Comparative studies on the nervous system of the Chilopoda with emphasis on the organization of deutocerebral neuropils, sensory structures and olfactory behavior

Vergleichende Studien des Nervensystems der Chilopoden mit dem Schwerpunkt auf der Organisation deutocerebraler Neuropile, sensorischer Strukturen und Verhaltensbiologie

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Abkürzungsverzeichnis

AMMC antennales mechanosensorisches und motorisches Zentrum

AnN Antenne 2 Neuropil der Crustacea

CL Corpus lamellosum

clc contralaterale Verbindung

DC Deutocerebrum

laL laterale akzessorische Loben

LAN laterales Antenne 1 Neuropil

MAN medianes Antenne 1 Neuropil

na Nervus antennalis

np Neuritenprojektion

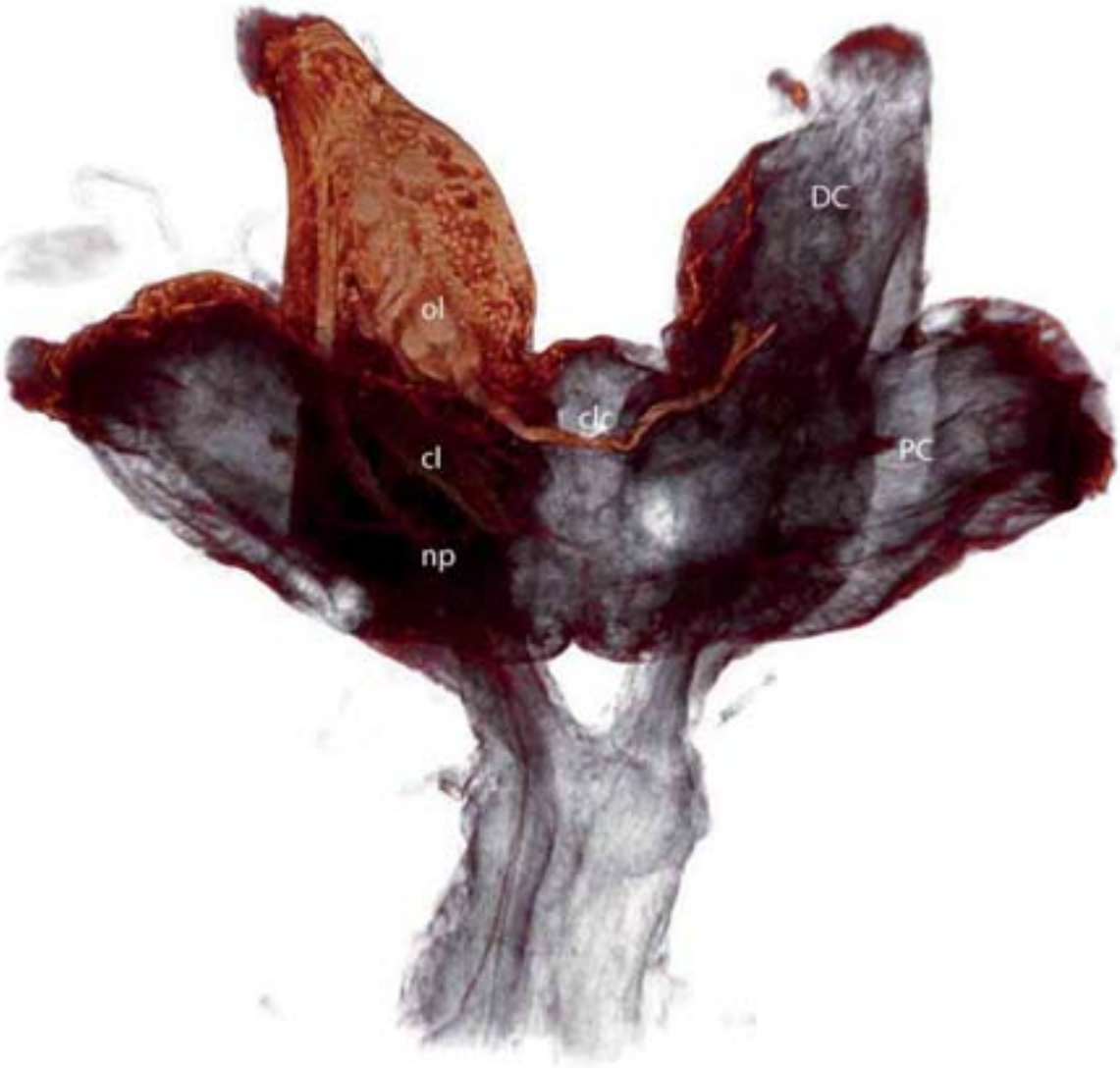
OG olfaktorische Glomeruli

OL olfaktorischer Lobus

ORN olfaktorische Rezeptorneuriten

PC Protocerebrum

USG Unterschlundganglion = Subösophagealganglion


DC
ol
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I Zusammenfassung

Im Gegensatz zu den Hexapoda und Crustacea (Tetraconata) liegen nur wenige Daten zur Architektur des Nervensystems der Chilopoda vor. Ein besonderer Fokus in neuroanatomischen Studien der Arthropoda liegt auf der internen Organisation des Deutocerebrum. Das Deutocerebrum ist ein primärer Verschaltungsort antennaler Sinnesmodalitäten. Es wurde von Schachtner et al. (2005) gezeigt, dass bei Vertretern der Hexapoda und Crustacea spezifische Synapomorphien in Bezug auf die olfaktorischen Glomeruli festzustellen sind. Durch den Einsatz verschiedenster histologischer Techniken, immunhistochemischer sowie histochemischer Methoden, anterograder Backfill-Anfärbungen und der dreidimensionalen Rekonstruktion wurde das Deutocerebrum der Chilopoda in dieser Dissertation untersucht um zu verifizieren, ob das Deutocerebrum ähnlich zu dem der Tetraconata ausgeprägt und ob diese innerhalb der Mandibulata homologisierbar sind. Zudem wurden die gewonnen Daten mit neuroanatomischen Studien zu den Chelicerata verglichen. Das Deutocerebrum der Chilopoda ist durch mehrere Merkmale charakterisiert: (1) Innervierung durch antennale sensorische Neuriten, (2) ein anterior gelegener olfaktorischer Lobus, (3) der posterior gelegene Corpus lamellosum, (4) afferente Projektionen aus der Antenne die in das Unterschlundganglion projizieren sowie (5) Projektionstrakte zwischen dem Protocerebrum und dem olfaktorischen Lobus. Neuroanatomische Daten zeigen, dass ein Schwestergruppenverhältnis zwischen Myriapoda und Chelicerata höchst unwahrscheinlich ist, da das durch sensorische Anhänge innervierte Neuromer bei den Chelicerata nicht durch ein mechanosensorisches Neuropil charakterisiert ist. Basierend auf den Befunden der untersuchten Chilopoda ergibt sich als Apomorphie der Mandibulata, dass der sensorische Eingang durch die homologe deutocerebrale Antenne zwei distinkte Neuropilbereiche innerviert.

Sensorische Informationen werden hauptsächlich von antennalen Sensillen wahrgenommen. Mit Ausnahme der Scutigeromorpha, lagen für alle höheren Taxa der Chilopoda Daten zur Struktur und Diversität antennaler Sensillen vor. In der vorliegenden Arbeit konnte diese Lücke geschlossen werden und ein Vergleich der antennalen Sensillen innerhalb der Chilopoda durchgeführt werden. Innerhalb der Chilopoda lassen sich für die Scutigeromorpha drei einzigartige antennale Strukturen feststellen: (1) der Besitz von langen Antennen mit Noden, die „sensory cones" tragen, (2) der Besitz eines zweigliedrigen Schaftes, der das Schaftorgan trägt und (3) der Besitz des Beak-like Sensillums.

Ein dritter Aspekt dieser Arbeit behandelt verhaltensbiologische Untersuchungen bei Vertretern der Chilopoda. Zusammenfassend zeigen die durchgeführten Experimente, dass die Chilopoda (im Speziellen *Scutigera coleoptrata*) Sinnesreize über die Antenne wahrnehmen kann, spezifische neuronale Strukturen für die Verarbeitung besitzen und auf olfaktorische Reize reagieren.

II Summary

Contrary to hexapods and crustaceans (Tetraconata), only few data on the architecture of the nervous system of chilopods are present. A focus in neuroanatomical studies of arthropods is the deutocerebrum which is a primary processing area of sensory input from the first antennae. Schachter et al. (2005) compiled several synapomorphic characters of the olfactory glomeruli of hexapods and crustaceans. To test whether the architecture of the deutocerebrum of chilopods is homologue to that of the Tetraconata, different techniques such as histology, immunhistochemistry and histochemistry, anterograde backfills and threedimensional reconstructions were conducted. Furthermore, the achieved neuroanatomical data were compared to available studies on the nervous systems of chelicerates. The deutocerebrum in Chilopoda is characterized by several features: (1) innervation by antennal sensory neurites, (2) an anterior olfactory lobe, (3) a posterior Corpus lamellosum, (4) afferent neurite projections into the subesophageal ganglion and (5) projection tracts between protocerebral neuropils and the olfactory lobe. Neuroanatomical data show that a sistergroup relationship between Myriapoda and Chilopoda is highly unlikely because in the Chelicerata the innervated neuromere is not equipped with a mechanosensory neuropil. In summary, in the Mandibulata the sensoric neurites innervate two distinct neuropilar regions. This feature is postulated as an apomorphic character.

The majority of sensory information in Chilopoda is perceived by antennal sensilla. With exception of the Scutigeromorpha, for all other higher taxa of the Chilopoda information on the external structure and distribution of antennal sensilla is available. Within the Chilopoda, three unique characters were found for the Scutigeromorpha: (1) the presence of long antennae with nodes bearing sensory cones, (2) the presence of a bipartite shaft including the shaft organ, and (3) the presence of beak-like sensilla.

A third aspect of this dissertation deals with ethological investigations of representatives of the Chilopoda. To sum up, ethological experiments show that chilopods (and especially *Scutigera coleoptrata*) can perceive olfactory stimuli via the antennae, possess distinct nervous system structures to process these information, and are able to react to olfactory stimuli.

1. Generelle Einleitung und Ziele der Arbeit

1.1 Die Chilopoda – phylogenetische Vorbetrachtung

Die Hunderfüßer (Chilopoda) sind eine phylogenetisch sehr alte Tiergruppe der Gliederfüßer (Arthropoda), deren Vertreter für mehr als 400 Millionen Jahre (paläozoisches Silur) fossil belegt sind (Rosenberg 2009, Shear und Edgecombe 2010). So stammen die ältesten Belege von Extremitätenteilen von einem Scutigeromorphen, mit einem ungefähren Alter von 414 Millionen Jahren (Jeram et al. 1990). Weltweit sind etwa 3.300 Arten der Chilopoda mit einer ausschließlich terrestrischen Lebensweise bekannt.

Abbildung 1: Ausgewählte Vertreter der Chilopoda: **A** *Scutigera coleoptrata* (Linneaus, 1758). **B** *Lithobius forficatus* (Linneaus, 1758). **C** *Cryptops hortensis* (Donovan, 1810). **D** *Geophilus carpophagus* Leach,1814. (Originale)

Die Monophylie der Myriapoda (Tausendfüßer) ist durch zahlreiche Untersuchungen und komplexe apomorphe Merkmale gesichert (Zusammenfassung in Rosenberg 2009). Das Taxon setzt sich aus den Chilopoda und Progoneata zusammen (Abbildung 2). Die wichtigsten Autapomorphien sind: (1) der Verlust der Medianaugen und Besitz von Ommatidien mit konstanter Zellzahl der proximalen Retinula [n= 4 bzw. 5] und Kristallkegelkompartimente [n= 3 bzw. 4], deren Matrizes von einem deutlichen elektronendichten Cytoplasmasaum umkleidet werden (Müller et al. 2003, 2007; Müller 2008), (2) schwingbare Kopftentorien (Koch 2003), (3) Postantennalorgane (Manton 1977, Fanenbruck 2003) und (4) der spezifische Aufbau epidermaler Drüsen mit Intermediärzelle (Hilken et al. 2003, Müller et al. 2003, Hilken et al. 2005, Hilken und Rosenberg 2006, Rosenberg und Hilken 2006, Müller et al. 2009). Auch in

molekulargenetischen bzw. kombinierten Studien (*„total evidence"*), wird die Monophylie der Myriapoden unterstützt (beispielsweise Ballard et al. 1992, Wheeler et al. 1993, Friedrich und Tautz 1995, Regier und Shultz 1997, Shultz und Regier 1997, Giribet et al. 2001, Burmester 2001, 2002; Edgecombe und Giribet 2002, Kusche et al. 2002, 2003, Regier et al. 2010).

