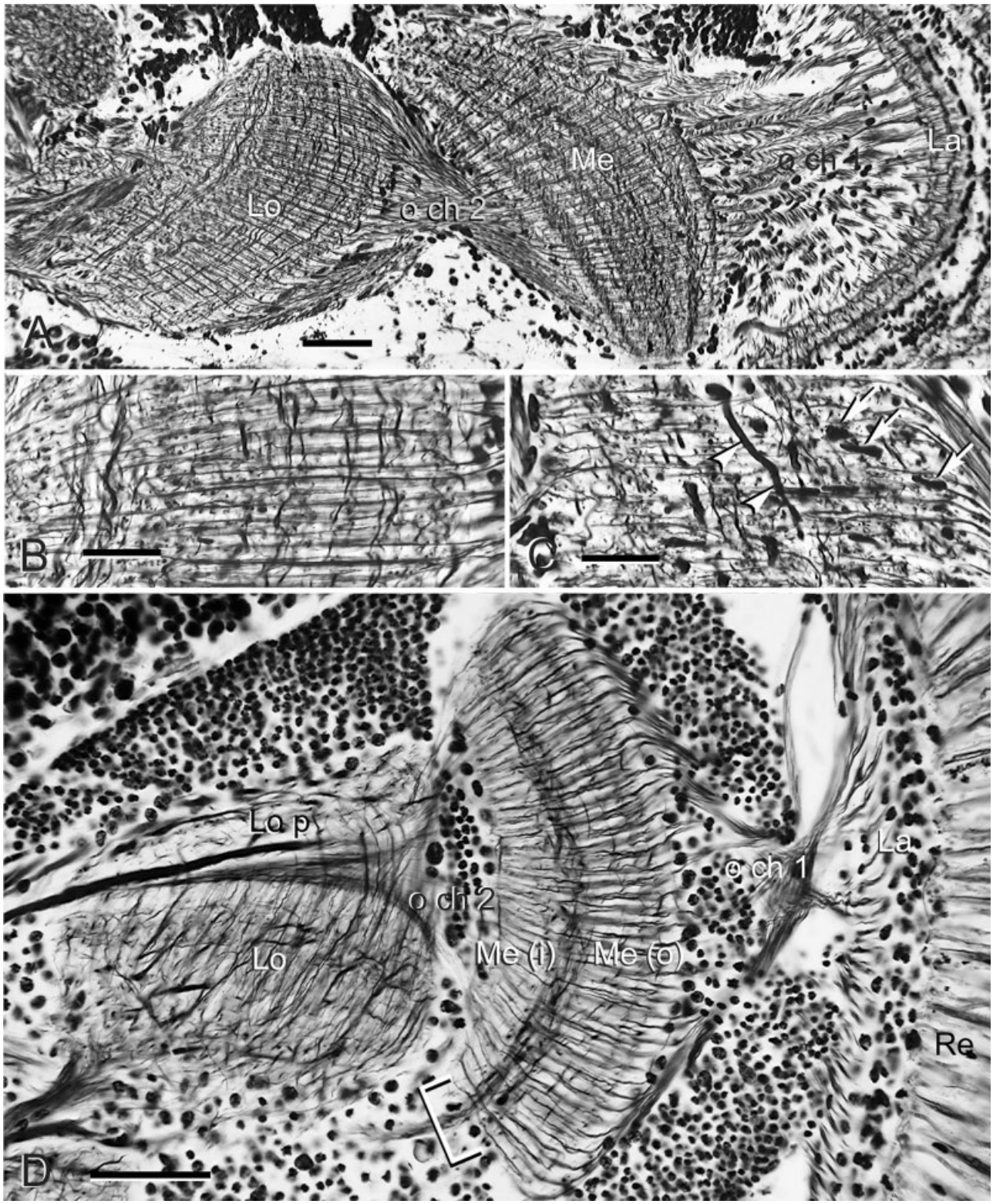

Fig. 11. Reduced-silver comparisons of medulla and lobula retinotopy in the optic lobe neuropils of the spiny lebbeid shrimp *Lebbeus groenlandicus* (A–C) and the house fly *Musca domestica* (D). **A–C:** The characteristically multistratified medulla (Me) and densely columnar lobula (Lo) of decapod crustaceans. The horizontal axis of the lobula is twisted relative to that of the medulla so that the planes of crossover by axons in the first and second optic chiasmata appear to be (but are not) at right angles to each other. B,C: Comparison of retinotopic packing in the lobula (B) and medulla (C). Note the many glial cell bodies in the medulla (three arrows), in addition to blood vessels (two arrowheads). Both of these non-neural elements are lacking from the lobula. **D:** Optic lobe neuropils of *Musca domestica*. Columnar organization in the lobula (Lo) appears coarsened, in comparison with that of the medulla. The prominent Cuccati bundle, which extends into the posterior optic tract (origin bracketed), divides the insect medulla into an outer and inner component [Me (o), Me (i)]. This section shows the lobula plate (Lo P) with one of its large horizontal tangential neurons. The lack of glial cell bodies within the medulla is typical of insect but not decapod optic lobes. o ch 1, 2, first and second optic chiasmata; Re, retina. Scale bars = 50 μm in A; 20 μm in B,C; 50 μm in D.

tion of restricted character sets, such as elements of the compound eyes, has been used to provide morphological support for a crustacean–insect relationship (Paulus, 2000) and Dohle (2001) has suggested that even a single shared character, that of four cone cells in an ommatidium, allows insects and crustaceans to be considered as monophyletic. However, this feature is not ubiquitous (see Meinertzhagen, 1991), and as cautioned by Richter (2002), it is still debated whether crustaceans themselves are mono- or paraphyletic. This debate raises the further question of whether there are characters shared by a subgroup of crustaceans (e.g., Malacostraca) and insects.