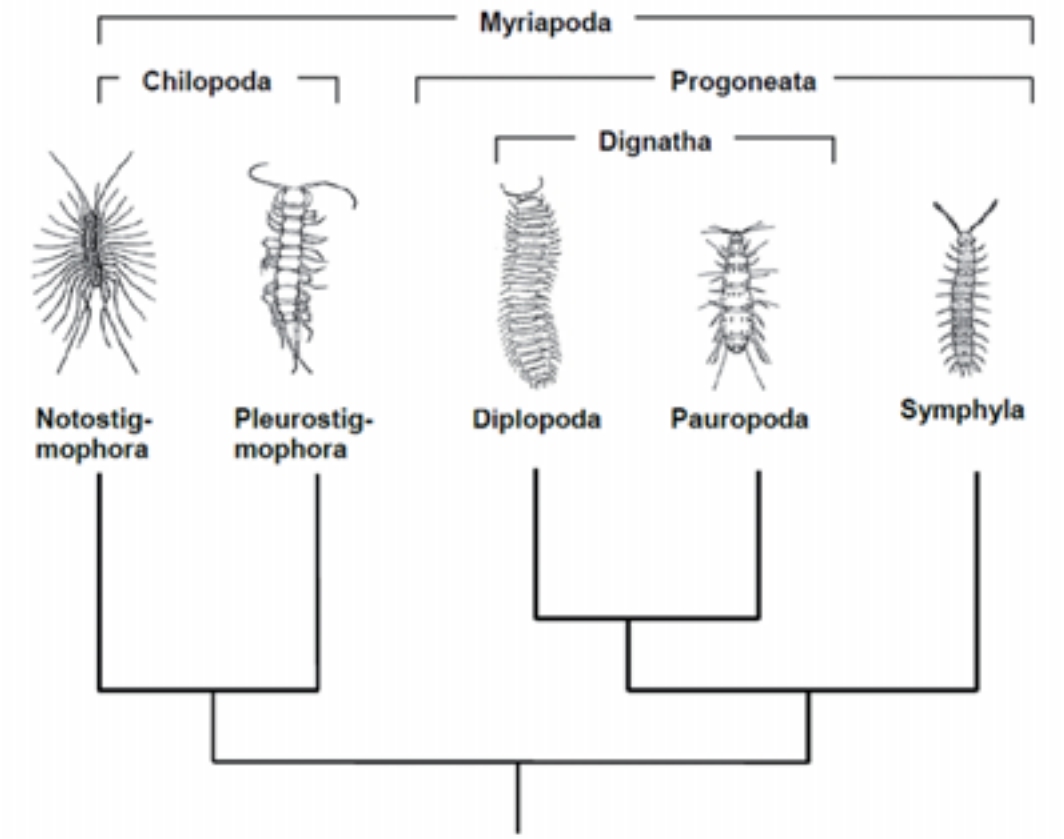


Abbildung 2: Verwandtschaftsbeziehungen innerhalb der Myriapoda. Verändert nach Rosenberg (2009).

Die Position der Myriapoda innerhalb der Arthropoda wird dagegen kontrovers diskutiert (Zusammenfassungen in Harzsch et al. 2005, Edgecombe und Giribet 2007, Edgecombe 2010). Traditionell wurden die Myriapoden als Schwestergruppe der Hexapoda innerhalb der Tracheata angesehen (z.B. Kraus 1997, 2001; Ax 1999, Waloszek 1999, Nielsen 2001) (Abbildung 3). In den letzten Jahren wurde diesem Konzept allerdings durch molekulare Studien, welche das Schwestergruppenverhältnis der Tetraconata oder Pancrustacea (Hexapoda + Crustacea) favorisieren (Shultz und Regier 2000, Cook et al. 2001, Friedrich und Tautz 2001, Giribet et al. 2001, Hwang et al. 2001, Burmester 2002, Kusche et al. 2002, Pisani et al. 2004, Giribet et al. 2005, Regier et al. 2010) (Abbildung 3) widersprochen. Auch in morphologischer Hinsicht wurde das Taxon der Tetraconata aufgrund des vierteiligen Kristallkegels der Ommatidien gestützt (Dohle 2001, Richter 2002). Detaillierte Vergleichsanalysen des namensgebenden Merkmals der Tracheata (die Tracheen) nährten zudem Zweifel am Tracheata-Konzept (Hilken 1998). Den oben genannte Studien folgend, bilden die Myriapoden die

Schwestergruppe zu den Tetraconata im Taxon Mandibulata, welches sich morphologisch unter anderen durch den Besitz einer gnathobasischen Mandibel (Edgecombe et al. 2003), der Struktur der Ommatidien (Müller et al. 2003, 2007) sowie durch die Entwicklung des Nervensystems (Harzsch et al. 2005) auszeichnet. Alternativ wurde jedoch ein Schwestergruppenverhältnis zwischen Myriapoda und Chelicerata vorgeschlagen (Abbildung 3). Dieses Taxon namens Paradoxopoda (Mallatt et al. 2004) oder auch Myriochelata (Pisani et al. 2004) wurde bisher einzig mit molekularen Daten bezüglich kernribosomaler Gene (Mallatt et al. 2004), mitochondrialer Gene (Podadic et al. 1998, Hwang et al. 2001, Negrisolo et al. 2004), Hox Gene (Cook et al. 2001) sowie Analysen aus 18S und 28S rRNA (v. Reumont et al. 2009) belegt. In weiteren phylogenetischen Studien wurden allerdings auch die Mandibulata molekulargenetisch unterstützt (Regier et al. 2010, Rota-Stabelli et al. 2011).

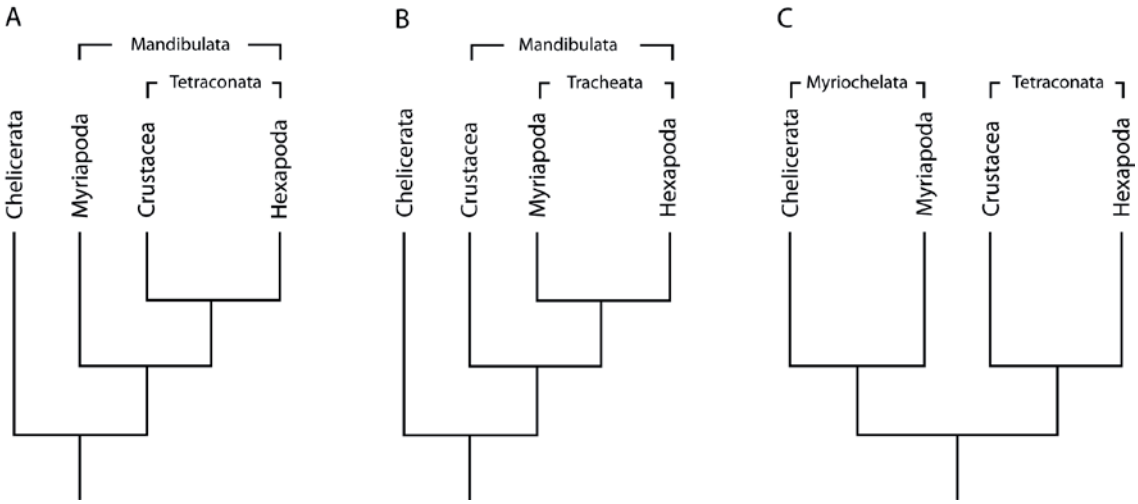
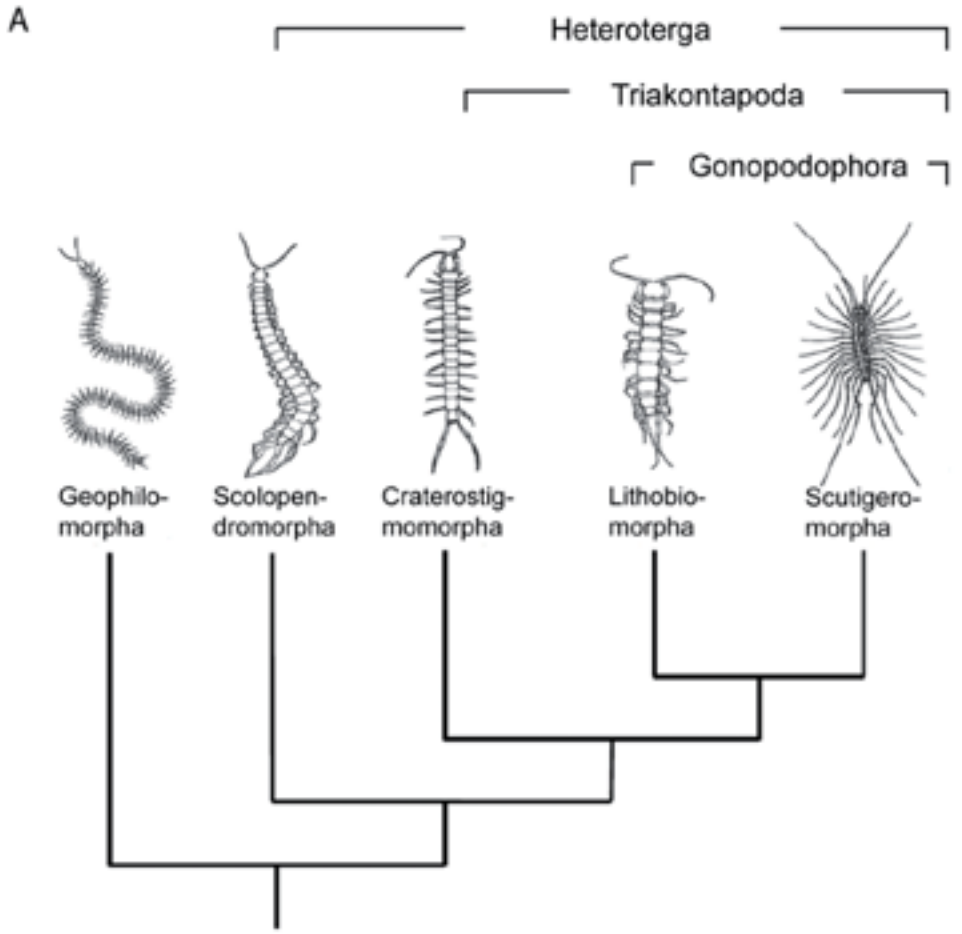


Abbildung 3: Konkurrierende Verwandtschaftsverhältnisse innerhalb der Euarthropoda. Verändert nach Müller (2008). **A** Tetraconata-Konzept. **B** Tracheata-Konzept. **C** Myriochelata-Konzept.

Die Chilopoda werden durch eine Reihe von Autapomorphien als monophyletische Gruppe gestützt (Zusammenfassung in Rosenberg 2009, Edgecombe 2011). Als Beispiele sind hier das zu Giftklauen umgebildete erste postcephale Extremitätenpaar (Maxillipeden) (Dohle 1985, Borucki 1996), die Spermienmorphologie (Jamieson 1986), die Heterotergie (Dohle 1985, Borucki 1996), die Ausbildung eines Maxillipedbogens der Dorsal- und Ventralgefäß miteinander verbindet (Wirkner und Pass 2002), die Anordnung der Stigmen (Hilken 1997, 1998), fusionierte Maxillarnephridien (Fahlander 1938, Rosenberg 1979, Rosenberg et al. 2009) sowie die Ausbildung von Coxal- und Analorganen (Rosenberg 1985) zu nennen. Innerhalb der Chilopoda konkurrieren mehrere Hypothesen zu möglichen Verwandtschaftsbeziehungen: unter diesen (1) das Anamorpha-Konzept, (2) das Heteroterga-Konzept und (3) das Pleurostigmophora-Konzept.


A
Heteroterga
Triakontapoda
Gonopodophora
Geophilo-
morpha
Scolopen-
dromorpha
Craterostig-
momorpha
Lithobio-
morpha
Scutigero-
morpha

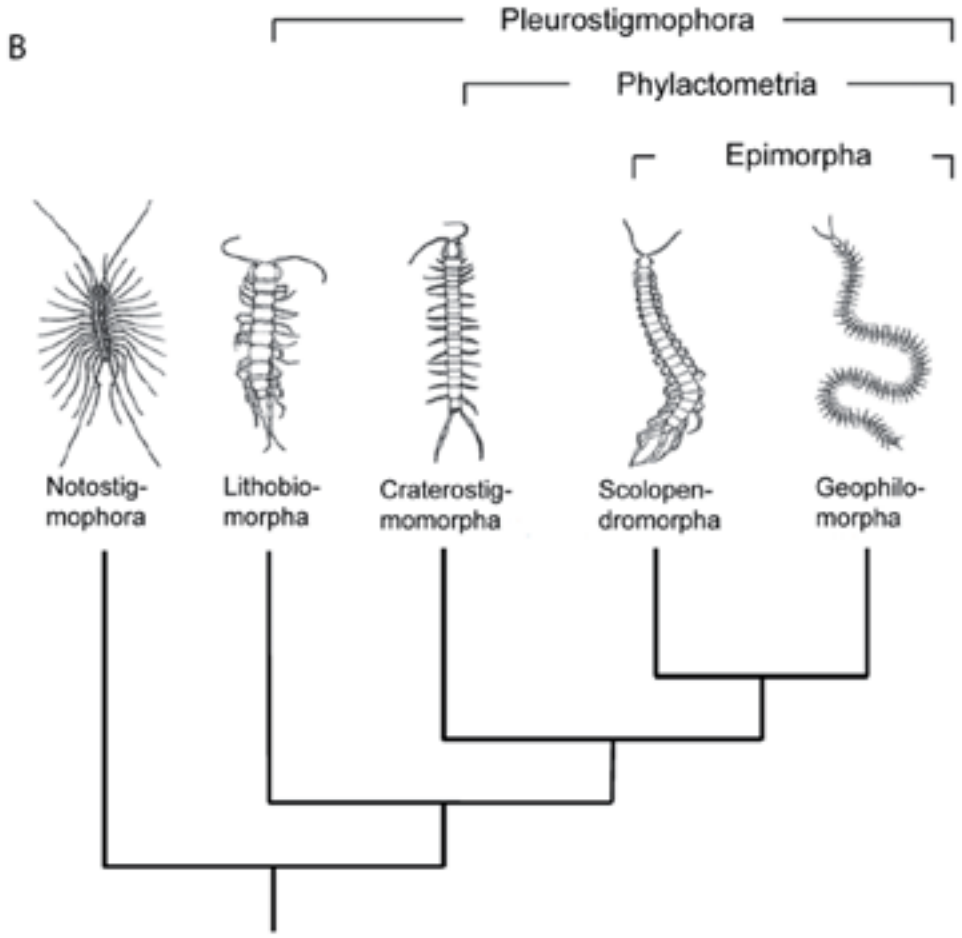

B
Pleurostigmophora
Phylactometria
Epimorpha
Notostig-
mophora
Lithobio-
morpha
Craterostig-
momorpha
Scolopen-
dromorpha
Geophilo-
morpha

Abbildung 4: Verwandtschaftsbeziehungen innerhalb der Chilopoda. **A** Heteroterga-Konzept. **B** Pleurostigmophora-Konzept. Verändert nach Ax (1999), Rosenberg (2009) und Edgecombe (2011).

Das Anamorpha-Konzept (Attems 1926-30, Manton 1965) gründet sich auf Unterschiede in der postembryonalen Entwicklung und gruppiert die Scutigeromorpha zu den Lithobiomorpha innerhalb des Taxons Anamorpha, welches dem Taxon Epimorpha (Craterostigmomorpha, Scolopendromorpha und Geophilomorpha) gegenübersteht. Beim Heteroterga-Konzept (Ax 1999) (Abbildung 4 A) bilden die Geophilomorpha die Schwestergruppe zu den Heteroterga (dorsal heteroterger Rumpf). Innerhalb letztgenannter Gruppe sind die Scolopendromorpha die Schwestergruppe der Triakontapoda, in welcher die Craterostigmomorpha die Schwestergruppe zu den Gonopodophora (Lithobiomorpha und Scutigeromorpha) darstellen. Das Pleurostigmophora-Konzept (Brandt 1841, Verhoeff 1902-25, Pocock 1902, Dohle 1985, Borucki 1996, Hilken 1997, 1998; Wirkner und Pass 2002, Edgecombe und Giribet 2007) (Abbildung 4 B) wird durch morphologische und molekulargenetische Studien gestützt. Hier bilden die Notostigmophora (Scutigeromorpha) die Schwestergruppe zu den Pleurostigmophora, in denen die Lithobiomorpha die Schwestergruppe der Phylactometria bilden. In letztgenanntem Taxon bilden die Craterostigmomorpha die Schwestergruppe zu den Epimorpha, welche sich aus den Scolopendromorpha und den Geophilomorpha zusammensetzt (Edgecombe 2011).

1.2 Neurophylogenie

Ein wichtiger Merkmalskomplex zum Verständnis der Verwandtschaftsbeziehungen innerhalb der Arthropoda ist das Nervensystem (Strausfeld et al. 1995, Strausfeld 1998, Paulus 2000, Dohle 2001, Harzsch 2001, 2003, 2004; Richter 2002, Schachtner et al. 2005, Harzsch et al. 2005, 2006, 2007). Die Neurophylogenie vereint neurobiologische Daten, um Beiträge zu phylogenetischen Fragestellungen zu liefern (Harzsch 2006, 2007). Strausfeld und Andrew (2011) postulieren, dass neuronale Merkmale eine gewisse Stabilität besitzen, die von der hohen Diversität externer Merkmale unabhängig sind. Neurophylogenie ist auf verschiedensten Komplexitätsebenen anwendbar (Zusammenfassung in Harzsch 2006, 2007). Aus vergleichend morphologischen und entwicklungsbiologischen Studien des Nervensystems der Arthropoda resultieren eine Reihe von Argumenten für das Taxon Tetraconata (Nilsson und Osorio 1997, Whitington und Bacon 1997, Strausfeld 1998, Dohle 2001, Harzsch 2001, Richter 2002, Harzsch et al. 2005). Ebenso wurde das Taxon Mandibulata unterstützt (Harzsch et al. 2005, Müller et al. 2007). Als spezifische Beispiele für Merkmalskomplexe aus neurophylogenetischen Studien innerhalb der Arthropoda sind weiterhin die homologe Architektur optischer Neuropile der Mandibulata (Strausfeld

1998, 2009, Fanenbruck et al. 2004), des Zentralkörpers der Euarthropoda (Loesel et al. 2002) und des olfaktorischen Systems bei Hexapoda und Crustacea (Schachtner et al. 2005) zu erwähnen. Als eines der bestuntersuchten Nervensystemstrukturen innerhalb der Crustacea und Hexapoda ist das primäre olfaktorische System zu nennen. Bereits in den klassischen Arbeiten von Holmgren (1916) und Hanström (1928, 1929) lag ein Fokus auf dem Deutocerebrum der Arthropoda. In den letzten Jahren erschien eine Fülle von Publikationen, die sich vor allem mit der Organisation deutocerebraler Neuropile beschäftigte. Herausragend ist hier insbesondere der Vergleich und die Homologisierung der olfaktorischen Zentren innerhalb der Tetraconata von Schachtner et al. (2005). Allerdings konnte die Frage, ob die spezifische Struktur olfaktorischer Zentren innerhalb der Tetraconata eine Autapomorphie oder eine Symplesiomorphie darstellt, nicht endgültig beantwortet werden. Für die Myriapoden im Allgemeinen und die Chilopoden im Speziellen liegen zu dieser Thematik nur sehr wenige und fragmentarische Daten vor (siehe Kapitel 2). Vor dem Hintergrund der genannten konkurrierenden Hypothesen der Verwandtschaftsbeziehungen innerhalb der Euarthropoda liegt der Fokus dieser Arbeit daher auf dem Versuch, neuroanatomische Daten als Beitrag zur Rekonstruktion der Phylogenie der zu gewinnen. In diesem Zusammenhang lassen sich Fragen auf verschiedenen Ebenen formulieren: (1) Wie ist das Deutocerebrum der Chilopoda aufgebaut? (2) Ist diese Architektur zu der der Tetraconata homolog? (3) Wie fügen sich diese Daten in die vorgeschlagenen Verwandtschaftsbeziehungen im Sinne des Mandibulata- bzw. Myriochelata-Konzepts ein?

1.3 Die Sinneswelt der Chilopoda

Die Chilopoda sich ein exklusiv terrestrisches Taxon der Euarthropoda. Ein erfolgreicher Übergang vom Wasser- zum Landleben erfordert eine Reihe von physiologischen Anpassungen. Als Beispiele sind Salz- und Wasserhaushalt, die Thermoregulation sowie die Reproduktion zu nennen (Powers und Bliss 1983, Burggren und McMahon 1988, Rosenberg et al. 2009). Vom Landgang besonders betroffen ist die Perzeption chemischer Stoffe, da diese im aquatischen Milieu in Lösung vorkommen, während sie an Land über die Gasphase detektiert werden. Zudem verändern sich die olfaktorischen Stimuli von hauptsächlich hydrophilen Molekülen im Wasser zu hauptsächlich hydrophoben an der Luft (Burggren und McMahon 1988). Die bedeutet, dass eine Anpassung an das Trägermedium eine morphologische und physiologische Modifikation der Perzeptionsorgane erfordert.

Die meisten Vertreter der Chilopoda leben mehr oder weniger kryptisch als räuberische, photophobe und hygrophile Bodenbewohner in der Laubstreu bzw. in den oberen Bodenschichten (Rosenberg 2009). Hundertfüßer leben ausschließlich räuberisch. Ihre Nahrung setzt sich aus kleineren Arthropoden zusammen. Wie für viele räuberische Tiere typisch, können Hungerzeiten von mehreren Wochen gut überstanden werden

(Pfleiderer-Gruber 1986). Untersuchungen zu Fähigkeiten der Olfaktion bei Chilopoda sind nur in sehr geringer Zahl durchgeführt worden (siehe Kapitel 9). Für die chemosensorische Perzeption müssen adäquate sensorische Strukturen vorhanden sein, welche mit dem Nervensystem verschaltet sind. Nicht nur die Ausstattung mit spezialisierten antennalen Sensillen (neben den sensorischen Strukturen an Extremitäten oder den Mundwerkzeugen) sondern auch die Architektur des Nervensystems ermöglicht es, Aussagen über die sensorischen Fähigkeiten von Organismen zu treffen. Das optische System ist besonders bei den Scutigeromorpha ausgeprägt (Müller et al. 2003). Bei *Scutigera coleoptrata, Lithobius forficatus* und *Scolopendra subspinipes* Leach, 1815 wurde eine positive Skototaxis nachgewiesen (Klein 1934, Görner 1959, Müller et al. 2011). Dies scheint ebenso für die blinden Cryptopidae (Scolopendromorpha) und Geophilomorpha zuzutreffen (Plateau 1886, 1887). Eine negative Phototaxis wurde für *L. forficatus* beschrieben (z.B. Scharmer 1935, Meske 1961). Das optische System von S. *coleoptrata* besitzt zudem eine hohe UV-Sensitivität (Meyer-Rochow et al. 2006). Als Funktion wurde die Detektion von Versteckmöglichkeiten vorgeschlagen (Meyer-Rochow et al. 2006). Laut Klingel (1960) soll der optische Sinn bei *Scutigera coleoptrata* nur eine geringe Rolle in der Beutedetektion spielen. Da die meisten Chilopoda dämmerungs- und nachtaktiv sind (Taguchi und Makiya 1982, Little 1983, Rosenberg 2009), scheinen Beutefang und auch innerartliche Kommunikation über mechanische und chemische Signale stattzufinden (Sombke et al. 2001a). Hierbei scheinen die Antennen eine wichtige Rolle zu spielen. Eine ausführliche Bewertung antennaler Sinnesstrukturen sowie ethologischer Untersuchungen finden sich dazu in den Kapiteln acht und neun.

1.4 Ziele der Dissertation

Aus diesen Vorbetrachtungen ergeben sich für die vorliegende Dissertation die folgenden primären Ziele:

1. Die Aufklärung der Architektur und Organisation deutocerebraler Neuropile bei ausgewählten Vertretern der Chilopoda.
2. Die Bewertung und der Vergleich der gewonnenen Daten mit Vertretern der Diplopoda, Tetraconata sowie innerhalb der Arthropoda.

Es ist das Ziel dieser Arbeit durch den gezielten Einsatz von histologischen Methoden, Autofluoreszenspräparationen, immunhistochemischen sowie histochemischen Methoden, anterograden Backfilltechniken sowie der dreidimensionalen Rekonstruktion die vorigen Erkenntnisse in Bezug auf das Deutocerebrum der Chilopoden zu bestätigen, gegebenenfalls zu berichtigen und vor allen Dingen zu ergänzen.

Sekundär lag der Fokus der vorliegenden Dissertation auf den Zielen:

3. Morphologische Untersuchung antennaler Sensillen von *Scutigera coleoptrata* und Vergleich innerhalb der Chilopoda.
4. Verhaltensbiologische Experimente, um die Daten aus Sinnesmorphologie und Neuroanatomie mit ethologischen Ergebnissen zu untermauern.