Recent discussion about insect–crustacean relationships has once again turned to neuroanatomical data. Osorio (1991) proposed that, because the optic lobes of insects and malacostracans are similarly constructed and have such similar cell types, they are unlikely to have evolved independently and convergently, a view subsequently modified to focus on lamina-medulla organization and cell types (Osorio and Bacon, 1994; Nilsson and Osorio, 1997). Harzsch (2002) argued that three nested optic neuropils connected by two successive chiasmata are so similar in morphology and cell type that these alone are grounds for concluding that the lamina, medulla, and lobula are synapomorphic characters uniting malacostracan crustaceans and insects. Anatomical support mustered for such discussion referred to descriptions (Strausfeld and Nässel, 1980) that showed obvious similarities in the cellular organization in the lamina of malacostracans and insects but fewer similarities between their deeper regions. A comparative study (Strausfeld, 1998) has been similarly recruited to support insect–malacostracan relationships on the basis of optic lobe similarities (Harzsch, 2002), although in Harzsch and Walossek (2001) this 1998 account was cited because of the differences described between the malacostracan and insect optic lobes. Harzsch (2002) underpins his view of a malacostracan–insect sister relationship with developmental studies (Wildt and Harzsch, 2002) that show the lobulas in insects and malacostracans derive from a protocerebral cluster of proliferative cells.

Meinertzhagen's thoughtful 1991 review of visual system evolution cautioned that the representation of the receptor layer of the compound eye in successive neuropils involves developmental constraints. Thus, independently of phylogenetic factors, the imposition of retinotopy must itself influence the organization and shapes of neurons. Such considerations are, unfortunately, less emphasized in much of the subsequent debate about optic lobe homologies, even though for another sensory system it has been shown that modality-specific receptor organization, in which odortypic receptor terminals cluster together, imposes such constraints (reviewed in Strausfeld and Hildebrand, 1999). Across disparate Phyla, first-order olfactory neuropils can share analogous glomerular organization and neuronal circuitry (reviewed in Hildebrand and Shepherd, 1997) that follows a basic blueprint also typical of circuitry at the level of photoreceptors (Strausfeld and Campos-Ortega, 1977; Strausfeld, 1989).

However, retinotopy may not be the single constraining factor leading to comparable neuron shapes. Variations in the shape and branching domains of neurons in the lamina of different insects might be adaptations that allow computation by the lamina to be independent of ecology and light-gathering properties of the retina. Such adaptations may be manifested by the shapes and extents of GABAergic centrifugal neurons. In *Ligia* these are within the range of morphologies demonstrated in insects. Even the distinctive planar arrangement of GABAergic centrifugal neurons in the laminas of shrimps is comparable to planar arrangements in certain insects, such as the honey bee. Such similarities between GABAergic centrifugal neurons suggest that the same *range* of morphologies has been conserved across malacostracan crustaceans and insects. However, the exceptionally narrow-field C2 and C3 endings found so far only in cyclorrhaphan flies are here interpreted as derived features that have evolved in tandem with the acquisition of a neural superposition retina and, concomitantly, the structural isolation of retina to lamina projections to glia-ensheathed optic cartridges (Shaw, 1989, 1990; Shaw and Moore, 1989).

The present study also demonstrates similarities in the stratification and morphology of local centrifugal neurons in the outer medulla of insects and through the depth of the medulla of *Ligia*. However, we have been unable to demonstrate structural equivalence between the inner medulla of insects, lying under the Cuccati bundle, and levels in the medulla of *Ligia* or other malacostracans. Although the malacostracan medulla provides and receives wide-field tangential neurons to and from the protocerebrum, the axons of these neurons do not obviously bundle into a discrete stratum but exit in a distributed manner from the anterior and dorsal rim of the medulla. Furthermore, few if any such tangentials are GABAergic, whereas in insects many tangential cells that contribute to the Cuccati bundle are. The existence of a discrete inner medulla in insects, but its apparent absence in malacostracans, is here considered to be an important distinction between these two groups of arthropods.

This difference, if confirmed by developmental studies, would be significant: whereas it supports the origin of the insect medulla from two Anlagen, it would speak against a dual origin of the malacostracan medulla. In insects, the lamina and outer medulla are known to originate from neuroblasts in the outermost of two Anlagen, whereas the inner medulla (that part beneath the Cuccati bundle) derives from neuroblasts of the inner Anlage, which also gives rise to the lobula complex (Meinertzhagen, 1973; Meinertzhagen and Hanson, 1993; Hofbauer and Campos-Ortega, 1990). Studies of the *sine oculis* mutant of *Drosophila*, which partially or wholly lacks compound eyes, demonstrate that, even if much of the lamina and outer medulla are lacking, neuropils representing the inner medulla and lobula plate (and lobula) are still present and stratification in the lobula complex is independent of compound eye development (Fischbach, 1982). Laser ablation studies of the developing optic lobes of the fly *Musca domestica* have also shown interdependence between the inner medulla and lobula plate (Nässel and Geiger, 1983). In contrast, studies on malacostracan optic lobe development are ambivalent with regard to what contribution the inner Anlage makes to the developing medulla (Wildt and Harzsch, 2002). If such a contribution does exists, then it does not obviously provide the same layered divisions as that seen even in the Machilidea.

The observation that stratification appears to be independent of compound eye development (Fischbach, 1982) provides a baseline for comparison between taxa that is independent of retinotopic constraints. It is, therefore, significant that the widths, levels, and morphologies of

GABAergic strata reflect differences of organization in the eumalacostracan and insect lobula complexes. GABAergic strata in the lobula complex of *Ligia* and *Hemigrapsus* contrast with those of *Phaenicia*, whereas those of *Phaenicia* and *Manduca* are similar. A further disparity between pterygotes and eumalacostracans refers to projections into the lobula plate. In pterygotes, uncrossed sheets of axons map each medullary column into the lobula plate so that it faces the representation of the same column in the lobula. Consequently, in insects, the class of Y-cells from the medulla (Strausfeld and Blest, 1970) that provide bifurcating axons do so to optically equivalent loci lying opposite each other in the lobula and lobula plate. In *Ligia*, axons extending from the medulla into the lobula plate are bundled, and uncrossed, and then spread out into retinotopic loci. To date, we have not identified Y-cells in *Ligia*, where the medulla appears to send undivided axons separately to the two deeper neuropils.

Synapomorphies of malacostracans and insects

The evidence thus far allows only two neuropils to be confidently considered as homologous. We propose that the lamina and the outer medulla of insects are homologous to the lamina and the whole depth of the medulla of malacostracans. Even in the Machilidea, which are claimed to be modern representatives of the earliest terrestrial insect known from the fossil record (Labandeira et al., 1988; but of interest is the enigmatic taxon described by Haas et al., 2003), the medulla is clearly divided into two distinct layers by a bundle of tangential axons. This finding contrasts with the medulla of the phyllocarid *Nebalia*.

However, in *Nebalia* the medulla sends crossed axons into a clearly defined dorsolateral protrusion from the lateral protocerebrum, which Hanström (1926b) even called the "medulla interna." This structure is obviously retinotopic. With respect to its location and chiasmal supply, the protolobula of *Nebalia* is comparable to a machilid-like third optic neuropil. However, in machilids, the layered and retinotopic protolobula is hallmarked by a deep layer containing dendrites of giant tangential neurons. This arrangement is comparable to that seen in those pterygote groups in which the lobula complex lacks a discrete lobula plate but has a deep layer of tangential neurons. An example of such an arrangement is in Hymenoptera and certain Odonata (Strausfeld, 1976), although in some odonates there is a clear separation of this deeper component into a separate neuropil supplied by uncrossed axons from the lobula (Strausfeld, unpublished). In the honey bee lobula, the tangential neurons of this deep layer receive the terminals of T5 cells, the dendrites of which reside in the lobula's distal-most stratum (Cajal and Sanchez, 1915). In Diptera (and other insects possessing a lobula plate), T5 neurons extend their axons from the eponymous layer of the lobula across to giant motion-sensitive tangential neurons located in the lobula plate (Strausfeld and Lee, 1991). We have not seen any similar system of tangentials in the *Nebalia* protolobula, although it is possible that their absence is a secondarily derived feature.

The question next arises whether the small satellite optic neuropil in *Nebalia* (Fig. 10B), which is separate from the lateral protocerebrum and which receives uncrossed projections from the medulla, represents an ancestral fourth optic neuropil, which in *Ligia* provides the lobula plate. This possibility finds support from observations of other eumalacostracans, in which satellite neuropils have now been identified, as in *Palaemonetes pugio* (Fig. 12A,B). As in *Ligia* (Fig. 12C,D), this retinotopic fourth optic neuropil receives uncrossed axons from the medulla.

Similarity with the lobula plate of insects, however, is problematic, because if the pterygote lobula plate is suggested to be homologous to the fourth optic neuropil of crustaceans, then why is there no satellite neuropil observed in the most primitive apterygotes? Although contiguous with the lateral protocerebrum, the archaeognathan lobula is constructed like the hymenopteran lobula with its wide-field tangential cells contiguous with the lobula rather than in a separate fourth neuropil, as in the Diptera. Does the presence in only some pterygote orders of a fourth neuropil receiving uncrossed axons suggest that this neuropil arose independently, possibly from a deep layer of the lobula? Or, conversely, is the deep level of the archaeognathan-type lobula secondarily derived from a fusion of a separate fourth neuropil to the base of the third?