2. Das Nervensystem der Chilopoda

Die folgenden Kapitel beinhalten eine Zusammenfassung von Literaturdaten, die mit eigenen Daten ergänzt und zusammenfassend diskutiert werden.

Das Nervensystem der Chilopoda gliedert sich in das Gehirn, die ventrale Ganglienkette und das periphere Nervensystem. Wie bei den Crustacea und Hexapoda unterteilt sich das Gehirn (Synonyme: Oberschlundganglion oder auch Syncerebrum) in die Neuromere Proto-, Deuto- und Tritocerebrum (Abbildung 5) (Sombke et al. 2011b). Bei den drei Taxa der Mandibulata beinhaltet das Protocerebrum die optischen Neuropile, den Zentralkörper und die sekundären Verarbeitungszentren (Globuli = Pilzkörper, Hemielipsoidkörper, Corpora pedunculata). Das Deutocerebrum (auch Riechzentrum genannt) beinhaltet die Verschaltungseinheiten für chemo- und mechanosensorische Informationen. Das Tritocerebrum ist assoziiert mit dem stomatogastrischen Nervensystem und stellt über die circumösophagealen Konnektive die Verbindung zum Unterschlundganglion her. Bei den Crustacea wird das Tritocerebrum durch die zweite Antenne innerviert. Auch wenn der Verschmelzungsgrad der drei Gehirnteile innerhalb der Chilopoda variiert, stellt das Gehirn der Chilopoda ein komplettes Syncerebrum dar, das heißt, eine klare Grenze zwischen den einzelnen Gehirnteilen ist nicht erkennbar. Das periphere Nervensystem setzt sich aus den Sinnesorganen sowie den Nerven, die Sinnes- und Lokomotionsorgane innervieren, zusammen.

Im Vergleich zu den übrigen Taxa der Euarthropoda (vergleiche Abbildung 3) sind die Chilopoda in Bezug auf anatomische, morphologische und physiologische Aspekte des Nervensystems eine wenig untersuchte Tiergruppe (Rosenberg 2009). Die ältesten neuroanatomischen Studien gehen auf Treviranus & Treviranus (1817), Léon-Dufor (1824) und Saint-Rémy (1887, 1889) zurück. Detailliertere Untersuchungen erschienen zu Beginn des 20. Jahrhunderts. Diese waren allerdings, wie die vorher genannten, methodisch auf histologische Paraffinschnitte mit Schnittdicken von 5 bis 10 µm (z.B. Fahlander 1938) begrenzt. Zudem hatten diese klassischen Arbeiten zumeist die Struktur des Protocerebrums im Fokus (Holmgren 1916, Hanström 1928, Hörberg 1931, Fahlander 1938). In seinem epochalen Werk „Vergleichende Anatomie des Nervensystems der wirbellosen Tiere unter Berücksichtigung seiner Funktion" schreibt Hanström (1928) über das Nervensystem der Myriapoden:

„Die Myriapoden sind die in bezug auf den Bau des Nervensystems am wenigsten untersuchte Gruppe der Arthropoden. Zum Teil beruht das wohl darauf, daß ihr sehr hartes Chitinskelett beim Schneiden große Schwierigkeiten macht, zum Teil darauf, daß das Nervensystem der Myriapoden ungewöhnlich schwer zu fixieren ist."

Dennoch steht, besonders im Vergleich zu oben genannten Arbeiten, eine weitere Untersuchung der Anatomie des Protocerebrums aus. Hierzu sind bislang nur wenige

Studien anhand weniger ausgewählter Taxa veröffentlicht worden (Melzer et al. 1996, Loesel et al. 2002, Strausfeld 2005).

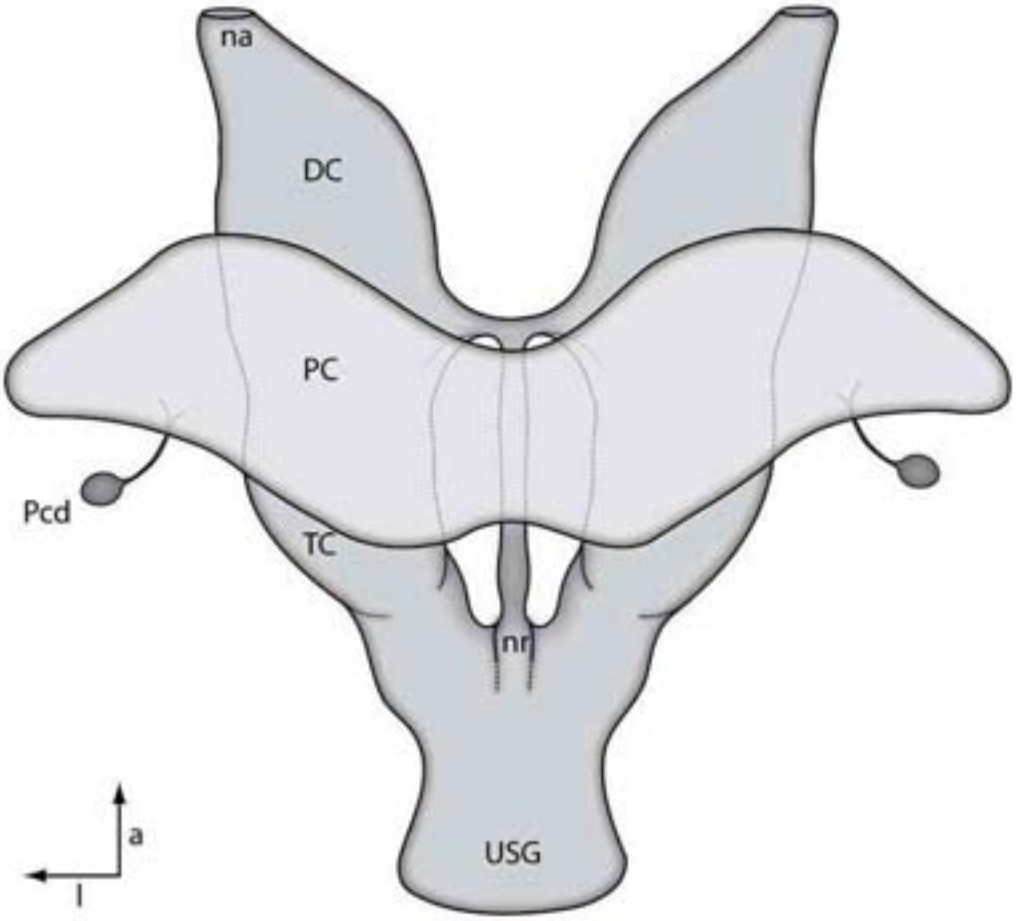


Abbildung 5: Schematische Darstellung des Gehirns von *Lithobius forficatus*. Das Protocerebrum (Pc; heller dargestellt) befindet sich dorsal des Deutocerebrum. Ansicht von dorsal. (Original).

a anterior, **DC** Deutocerebrum, **l** lateral, **na** Nervus antennalis, **nr** Nervus recurrens, **PC** Protocerebrum, **Pcd** Protocerebraldrüse, **TC** Tritocerebrum, **USG** Unterschlundganglion.

2.1 Protocerebrum

Die Scutigeromorpha stechen aufgrund mehrerer Eigenschaften des Nervensystems aus der Gruppe der Chilopoden heraus. Nach eigenen Untersuchungen ist das auffälligste Merkmal die runde Kopfform, die sich in gewisser Weiser auch in der Form des Nervensystems widerspiegelt. Bei *Scutigera coleoptrata* verläuft die Neurachse in einem Bogen nach posteriodorsal, wodurch das Protocerebrum nach posterior geklappt erscheint (Abbildung 8, 10, 16). Ein ähnlicher Verlauf der Neurachse nach posteriodorsal tritt bei verschiedensten Vertretern der Hexapoda auf (vergleiche Burrows 1996). Die Kopfform der Pleurostigmophora ist generell dorsoventral abgeflacht. Dies spiegelt sich ebenso in der Form des Nervensystems wider. Bei *Lithobius forficatus*, *Craterostigmus tasmanianus* Pocock, 1902, *Scolopendra* spp. und *Cryptops hortensis* erscheint das Gehirn dorsoventral abgeflacht. Bei den von mir untersuchten Vertretern der Geophilomorpha (*Geophilus carpophagus*, *Haplophilus subterraneus* (Shaw, 1789), *Stigmatogaster dimidiatus* (Meinert, 1870)) erscheint das Gehirn aufgrund des kleineren Protocerebrum rundlicher. Generell erstreckt sich das Protocerebrum dorsal des Deutocerebrum über die gesamte Kopfbreite (Abbildung 5, 6, 16). Bei den augenbesitzenden Chilopoda verschmälert sich das Protocerebrum lateral und tritt über robuste/ kompakte (*S. coleoptrata* und *L. forficatus*) oder separate (*Scolopendra* spp.) optische Nerven in Kontakt mit den Augen (Abbildung 6 A, C, 13 C, 16). Das laterale Protocerebrum enthält die beiden optischen Neuropile (visuelles/optisches Tektum und Lamina) (Melzer et al. 1996, Strausfeld 2005). Auch wenn die Augen bei den Cryptopidae reduziert sind, ist das Protocerebrum bei *C. hortensis* (Abbildung 6 B) lateral verbreitert. Die Protocerebraldrüsen sind mit dem posterioren lateralen Protocerebrum über den Nervus glandulae cerebralis verbunden (Abbildung 5, 16). Diese Nerven sind bei *Lithobius forficatus* und den Geophilomorpha nach Seifert (1967) paarig vorhanden. Der Zentralkörper erstreckt sich als breites spindelförmiges Neuropil, welches bei *S. coleoptrata* und *C. tasmanianus* mit den dorsolateral gelegenen lateralakzessorischen Loben (laL) verbunden ist (Abbildung 17). Bei den übrigen von mir untersuchten Taxa konnte der Zentralkörper, nicht aber die laL festgestellt werden. Die Pilzkörper oder Corpora pedunculata befinden sich ebenfalls im Protocerebrum. Eine genaue Darstellung dieser Strukturen liegt nur in älteren Arbeiten vor (z.B. Holmgren 1916, Hanström 1928, Hörberg 1931). Strukturelle Untersuchungen der Corpora pedunculata wurden in der vorliegenden Arbeit nicht weiter vertieft. Der Nervus tömösváryi (Abbildung 8), der das Postantennalorgan (Synonyme: Temporal- oder Tömösváryorgan) bei Scutigeromorpha und Lithobiomorpha innerviert, zieht bei *L. forficatus* posteriomedian zu den optischen Neuropilen (Petykó et al. 1996).