Thus, if it is proposed that the lobula unites malacostracan crustaceans and insects (Harzsch, 2002), it is still necessary to recognize these major differences in lobula complex organization and to determine the origin of the lobula plate in insects. A schematic suggesting possible relationships that allow comparisons between the insect lobula plate and malacostracan satellite neuropils is shown in Figure 12E.

One further anatomical distinction between pterygotes and eumalacostracans needs to be considered, however. Namely, in the eumalacostracan crustaceans, the retinotopic spacing of the lamina and medulla is preserved in the lobula (Fig. 11A–C), whereas the mosaic is coarsened in the lobulas of pterygote insects (Fig. 11D). First documented for cyclorrhaphan Diptera (Braitenberg, 1970; Strausfeld and Hausen, 1977; Strausfeld and Nässel, 1980), this coarsening appears to be ubiquitous as it has now been observed in species belonging to the Hymenoptera, Lepidoptera, Odonata, and Hemiptera. Possibly, this difference of architecture between insects and eumalacostracans explains why GABAergic organization appears so different in the lobula of *Phaenicia* compared with that of *Ligia*, but so similar between the lobulas of *Phaenicia* and *Manduca*.

Are such differences a derived feature of pterygote insects or might they speak for independent origins of the lobula complex? Differences in the arrangements, shapes, and layers of neurons in the lobulas of malacostracans and insects (see Strausfeld and Nässel, 1980; Strausfeld, 1998) could still indicate independent origins of these deep optic lobe neuropils. And although *similarities* in neuronal shapes have been recruited for arguing a malacostracan+insect clade (Harzsch, 2002), such similarities may be, as Meinertzhagen (1991) has cautioned, due to developmental constraints rather than to common origins.

The suggestion that insects may be paraphyletic (Nardi et al., 2003) demonstrates that insect–crustacean phylogenetic relationships are by no means resolved (see Richter, 2002), even though several cladistic analyses by using molecular data sets propose a sister-group relationship between malacostracan crustaceans and insects (Wilson et al., 2000; Hwang et al., 2001). This relationship awaits

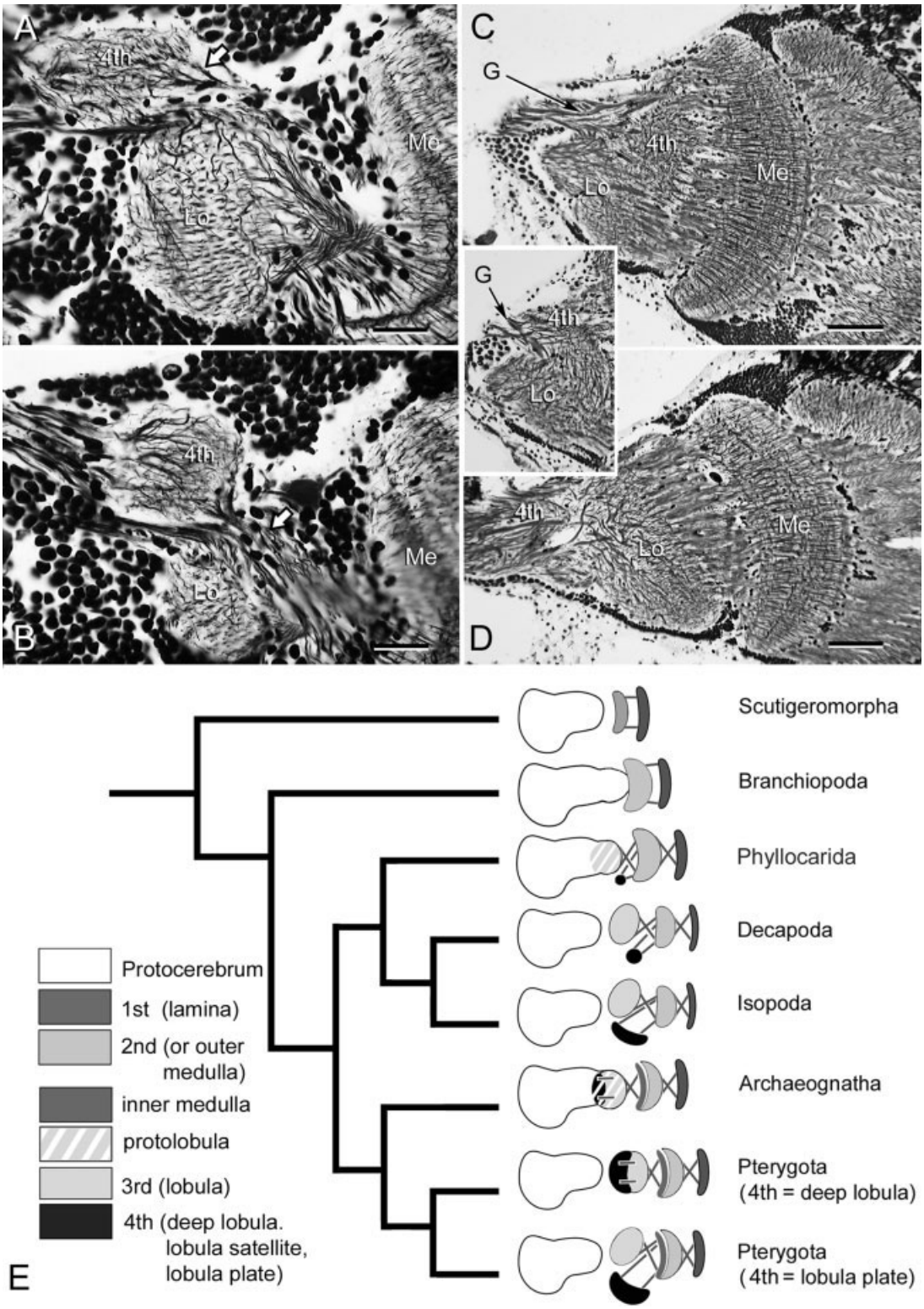

Fig. 12. A–D: Comparisons of fourth optic neuropils in decapod and peracarid eumalacostracans. **A,B:** *Palaeomonetes pugio*. Consecutive sections showing the fourth optic neuropil (4th) supplied by bundles of uncrossed axons (arrowed) from the medulla (Me). The lobula (Lo) receives crossed axons from the medulla. **C,D:** *Ligia occidentalis*. The fourth optic neuropil (lobula plate) is similarly disposed above and behind the lobula. In *Ligia*, this neuropil contains giant tangential neurons (G). Inset shows continuation of giant axon in next section. **E:** Proposed relationships of crustaceans and insects with reference to optic lobe organization. A hypothetical ancestor is proposed to have given rise to scutigeromorph-type optic lobes—the second neuropil of which is detached from the protocerebrum (unshaded outline) and is connected to the first optic neuropil by uncrossed fibers (Strausfeld, unpublished observations)—and crustacean–insect type optic lobes, which in the branchiopods comprise two neuropils, the second contiguous with the protocerebrum. Malacostracans (here, phyllocarids, decapods, isopods) and insects (archaeognathans and pterygotes) have at least three retinotopic optic neuropils. A fourth neuropil is seen in phyllocarids, certain pterygote orders, and eumalacostracans. A structurally equivalent neuropil (see text) is attached beneath the lobula of archaeognathans and certain pterygote orders (e.g., Hymenoptera). Pterygotes with distinct lobula plates receiving uncrossed axons from the medulla are exemplified by Diptera, Coleoptera, and Lepidoptera. Insects are united by possessing a divided medulla. Insects and malacostracans are united by two chiasmata and by the presence of a protolobula. Scale bars = 25 μm in A,B; 50 μm in C,D.

definitive corroboration by morphological and anatomical characters. Here, we suggest that an optic lobe comprising two nested visual neuropils and a protolobula, which is still fused to the lateral protocerebrum, and involving successive optic chiasmata, is characteristic for the ground plans of malacostracans and insects. Thus, the occurrence of a second chiasma and a protolobula is suggested to be synapomorphic for a malacostracan/insect clade, because this conjunction of structures is unknown from any other arthropod group. What is still unresolved is whether fourth optic neuropils (satellite neuropil, lobula plates) and lobulas possessing two distinctive architectures have evolved independently within malacostracans (Eumalacostraca) and insects (Pterygota).

ACKNOWLEDGMENTS

We thank Patricia Jansma, M.S., for help and advice regarding confocal microscopy. Charles Hedgcock, R.B.P., took the photographs for Figures 1A,D and 8A and Pavel Masek (University of Würzburg, Germany) contributed the photograph for Figure 9B. Michael Zimmerman, B.S., provided technical assistance. We also thank Prof. D.R. Nässel (University of Stockholm, Sweden) for the generous gift of anti-tachykinin antibodies. Prof. John S. Edwards (University of Washington, Seattle) kindly supplied living specimens of *Petrobius*. The Deutsche Forschungsgemeinschaft supported the visit of G. Scholtz to the Friday Harbor Marine Laboratory (Scho 442/5-2). A visiting fellowship to N.J Strausfeld, provided by the Research School of Biological Sciences, Australian National University, was instrumental in obtaining *Allomachilis*, which would not have been possible without the exertions of Dr. Jochen Zeil (RSBS, Australian National University) and the hospitality of Drs. A.D. Blest and Sally Stowe (RSBS, ANU). The latter kindly organized facilities and materials for fixing material for immunohistology. A significant part of studies of crustaceans was done at the University of Washington's Friday Harbor Laboratories. We thank the Director and staff for their support and assistance and especially to David Duggins for his navigational and trawling skills. As always, we thank Dr. Camilla Strausfeld for commenting on and meticulously editing the article.

LITERATURE CITED

Averof M, Akam M. 1995. Insect-crustacean relationships: insights from comparative developmental and molecular studies. Philos Trans R Soc Lond B 347:293–303.

Bodian D. 1937. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat Rec 69:153–162.

Boore JL, Lavrov DV, Brown WM. 1998. Gene translocation links insects and crustaceans. Nature 392:667–668.

Braitenberg V. 1970. Ordnung und Orientierung der Elemente im Sehsystem der Fliege. Kybernetik 7:235–242.

Brusca RC, Wilson GDF. 1991. A phylogenetic analysis of the Isopoda with some classificatory recommendations. Mem Queensland Mus 31:143–204.