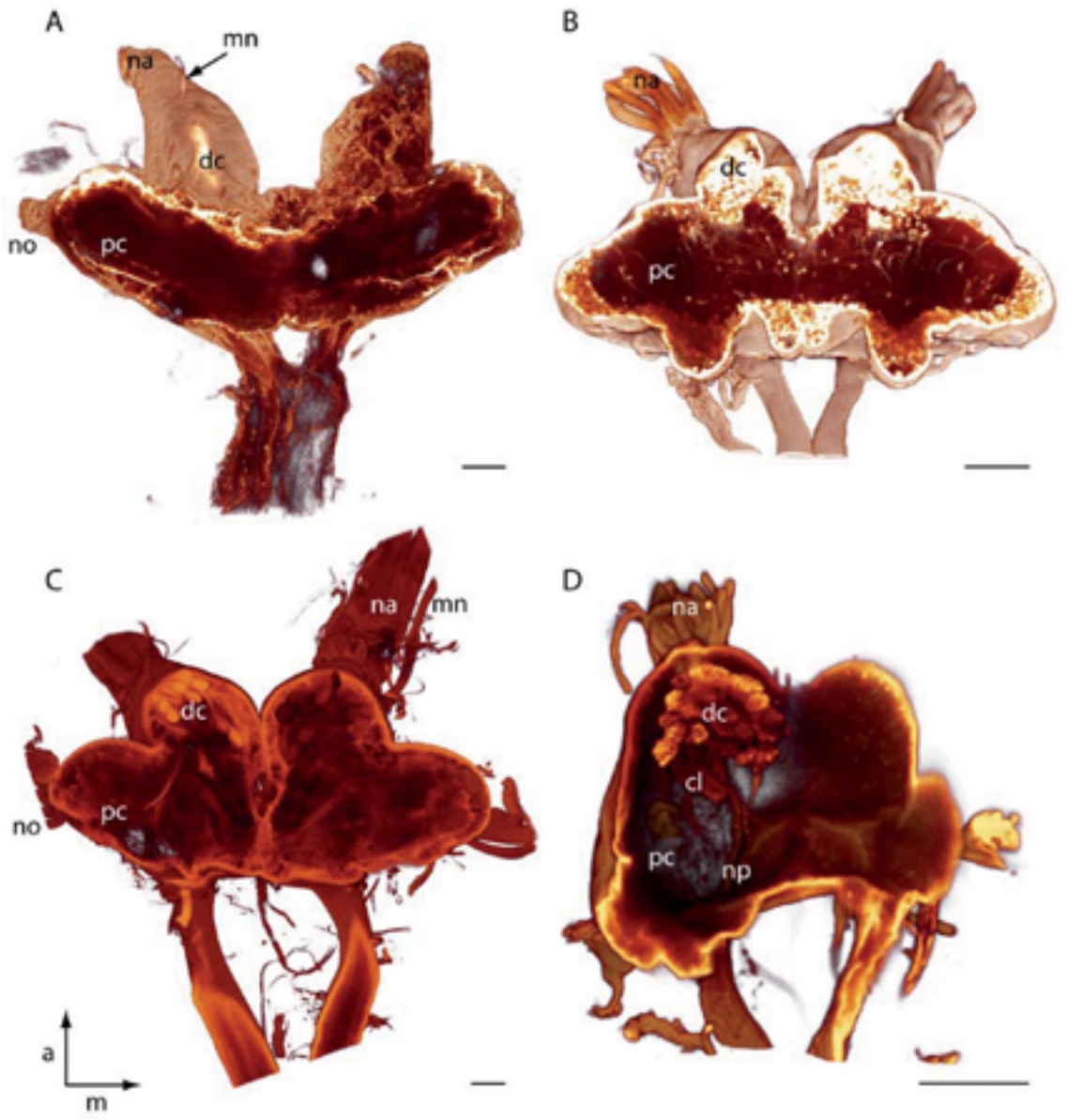


Abbildung 6: 3D Voltexrendering verschiedener Gehirne der Chilopoda auf der Basis confokaler Scans von Autofluoreszenzpräparationen. Ansichten von dorsal mit angeschnittenem Protocerebrum und zum Teil sichtbaren deutocerebralen Neuropilen und Projektionen. **A** *Lithobius forficatus*. Die mächtigen deutocerebralen Loben sind nach frontal abgesetzt und werden von robusten Antennalnerven innerviert. Lateral ist der kompakte Nervus opticus zu erkennen. Ein kleiner dorsaler Nerv ist auf den Deutocerebralloben erkennbar – hierbei handelt es sich höchstwahrscheinlich um den efferenten, motorischen Antennennerv. **B** *Cryptops hortensis* mit charakteristischer Morphologie einzelner Antennalnerven. Aufgrund von dorsoventral verlaufenden Muskeln ist das Protocerebrum posterior ausgebuchtet. **C** *Scolopendra oraniensis*. Die Unterteilung des Antennalnervs ist nur schwach erkennbar. Auf der rechten Seite zieht lateral der kräftigen Antennalnerven der mutmaßliche efferente, motorische Antennalnerv. **D** *Stigmatogaster dimidiatus* mit nahezu verschmolzenen Deutocerebralloben. Im Deutocerebrum sind anterograd angefärbte olfaktorische Glomeruli in einem konkaven olfaktorischen Lobus erkennbar. Weiter ventral ist der Corpus lamellosum (cl) sowie projizierende Neuritenbündel (np) erkennbar, Das Protocerebrum ist in seiner lateralen Ausdehnung stark reduziert. (Originale).

a anterior, **cl** Corpus lamellosum, **dc** Deutocerebrum, **m** median, **mn** motorischer Antennennerv, **na** Nervus antennalis, **no** Nervus opticus, **np** Neuritenprojektion, **pc** Protocerebrum. Maßstäbe = 100 µm.

2.2 Deutocerebrum

Das Deutocerebrum bildet gemäß der Körperachse die anteriore Grenze des Nervensystems und wird durch die Antennalnerven frontal bzw. frontolateral innerviert (Abbildung 5, 6, 16). Bei *Scutigera coleoptrata* verlaufen die kräftigen Antennalnerven zunächst parallel zur Körperache und biegen dann in einem stumpfen Winkel zur Medianen (Abbildung 8, 16) (Sombke et al. 2011a). Bei den übrigen von mir untersuchten Chilopoda ist der Eintritt der Antennalnerven, welche mehr oder weniger parallel zueinander verlaufen, frontal (Abbildung 6, 16). Bei den Scutigeromorpha, Lithobiomorpha und Craterostigmomorpha ist der Antennalnerv als einzelner, robuster/ kompakter Nerv ausgeprägt (Abbildung 6 A). Bei den Scolopendromorpha und Geophilomorpha ist der Antennalnerv in einzelne Nervenbündel untergliedert bzw. aufgegliedert (Abbildung 6 B – D). Diese Befunde bestätigen die Darstellung von Seifert (1967). Die Beschreibung von Lorenzo (1960) von 10 bis 15 Bündeln sensorischer Axone bei *Arenophilus bipuncticeps* (Geophilomorpha) wurde für die hier untersuchten Geophilomorpha von mir ebenfalls bestätigt (*Haplophilus subterraneus* ca. 15, *Stigmatogaster dimidiatus* >20). Bei *Cryptops hortensis* treten ca. zehn separate Nerven auf.

Nach Seifert (1967) spaltet sich der Antennalnerv kurz vor Eintritt in die Antenne bei den Scutigeromorpha und Lithobiomorpha in einen dickeren, sensorischen und einen dünneren, motorischen Ast. Diese Darstellung ist etwas irreführend, weil zum einen die Aufspaltung zum olfaktorischen Lobus und zum Corpus lamellosum gemeint sein kann (Sortierungszone), zum anderen aber auch die Präsenz eines efferenten, motorischen Antennalnervs (Abbildung 6 A, C: mn). Nach eigenen Untersuchungen sind beide Zustände bei den Chilopoden realisiert. Eine Sortierungszone beschreibt den Bereich, in dem die zunächst parallel laufenden sensorischen Neuriten nach oder beim Eintritt in das Deutocerebrum ihre Projektionsrichtung zu ihren Zielstrukturen ändern. Somit ist die Sortierungszone von sich überkreuzenden Neuriten gekennzeichnet. Bei *S. coleoptrata* konnte ich diese Zone mit Golgi-Imprägnationen nachweisen. Deutlich separieren sich hier die Neuriten, die die olfaktorischen Glomeruli und den Corpus lamellosum innervieren. Durch antennale Backfill-Experimente konnten diese Strukturen ebenfalls dargestellt werden (Abbildung 7, 13 B, 16 B). Abbildung 7 zeigt 3D-Voltexrenderings des rechten Deutocerebrums von *Cryptops hortensis* in verschiedenen horizontalen Ebenen sowie verschiedenen Darstellungsarten. Aus diesen eigenen Befunden sind deutlich überkreuzende Axone erkennbar die einen olfaktorischen Glomerulus als Zielstruktur innervieren (Abbildung 7 A, 16), weshalb eine uniglomeruläre Innervierung angenommen wird. Ähnliche Sortierungszonen lassen sich auch bei den Tetraconata feststellen, wie zum Beispiel beim Tabakschwärmer *Manduca sexta* (Rössler et al. 1999) oder bei der karibischen Languste *Panulirus argus* (Schmidt und Ache 1992). Bei letzterer konnte nachgewiesen werden, dass dünnere Axone in die olfaktorischen Loben und dickere Axone in das laterale Antenne 1 Neuropil (LAN)

projizieren. Eine ähnliche Separierung von dünneren und dickeren Neuriten konnte für *Scutigera coleoptrata* nachgewiesen werden (Abbildung 12 B, 16 A-D). Die morphologische Divergenz sensorischer Neuriten, die das Deutocerebrum innervieren, ist ebenso bei den Hexapoda bekannt. Bei *Periplaneta americana* innervieren dünne Neuriten die Glomeruli im anterioventralen Bereich des Deutocerebrum, während dickere mechanosensorische Afferenzen den Dorsallobus und das Unterschlundganglion innervieren (Nishino et al. 2005).

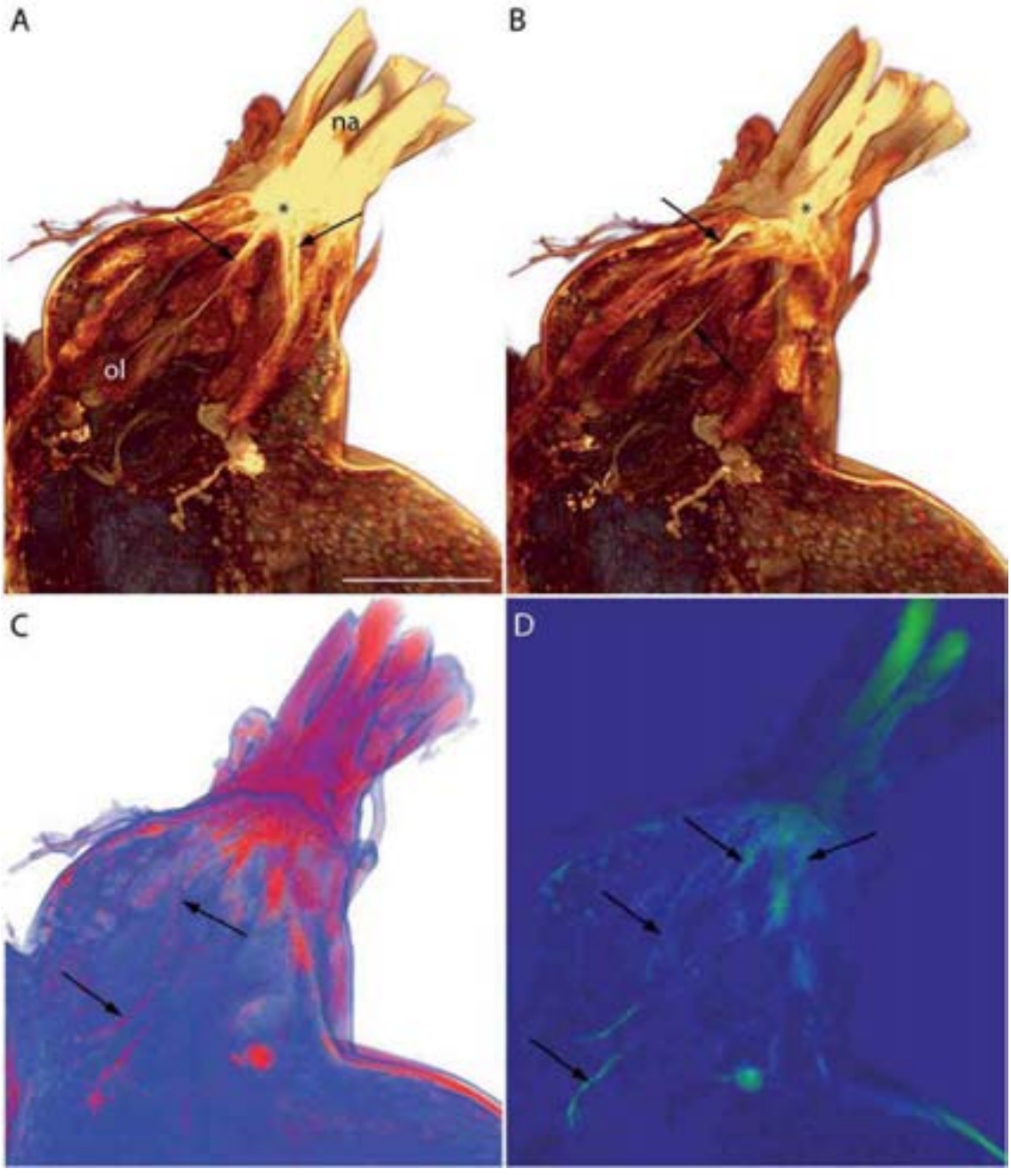


Abbildung 7: 3D Voltexrendering des rechten Deutocerebrums und des Antennalnervs von *Cryptops hortensis* (Neurobiotin Backfill). Ansichten von dorsal. **A** und **B:** Verschiedene Ebenen der Sortierungszone (Sternchen) und Innervation von olfaktorischen Glomeruli. Verschiedene Projektionen mit überkreuzenden Mustern sind erkennbar. **C** und **D:** Verschiedene Visualisierungen der gleichen Präparation. Axonale Projektionen in rot (C) und grün (D). Auch hier sind überkreuzende Projektionen sowie Innervationsmuster einzelner olfaktorischer Glomeruli erkennbar. (Originale).

na Nervus antennalis, **ol** olfaktorischer Lobus. Maßstab = 100 µm.