Buchner E, Bader R, Buchner S, Cox J, Emson PC, Flory E, Heizmann CW, Hemm S, Hofbauer A, Oertel WH. 1988. Cell-specific immunoprobes for the brain of normal and mutant *Drosophila melanogaster*. I. Wild-type visual system. Cell Tissue Res 253:357–370.

Buschbeck EK, Strausfeld NJ. 1996. Visual motion-detection circuits in flies: small-field retinotopic elements responding to motion are evolutionarily conserved across taxa. J Neurosci 16:4563–4578.

Buschbeck EK, Strausfeld NJ. 1997. The relevance of neural architecture to visual performance: phylogenetic conservation and variation in dipteran visual systems. J Comp Neurol 383:59–75.

Cajal SR, De Castro F. 1933. Elementos de Técnica micrografica del sistemo nervioso. Madrid: Tipografican Artistica Alameda.

Cajal SR, Sánchez SD. 1915. Contribución al conocimiento de los centros nerviosos de los insectos Parte I Retina y centros opticos. Trab Lab Invest Biol Univ Madrid 13:1–168.

Datum KH, Weiler R, Zettler F. 1986. Immunocytochemical demonstration of γ-amino butyric acid and glutamic acid decarboxylase in R7 photoreceptors and C2 centrifugal fibres in the blowfly visual system. J Comp Physiol A 159:241–249.

DeVoe RD, Kaiser W, Ohm J, Stone LS. 1982. Horizontal movement detectors of honeybees: directionally-selective visual neurons in the lobula and brain. J Comp Physiol A 147:155–170.

Dohle W. 2001. Are the insects terrestrial crustaceans? A discussion of some new facts and arguments and the proposal of the proper name 'Tetraconata' for the monophyletic unit Crustacea+Hexapoda. In: Deuve T, editor. Origin of the hexapoda. Ann Soc Entomol Fr 37:85–103.

Douglass JK, Strausfeld NJ. 1995. Visual motion detection circuits in flies: peripheral motion computation by identified small-field retinotopic neurons. J Neurosci 15:5596–5611.

Douglass JK, Strausfeld NJ. 2003a. Retinotopic pathways providing motion-selective information to the lobula from peripheral elementary motion detecting circuits. J Comp Neurol 457:326–344.

Douglass JK, Strausfeld NJ. 2003b. Retinotopic motion-sensitive pathways in dipteran optic lobes. Microsc Res Tech 62:132–150.

Elofsson R, Dahl E. 1970. The optic neuropils and chiasmata of Crustacea. Z Zellforsch Mikrosk Anat 107:343–360.

Fischbach KF. 1982. Neural cell types surviving congenital sensory deprivation in the optic lobes of *Drosophila melanogaster*. Dev Biol 95:1–18.

Fischbach K-F, Dittrich APM. 1989. The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure. Cell Tissue Res 258:441–475.

Franceschini N. 1975. Sampling of the visual environment by the compound eye of the fly: fundamentals and applications. In: Snyder AW, Menzel R, editors. Photoreceptor optics. Berlin, Heidelberg, New York: Springer. p 98–125.

Friedrich N, Tautz D. 1995. Ribosomal DNA phylogeny of the major extant arthropod classes and the evolution of the myriapods. Nature 376:165–167.

Fuller H, Eckert M, Blechschmidt K. 1989. Distribution of GABA-like immunoreactive neurons in the optic lobes of *Periplaneta americana*. Cell Tissue Res 255:225-233.

Giribet G, Edgecombe GE, Wheeler WC. 2001. Arthropod phylogeny based on eight molecular loci and morphology. Nature 413:157–161.

Haas F, Waloszek D, Hartenberger R. 2003. *Devonohexapodus bocksbergensis*, a new marine hexapod from the lower Devonian Hunsrück Slates, and the origin of Atelocerata and Hexapoda. Org Divers Evol 3:39–54.

Hanström B. 1926a. Untersuchungen über die relative Grösse der Gehirnzentren verschiedener Arthropoden unter Berücksichtigung der Lebensweise. Z Mikrosk Anat Zellforsch 7:135–190.

Hanström B. 1926b. Vergleichende Anatomie des Nervensystems der wirbellosen Tiere. 1968 reprint. Amsterdam: A. Asher.

Hariyama T, Meyer-Rochow VB, Kawauchi T, Takaku Y, Tsukahara Y. 2001. Diurnal change in retinula cell sensitivities and receptive fields. Two-dimensional angular sensitivity functions in the apposition eyes of *Ligia exotica*. Crustacea Isopoda. J Exp Biol 204:239–248.

Harzsch S. 2002. The phylogenetic significance of crustacean optic neuropils and chiasmata: a re-examination. J Comp Neurol. 453:10–21.

Harzsch S, Walossek D. 2001. Neurogenesis in the developing visual system of the branchiopod crustacean *Triops longicaudatus* (LeConte, 1846): corresponding patterns of compound-eye formation in Crustacea and Insecta? Dev Genes Evol. 211:37–43.

Hausen K, Egelhaaf M. 1989. Neural mechanisms of visual course control in insects. In: Stavenga GG, Hardie RC, editors. Facets of vision. Heidelberg, New York: Springer. p 391–424.

Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20:595–631.

Hofbauer A, Campos-Ortega JA. 1990. Proliferation pattern and early differentiation of the optic lobes in *Drosophila melanogaster*. Rouxs Arch Dev Biol 198:264–274.

Holmgren N. 1916. Zur vergleichenden Anatomie des Gehirns von Polychaeten, Onychophoren, Xiphosuren, Arachniden, Crustaceen, Myriapoden und Insekten. K Svenska Vetensk Akad Handl 56:1–303.

Homberg U, Kingan TG, Hildebrand JG. 1987. Immunocytochemistry of GABA in the brain and suboesophageal ganglion of *Manduca sexta*. Cell Tissue Res 248:1–24.

Hwang UW, Friedrich M, Tautz D, Park CJ, Kim W. 2001. Mitochondrial protein phylogeny joins myriapods with chelicerates. Nature 413:154–157.

Kirschfeld K. 1967. Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*. Exp Brain Res 3:248–270.

Labandeira CC, Beal BS, Hueber FM. 1988. Early insect diversification: evidence from a Lower Devonian bristletail from Quebec. Science 242: 913–916.

Land MF, Nilsson D-E. 2002. Animal eyes. Oxford: Oxford University Press.

Loesel R, Nässel DR, Strausfeld NJ. 2002. Common design in a unique midline neuropil in the brains of arthropods. Arth Struct Dev 31:77–91.

Meinertzhagen IA. 1973. Development of the compound eye and optic lobe of insects. In: Young D, editor. Developmental neurobiology of arthropods. Cambridge: Cambridge University Press. p 51–104.

Meinertzhagen IA. 1976. The organization of perpendicular fibre pathways in the insect optic lobe. Philos Trans R Soc Lond B 274:555–596.

Meinertzhagen IA. 1991. Evolution of the cellular organization of the arthropod compound eye and optic lobe. In: Cronly-Dillon JP, Gregory RL, editors. Vision and visual dysfunction. Vol. 2. Evolution of the eye and visual system. London: Macmillan Press. p 341–363.

Meinertzhagen IA, Hanson TE. 1993. The development of the optic lobe. In: Bate M, Martinez-Arias A, editors. The development of *Drosophila melanogaster*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. p 1363–1491.

Meyer EP, Matute C, Streit P, Nässel DR. 1986. Insect optic lobe neurons identifiable with monoclonal antibodies to GABA. Histochemistry 84: 207–216.

Meyerowitz EM, Kankel DR. 1978. A genetic analysis of visual system development in *Drosophila melanogaster*. Dev Biol 62:112–142.

Nardi F, Spinsanti G, Boore JL, Carapelli A, Dallai R, Frati F. 2003. Hexapod origins: monophyletic or paraphyletic? Science 299:1887–1889.

Nässel DR, Geiger G. 1983. Neuronal organization in fly optic lobes altered by laser ablations early in development or by mutations of the eye. J Comp Neurol 217:86–102.

Nilsson D-E, Osorio D. 1997. Homology and parallelism in arthropod sensory processing. In: Fortey RA, Thomas RH, editors. Arthropod relationships. London: Chapman and Hall. p 317–332.

Osorio D. 1991. Patterns of function and evolution in the arthropod optic lobe. In: Cronly-Dillon JP, Gregory RL, editors. Vision and visual dysfunction. Vol. 2. Evolution of the eye and visual system. London: Macmillan Press. p 203–229.

Osorio D, Bacon JP. 1994. A good eye for arthropod evolution. Bioessays 16:419–424.

Paulus HF. 2000. Phylogeny of the Myriapoda-Crustacea-Insecta: a new attempt using photoreceptor structure. J Zool Syst Evol Res 38:189–208.

Retzius G. 1891a. Zur Kenntnis des centralen Nervensystems der Würmer, Das Nervensystem der Annulaten. Biol Untersuch Neue Folge 2:1–28.

Retzius G. 1891b. Das Nervensystem der Lumbriciden. Biol Untersuch Neue Folge 3:1–16.

Ribi WA. 1975. The neurons of the first optic ganglion of the bee *Apis mellifera*. Adv Anat Embryol Cell Biol 50:1–43.

Richter S. 2002. The tetraconata concept: hexapod-crustacean relationships and the phylogeny of Crustacea. Org Divers Evol 2:217–237.

Richter S, Scholtz G. 2001. Phylogenetic analysis of the Malacostraca (Crustacea). J Zool Syst Evol Res 39:113–136.

Rodriguez J, Deinhardt F. 1960. Preparation of a semipermanent mounting medium for fluorescent antibodies studies. Virology 12:316–317.

Schäfer S, Bicker G. 1986. Distribution of GABA-like immunoreactivity in the brain of the honey bee. J Comp Neurol 246:287–300.

Scholtz G. 2000. Evolution of the nauplius stage in malacostracan crustaceans. J Zool Syst Evol Res 398:175–187.

Schram FR. 1970. Isopods from the Pennsylvanian of Illinois. Science 169:854–855.