2.3 Tritocerebrum und stomatogastrisches Nervensystem

Eine Grenze zwischen Proto- und Deutocerebrum sowie zwischen Deuto- und Tritocerebrum ist nicht klar erkennbar. Bei *Scutigera coleoptrata* ist die Grenze zwischen Deuto- und Tritocerebrum nur durch die abziehenden Nervi labrales (Abbildung 8: n6) und die Frontalkonnektive lokalisierbar. Die Frontalkonnektive projizieren leicht caudad und innervieren das median frei liegende Frontalganglion (Abbildung 8: fg). Vom Frontalganglion zieht caudad der Nervus recurrens ab, der dorsal des Ösophagus verläuft (Abbildung 5: nr, 8: n4). Weiter caudad bildet dieser ein kleines Hypocerebralganglion (nicht dargestellt). Die stomatogastrische Brücke inklusive des Nervus recurrens ist bei allen untersuchten Chilopoden ausgebildet (Seifert 1967, eigene Untersuchungen).

Detaillierte, vergleichend morphologische Untersuchungen der Nerven des Gehirns der Chilopoda wurden nur von Fahlander (1938) und Seifert (1967) durchgeführt. Leider sind diese nicht widerspruchsfrei und anhand der Zeichnungen und Beschreibungen ist es zum Teil nicht möglich, eine eindeutige Zuordnung zu treffen. So fehlt zum Beispiel eine einheitliche Terminologie, vor allem der tritocerebralen Nerven. Ein von Fahlander (1938) für *Thereuopoda clunifera* (Scutigeromorpha) beschriebener Frontalnerv, der von den Frontalkonnektiven ausgehen soll und von Seifert (1967) als Artefakt interpretiert wurde, konnte nach eigenen Befunden bei *S. coleoptrata* nicht verifiziert werden. Die Labralnerven konvergieren anteroventral und projizieren weiter ventrad in die clypeolabrale Region. Der Nervus connectivus II soll nach Seifert (1967) bei *Lithobius forficatus* vom Frontalganglion ausgehen. Das Innervationsziel ist allerdings unklar (Rilling 1968). Dieser Nerv konnte bei *S. coleoptrata* ebenfalls nachgewiesen werden (Abbildung 8: n5). Der Nervus connectivus I soll bei *L. forficatus* weiter posterior vom ventralen Bereich des Tritocerebrums abgehen. Auch dieser Nerv konnte nach eigenen Befunden bei *Scutigera coleoptrata* nachgewiesen werden (Abbildung 8: n8). Eine Reihe weiterer Nerven projiziert ventrad vom Tritocerebrum und innerviert die Region des Hypopharynx sowie Muskulatur (Abbildung 8: n7). Die kräftigen subösophagealen Konnektive verbinden sich weiter caudad und verbinden das Gehirn mit dem Unterschlundganglion (Abbildung 5: USG). Auch wenn in der vorliegen Arbeit die Architektur des stomatogastrischen System nicht vertiefend thematisiert wurde, lassen sich aus den Rekonstruktionen des Nervensystems dennoch einige Schlüsse ziehen.

Eine freie tritocerebrale Kommissur, wie von Fahlander (1938) beschrieben, konnte nicht nachgewiesen werden, was ebenfalls die Ergebnisse von Seifert (1967) bestätigt.

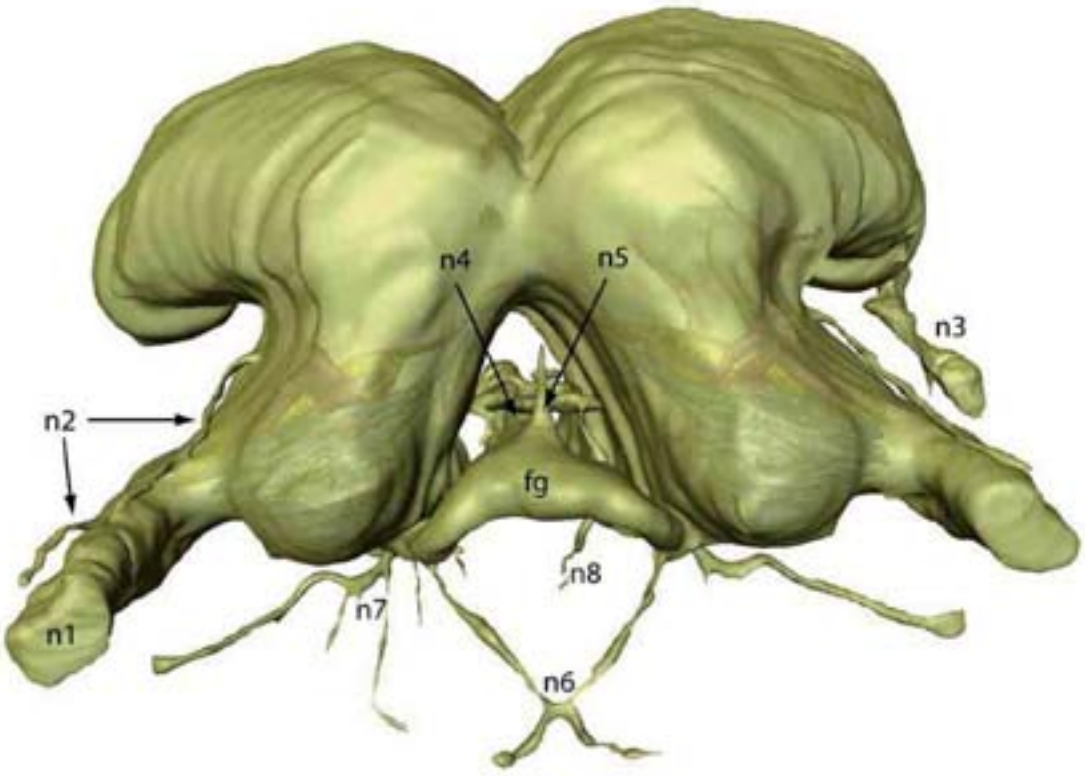


Abbildung 8: 3D Rekonstruktion des Gehirns von *Scutigera coleoptrata* mit abziehenden Nerven. Ansicht von frontal (Protocerebrum dorsal). (Original).

fg Frontalganglion mit den lateralen Frontalkonnektiven, **n1** Nervus antennalis, **n2** motorischer Antennennerv, **n3** Nervus tömösváryi, **n4** Nervus recurrens, **n5** Nervus connectivus II (*sensu* Seifert 1967), **n6** Nervi labrales, **n7** Innervierung der Clypeolabralregion inklusive der Muskulatur, **n8** Nervus connectivus I (*sensu* Seifert 1967).

3. Die olfaktorischen Loben

Das Deutocerebrum besteht bei den Chilopoda, wie bei den Crustacea und Hexapoda, aus zwei distinkten Neuropilbereichen: den olfaktorischen Loben (bei Hexapoden: Antennalloben) und dem mechanosensorischen Neuropil (siehe Kapitel 5). Der Begriff olfaktorischer Lobus (Fahlander [1938]: Lobus olfactorius) impliziert eine Verarbeitung von Geruchsinformationen (= Olfaktion) und schließt daher theoretisch den Geschmackssinn (= Gustation) aus. Betrachtet man primäre sensorische Strukturen, wie antennale Sensillen, wird oft deutlich zwischen Olfaktion und Gustation differenziert (siehe Kapitel 8). In Bezug auf Strukturen des Nervensystems kann diese Trennung allerdings nicht getroffen werden. Deshalb sollte konsequenterweise der Begriff chemosensorischer Lobus bevorzugt werden. Traditionell wird aber auch die Wahrnehmung und Verarbeitung chemosensorischer Reize bei aquatischen Tieren (zum Beispiel bei den Crustacea) als Olfaktion bezeichnet. In der vorliegenden Arbeit wird die anteriore Neuropilstruktur des Deutocerebrum weiterhin als olfaktorischer Lobus bezeichnet. Der Aussage von Strausfeld (1998), dass die olfaktorischen Loben der Chilopoden im dorsalen Teil des Unterschlundganglions lokalisiert sind, muss hier widersprochen werden, da diese Lokalisation älteren Darstellungen (z.B. Strausfeld et al. 1995) und den hier dargestellten Befunden eindeutig widerspricht.

Wie bereits erwähnt, bedingt der Eintrittswinkel der Antennalnerven in das Gehirn die Orientierung des olfaktorischen Lobus. Bei *Scutigera coleoptrata* bewirkt dies, dass die olfaktorischen Loben fast rechtwinklig zur Körperachse und damit mit ca. 180° zueinander verlaufen (Abbildung 8, 12 A) (Sombke et al. 2001a). Aufgrund der frontalen Innervierung und der posteriomedianen Projektion beträgt der Winkel bei den übrigen von mir untersuchten Chilopoda maximal 90° (Abbildung 6, 9, 16) bzw. stehen die Loben fast parallel zueinander. Der olfaktorische Lobus setzt sich aus diskreten Untereinheiten, den olfaktorischen Glomeruli, zusammen. Prinzipiell sind diese bei den Pleurostigmophora in einer versetzten, parallelen Anordnung im olfaktorischen Lobus verteilt. Dies bedeutet, dass sensorische Axone aus dem Antennalnerv ihre Zielglomeruli in einer dem Winkel des Lobus folgenden Bahn innervieren. Bei *S. coleoptrata* findet diese Innervierung ungefähr auf gleicher Höhe statt (Abbildung 8, 9 A). Hier erstrecken sich die langgezogenen Glomeruli zur dorsomedianen und füllen somit den gesamten anterioren Teil des Deutocerebrums aus. Bei *Lithobius forficatus* und *Craterostigmus tasmanianus* sind die Glomeruli überwiegend tropfenförmig und werden nicht auf einer einheitlichen Höhe innerviert (Abbildung 9; 10 B, C; 12 D, F). Das distale Ende ist zum Antennalnerv hin ausgezogen und die Glomeruli sind antero-posterior gegeneinander verschoben. Eine Ausnahme bilden die kontralateral verbunden Glomeruli bei *L. forficatus*, die ähnlich wie bei *S. coleoptrata* langezogen sind und nach dorsomedian ziehen. Bei *C. tasmanianus* sind die meisten olfaktorischen Glomeruli an den dorsomedianen Rand der Deutocerebralloben gerückt (Abbildung 10 C, 12 E), was zum Teil auch bei den Scolopendromorpha zu beobachten ist (Abbildung

13 B, C, G). Bei den Scolopendromorpha rücken die tropfenförmigen Glomeruli noch stärker zusammen und bilden zum Teil traubenförmige Muster (Abbildung 7, 9, 10 D, 13 A-C). Der Lobus selbst bildet dorsomedian eine leicht konkave Form aus. Dieses Muster findet sich auch bei den Geophilomorpha. Hier ordnen sich die zum größten Teil kugeligen Glomeruli in einem posterior konkaven olfaktorischen Lobus (Abbildung 6 D, 9, 10 E, F; 13 F, 16, 17).

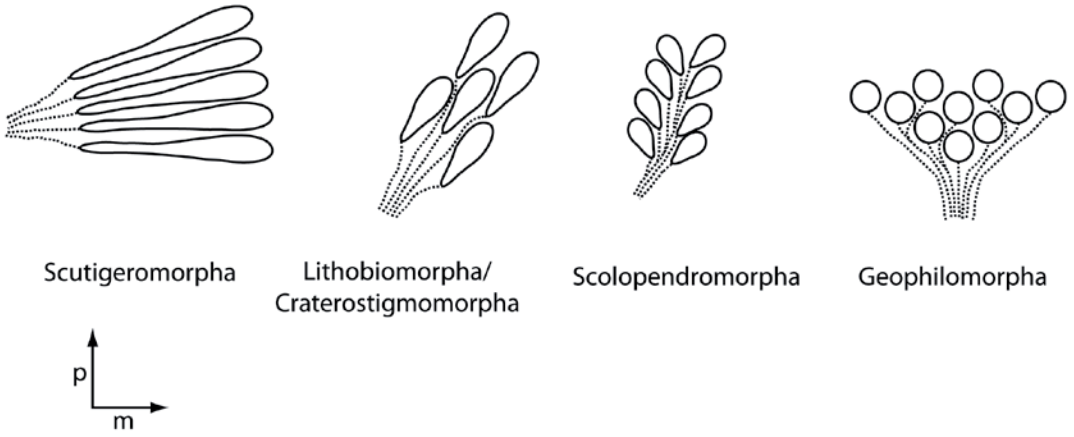


Abbildung 9: Schematische Darstellung von Projektionsrichtungen, Arrangement und Innervierung der olfaktorischen Glomeruli innerhalb einer theoretischen Ebene in den olfaktorischen Loben der Chilopoda. Ansicht von dorsal. (Original).

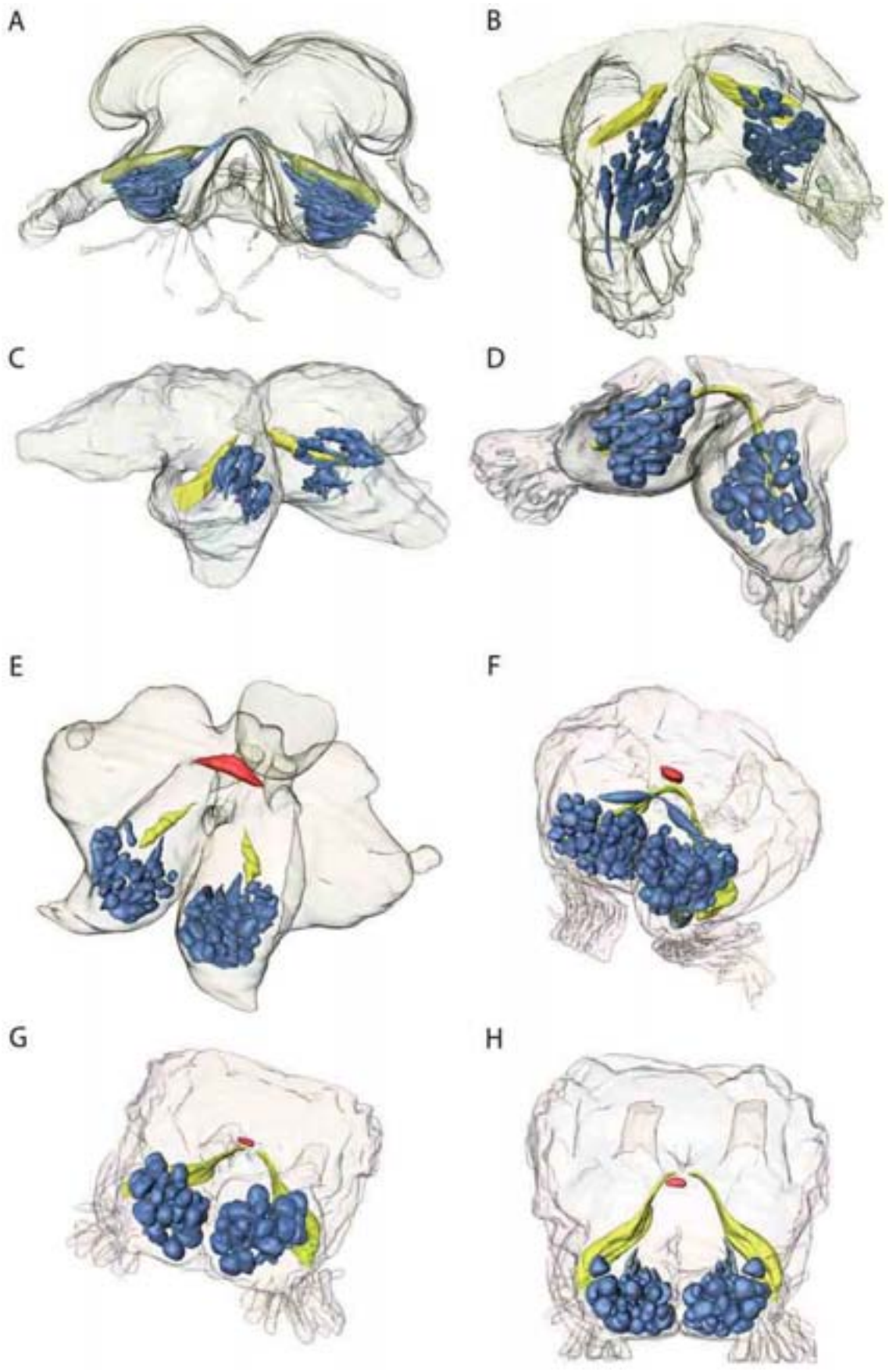
Die Form des olfaktorischen Lobus lässt sich daher mit der Lokalisation der Antenneninnervierung, dem Arrangement der olfaktorischen Glomeruli und mit ihrer Form und Innervation erklären. Im ersten Fall bedingt die Innervation den Winkel der Loben zueinander. Im zweiten Fall werden die Loben mit der Zunahme an olfaktorischen Glomeruli kompakter (Abbildung 7, 9, 10 C, D). Da die Glomeruli in Projektionsrichtung des Antennalnervs innerviert werden und ihre Form eine immer kompaktere Zusammenlagerung ermöglicht (Abbildung 9, 10), variiert die Form des olfaktorischen Lobus von einem Doppelkegel, dessen spitze Seiten am Antennalnerv und an der contralateralen Projektion liegen (Scutigeromorpha), bis zu einer kugelförmigen, posterior konkaven Form (Geophilomorpha).

4. Die olfaktorischen Glomeruli

Der olfaktorische Lobus setzt sich aus den olfaktorischen Glomeruli (OG) zusammen. Mittels Backfill-Techniken und Golgi-Imprägnationen wurde nachgewiesen, dass die OG bei *Scutigera coleoptrata* primäre Verschaltungsorte im Deutocerebrum darstellen und durch charakteristische, dünne sensorische Axone innerviert werden (Abbildung 12 B) (Sombke et al. 2011a). Anterograde Anfärbungen wurden auch für die meisten der hier dargestellten Taxa angewendet (mit Ausnahme von *Craterostigmus tasmanianus*). Bei *S. coleoptrata* wurden in der vorliegenden Studie pro deutocerebraler Hemisphäre 34 individuell identifizierbare langgestreckte OG gefunden deren Anzahl, Position, Arrangement und Form invariant ist (Abbildung 9, 10 A, 11, 12 A). Bereits Saint-Rémy (1887, 1889) und Hörberg (1931) beschrieben in kurzer Form die olfaktorischen Glomeruli. Hörberg (1931) benannte die Massen in den „gut entwickelten Lobi antennalis" als „unregelmäßige, wurmähnliche, stark gewundene Bänder" und erkannte deren kontralaterale Verbindung (clc). Fahlander (1938) beschrieb die olfaktorischen Glomeruli bei *Thereuopoda clunifera* (Scutigeromorpha) als „zahlreiche langgestreckte, im Querschnitt kreisförmige, dichte Glomerulimassen" und ergänzte weiterhin:

„Diese Partie des Lobus olfactorius ist mit der entsprechenden Partie in dem anderen Lappen durch die vordere Antennenkommissur verbunden."

Diese Kommissur konnte in der vorliegen Arbeit auch bei *Lithobius forficatus* und *Stigmatogaster dimidiatus* nachgewiesen werden (siehe Abbildung Seite 6: clc, Abbildung 10 B, F), wobei bei *L. forficatus* zwei contralaterale Glomerulipaare diese Verbindung herstellen (Abbildung 12 D), während bei *S. dimidiatus* nur ein contralaterales Paar vorhanden ist. In den übrigen untersuchten Chilopoda konnte diese anteriore Deutocerebralkommissur (*sensu* Fahlander 1938) nicht gefunden werden. Contralaterale Kommissuren der olfaktorischen Loben (OL) sind bei mehreren Crustacea von Hanström (1928, 1929, 1931) beschrieben worden. Viele dieser Beschreibungen konnten in aktuelleren Untersuchungen nicht bestätigt werden (zusammengefasst in Schachtner et al. 2005). Gesichert scheint die Existenz einer anterioren Deutocerebralkommissur allerdings für die Leptostraca (Kenning und Harzsch in Vorb.). Ein Vorhandensein bei den Stomatopoden und Euphausiacea ist fraglich. Innerhalb der Hexapoda sind contralaterale Verbindungen des OL zum Beispiel bei Diptera bekannt (Boeckh et al. 1970, Homberg et al. 1989). Ein grundlegender Unterschied scheint allerdings zu sein, dass bei Chilopoda diese Kommissur durch die OG realisiert wird (Abbildung 12 A, D, 18), und damit neuropilär ist, während bei den Tetraconata diese Verbindung nur über Neuriten verschaltet zu sein scheint.


A
B
C
D
E
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G
H

Abbildung 10: 3D Rekonstruktionen der Gehirne verschiedener Chilopoda. (Originale). Darstellung der olfaktorischen Glomeruli (blau), dem Corpus lamellosum (gelb) sowie des Zentralkörpers (rot) (nicht in allen Rekonstruktionen dargestellt). Contralaterale Verbindungen des CL wurden nicht in allen Rekonstruktionen dargestellt. **A** *Scutigera coeloptrata*, **B** *Lithobius forficatus*, **C** *Craterostigmus tasmanianus*, **D** *Scolopendra subspinipes*, **E** *Cryptops hortensis*, **F** *Stigmatogaster dimidiatus*, **G** *Haplophilus subterraneus* (von dorsolateral), **H** *Haplophilus subterraneus* (von dorsal). (Originale).

Nach Fahlander (1938) ähnelt die interne Organisation deutocerebraler Neuropile von *Lithobius forficatus* stark der der Scutigeromorpha, was allerdings nur teilweise richtig ist, da die Form und Anordnung der OG deutlich variiert. In einer kurzen Darstellung beschrieben Strausfeld et al. (1995) einen glomerulären Antennallobus für *Lithobius variegatus* Leach, 1913 und schematisierten in einer Zeichnung kugelige olfaktorische Glomeruli und einen posterioren Dorsallobus. Durch histologische Schnitte und anterograde Färbungen konnte allerdings in der vorliegenden Studie nachgewiesen werden, dass *L. forficatus* 43 OG pro deutocerebraler Hemisphäre besitzt, die eine längliche, zum Teil tropfenförmige Form aufweisen (Abbildung 10 B; 12 C, D) und der Corpus lamellosum aus einzelnen Lamellen inklusive einer contralateralen Kommissur besteht (siehe Kapitel 5). Bei *Craterostigmus tasmanianus* war es in eigenen Autofluoreszenz-Untersuchungen nicht möglich, die Anzahl der OG exakt zu ermitteln. Aus einer histologischen Schnittserie konnte allerdings die Anzahl von 36 OG pro deutocerebraler Hemisphäre rekonstruiert werden (Abbildung 10 C). Ähnlich den OG von *Lithobius forficatus*, sind sie bei *C. tasmanianus* langgestreckt und tropfenförmig sowie anteroposterior verschoben, wenngleich sie bei *L. forficatus* länger ausgezogen sind (Abbildung 12 F). Innerhalb der Scolopendromorpha zeigen die OG eine tropfenförmige und selten kugelige Form (Abbildung 13 A-D). Hier beträgt die Anzahl der OG pro deutocerebraler Hemisphäre bei *Scolopendra subspinipes* 51, bei *Cryptops hortensis* 56 und bei *Scolopendra oraniensis* 58. Die Reduktion der Augen bei *C. hortensis* (alle Cryptopidae sind augenlos) scheint keinen Einfluss auf die Anzahl der OG zu haben; die Anzahl der OG ist bei den untersuchten Scolopendromorpha in etwa gleich. Wie im vorigen Abschnitt dargelegt, liegen die OG im olfaktorischen Lobus der Scolopendromorpha dichter gepackt als bei *L. forficatus* und *C. tasmanianus* (Abbildung 6 C, 7, 9, 10, 13 A, C). Eine contralaterale Verbindung der olfaktorischen Loben, wie für *Scolopendra cingulata* von Fahlander (1938) beschrieben, konnte im Rahmen dieser Dissertation bei den untersuchten Scolopendromorpha nicht nachgewiesen werden. Im Unterschied zu den übrigen Chilopoda erscheint die Form der OG bei den Geophilomorpha zum überwiegenden Teil kugelig (Abbildung 13 E, F). Bei den untersuchten Taxa variiert die Anzahl der OG stark mit 49 bei *Haplophilus subterraneus* und 97 bei *Stigmatogaster dimidiatus*. Eine Ausnahme in der Form bilden hier die bereits erwähnten langgestreckten, contralateral verbundenen OG bei *S. dimidiatus* (Abbildung 10 F).