Seguela P, Geffard M, Buijs RM, Le Moal M. 1984. Antibodies against gamma-aminobutyric acid: specificity studies and immunocytochemical results. Proc Natl Acad Sci U S A 81:3888–3892.

Shaw SR. 1989. The retina-lamina pathway in insects particularly Diptera viewed from an evolutionary perspective. In: Stavenga PG, Hardie RC, editors. Facets of vision. Berlin, Heidelberg, New York: Springer Verlag. p 186–212.

Shaw SR. 1990. The photoreceptor axon projection and its evolution in the neural superposition eye of some primitive brachyceran Diptera. Brain Behav Evol 35:107–125.

Shaw SR, Meinertzhagen IA. 1986. Evolution of synaptic connections among homologous neurons. Proc Natl Acad Sci U S A 83:7961–7965.

Shaw SR, Moore D. 1989. Evolutionary remodeling in a visual system through extensive changes in the synaptic connectivity of homologous neurons. Vis Neurosci 3:405–410.

Sinakevitch I, Geffard M, Pelhate M, Lapied B. 1996. Anatomy and targets of dorsal unpaired median (DUM) neurons in the terminal abdominal ganglion of the male cockroach *Periplaneta americana* L. J Comp Neurol 367:147–163.

Sinakevitch I, Strausfeld NJ, Douglass JK. 2000. Comparisons of GABAergic organization in insects and isopods support divergent evolution of their visual neuropils. Soc Neurosci Abstr 26:697.

Strausfeld NJ. 1971. The organization of the insect visual system (light microscopy). I. Projections and arrangements of neurons in the lamina ganglionaris of Diptera. Z Zellforsch 121:377–441.

Strausfeld NJ. 1976. Atlas of an insect brain. Berlin, Heidelberg, New York: Springer.

Strausfeld NJ. 1989. Insect vision and olfaction: common design principles of neuronal organization. In: Singh RN, Strausfeld NJ, editors. Proceedings of the International Conference on the Neural Organization of Sensory Systems (ICONOSS). New York: Plenum. p 319–353.

Strausfeld NJ. 1998. Crustacean-insect relationships: the use of brain characters to derive phylogeny amongst segmented invertebrates. Brain Behav Evol 52:186–206.

Strausfeld NJ, Blest AD. 1970. Golgi studies on insects. I. The optic lobes of Lepidoptera. Philos Trans R Soc Lond B 258:81–134.

Strausfeld NJ, Campos-Ortega JA. 1977. Vision in insects: pathways possibly underlying neural adaptation and lateral inhibition. Science 195: 894–897.

Strausfeld NJ, Gilbert C. 1992. Small-field neurons associated with oculomotor and optomotor control in muscoid flies. Cellular organization. J Comp Neurol 316:56–71.

Strausfeld NJ, Hausen K. 1977. The resolution of neuronal assemblies after cobalt injection into neuropil. Proc R Soc Lond B 199:463–476.

Strausfeld NJ, Hildebrand JG. 1999. Olfactory systems: common design, uncommon origins? Curr Opin Neurobiol 9:634–639.

Strausfeld NJ, Lee JK. 1991. Neuronal basis for parallel visual processing in the fly. Vis Neurosci 7:13–33.

Strausfeld NJ, Meinertzhagen IA. 1996. The insect neuron: morphologies, structures and relationships to neuroarchitecture. In: Harrison FW, Locke M, editors. Microscopic anatomy of invertebrates. New York: Wiley-Liss. p 487–538.

Strausfeld NJ, Nässel DR. 1980. Neuroarchitecture of brain regions that subserve the compound eyes of crustacea and insects. In: Autrum HJ, editor. Handbook of sensory physiology VII/6B. Berlin, Heidelberg, New York: Springer. p 1–132.

Strausfeld NJ, Kong A, Milde JJ, Gilbert C, Ramaiah L. 1995. Oculomotor control in calliphorid flies: GABAergic organization in heterolateral inhibitory pathways. J Comp Neurol 361:298–320.

Wicklein M, Strausfeld N J. 2000. Organization and significance of neurons that detect change of visual depth in the hawk moth *Manduca Sexta*. J Comp Neurol 424:356–376.

Wildt M, Harzsch S. 2002. A new look at an old visual system: structure and development of the compound eyes and optic ganglia of the brine shrimp *Artemia salina* Linnaeus, 1758 (Branchiopoda, Anostraca). J Neurobiol 52:117–132.

Wilson K, Cahill V, Ballment E, Benzie J. 2000. The complete sequence of the mitochondrial genome of the crustacean *Penaeus monodon*: are malacostracan crustaceans more closely related to insects than to branchiopods? Mol Biol Evol 17:863–874.

Winther AME, Nassel DR. 2001. Intestinal peptides as circulating hormones: release of tachykinin-related peptide from the midgut of locust and cockroach. J Exp Biol 204:1269–1280.

Zawarzin AA. 1913. Histologische Studien über Insekten. 4. Die optischen Ganglien der Aeschnalarven. Z Wiss Zool 108:175–257.

Zawarzin AA. 1924. Zur Morphologie der Nervenzentren. Das Bauchmark der Insekten. Ein Beitrag zur vergleichenden Histologie. Z Wiss Zool 122:323–424.