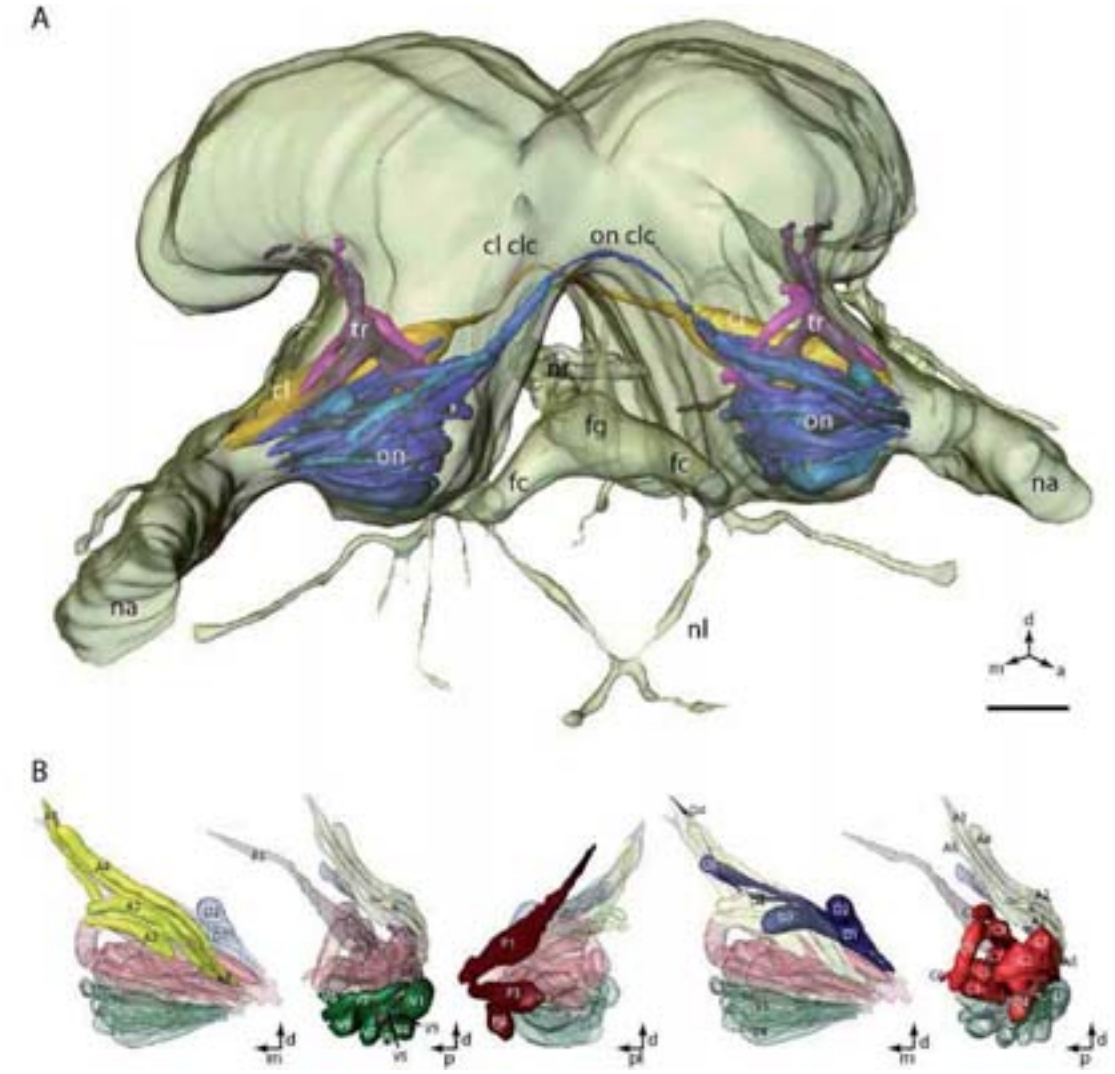

Abbildung 11: 3D Rekonstruktion der Gehirns und deutocerebraler Neuropile von *Scutigera coleoptrata* (aus Sombke et al. 2011a). **A** olfaktorische Glomeruli (on, blau), Corpus lamellosum (cl, gelb), Neuritenprojektionen (tr) violett. **B** Rekonstruktion der olfaktorischen Glomeruli des linken olfaktorischen Lobus aus verschiedenen Perspektiven. Darstellung verschiedener gruppierter OG mit Farbkodierung: anterior (gelb), ventral (grün), posterior (braun), dorsal (blau), zentral (rot).

a anterior, **cl** Corpus lamellosum, **clc** contralaterale Verbindung, **d** dorsal, **fc** Frontalkonnektive, **m** median, **na** Nervus antennalis, **nl** Nervus labralis, **nr** Nervus recurrens, **on** olfaktorische Glomeruli/Neuropile, **p** posterior, **pl** posteriolateral. Maßstab = 100 µm.

Die olfaktorischen Glomeruli weisen bei *Scutigera coleoptrata* und *Scolopendra subspinipes* in immunhistochemischen Experimenten eine starke Synapsin-Immunreaktivität auf, was das Vorhandensein einer erhöhten Anzahl an Synapsen kennzeichnet (Abbildung 15 A, B, E, F, G). Die Lokalisation von tyrosiniertem Tubulin (Microtubuli-Marker) bei *S. coleoptrata* zeigt, dass neben der Muskulatur und

Cytoskelettbestandteilen die olfaktorischen Glomeruli eine hohe Immunreaktivität besitzen (Abbildung 15 C, D). Ähnliche Ergebnisse lassen sich durch eine histochemische Färbung gegen f-Actin mit Phallotoxinen bei *S. subspinipes* erlangen (Abbildung 15 G). Die Lokalisation des Neuropeptides RFamid bei *S. coleoptrata* zeigt, dass hauptsächlich in Neuriten um die OG eine schwache Immunreaktivität besteht. Die Immunreaktivität in den OG selbst ist nur gering. Zudem zeigen einige mediolaterale Somata eine RFamid-Immunreaktivität (Sombke et al. 2011a). Allatostatin zeigt eine starke Immunreaktivität innerhalb der OG bei *S. coleoptrata* und *S. subspinipes* (Abbildungen 15 E-F). Zudem finden sich bei *S. coleoptrata* ca. 30 Allatostain-immunreaktive Somata anterior der OG. Bei *S. subsipnipes* befindet sich ein Cluster von Allatostain-immunreaktiven Somata posterior in der Region des Frontalganglions (Abbildung 15 E).

Zusammenfassend setzt sich bei den Chilopoda der olfaktorische Lobus aus einer unterschiedlichen Anzahl von olfaktorischen Glomeruli zusammen. Diese variiert bei den untersuchten Taxa von 34 bei *Scutigera coleoptrata* bis zu 97 bei *Stigmatogaster dimidiatus*. Bei den Hexapoda scheint die Anzahl der olfaktorischen Glomeruli taxonspezifisch konstant zu sein (Chambille und Rospars 1981, Rospars 1983, Rospars und Hildebrand 1992, Galizia et al. 1999, Laissue et al. 1999, Berg et al. 2002, Huetteroth und Schachtner 2005, Kirschner et al. 2006, Ghaninia et al. 2007, Zube et al. 2008, Dreyer et al. 2010). Hier variiert sie von ca. 40 bei Diptera und Orthoptera bis zu über 250 bei Formicidae (zusammengefasst in Schachtner et al. 2005). Bei den malakostraken Crustacea variiert die Anzahl von ca. 150 bis ca. 1300 und scheint nicht taxonspezifisch fixiert zu sein (Blaustein et al. 1988, Beltz et al. 2003, Schachtner et al. 2005, Krieger et al. 2010).

Der auffälligste Unterschied der OG innerhalb der Chilopoda ist die Form bzw. deren Veränderung innerhalb der höheren Taxa. Prinzipiell ist eine Transformation der OG von langestreckt bei den Scutigeromorpha, über langestreckt/tropfenförmig bei den Lithobiomorpha und Craterostigmomorpha, zu tropfenförmig/ovoid bei den Scolopendromorpha bis hin zu annähernd kugelig innerhalb der Geophilomorpha (Abbildung 9, 10, 16, 17) festzustellen. Bei den Hexapoda ist der olfaktorische Lobus (hier auch Antennallobus genannt) aus überwiegend kugelförmigen OG aufgebaut. Bei den phylogenetisch basalen Taxa variiert allerdings die Form: Während die Collembola 20 bis 30 kugelförmige OG besitzen (Kollmann et al. 2011), treten bei den Archaeognatha langestreckte OG auf (Mißbach et al. 2011).

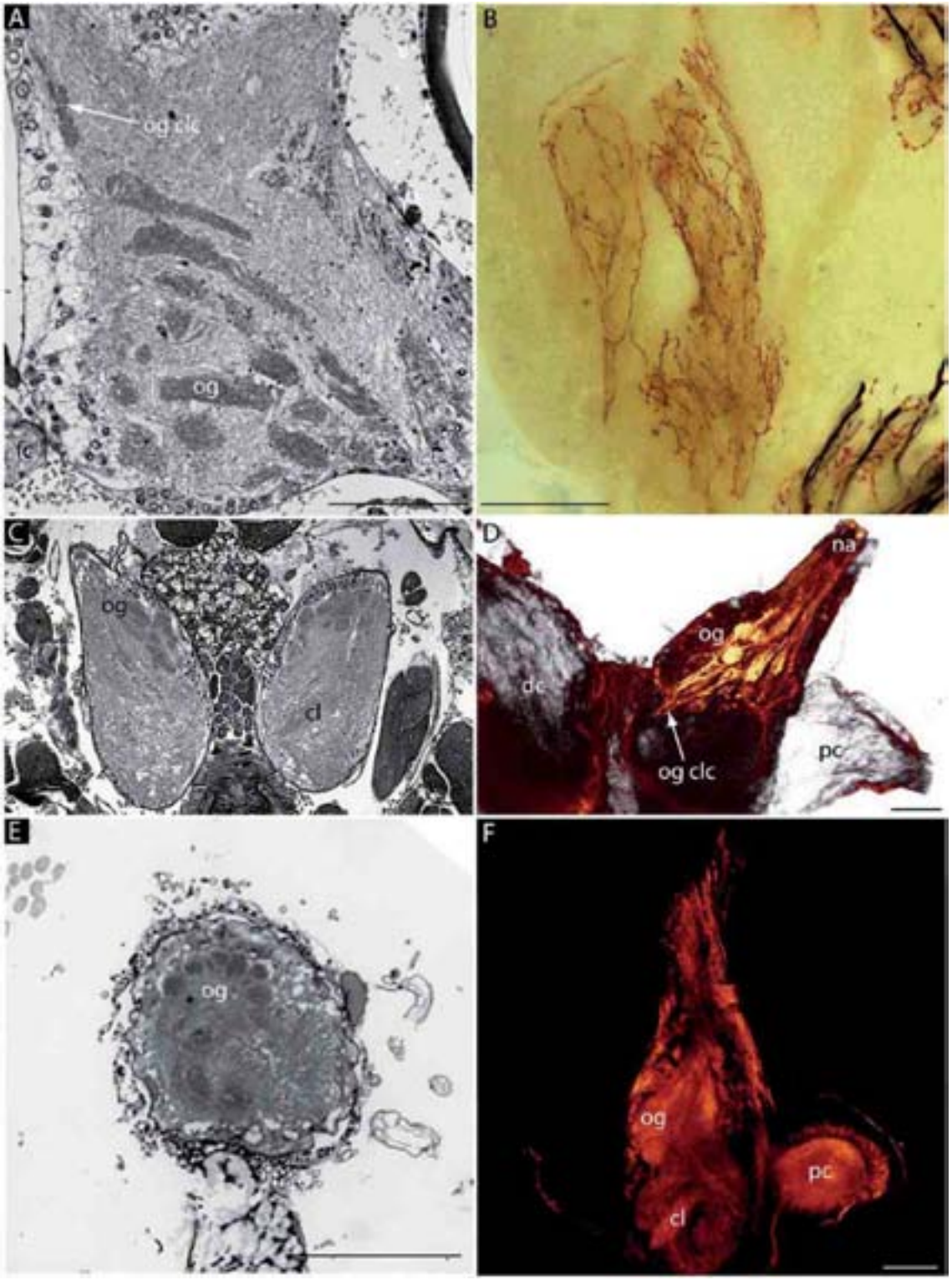

Abbildung 12: Verschiedene Präparationen und Darstellungen der olfaktorischen Glomeruli bei ausgewählten Vertretern der Chilopoden. (Originale). **A** Histologischer Querschnitt durch das linke Deutocerebrum von *Scutigera coleoptrata*. Neben den langgestreckten olfaktorischen Glomeruli (og) sind die contralateral ziehenden OG (og clc) sowie die tritocerebralen Frontalkonnektive (fc) gekennzeichnet. **B** Horizontalschnitt einer Golgi-Imprägnation des Deutocerebrums von *S. coleoptrata*. Dünne afferente Axone mit vermutlich synaptischen Anschwellungen innervieren einzelne olfaktorische Glomeruli. **C** Histologischer Horizontalschnitt durch den Kopf von *Lithobius forficatus*. Deutocerebrale Loben mit zum Teil langgestreckten olfaktorischen Glomeruli (og). Der linke Lobus erscheint anterior zum Antennalnerv ausgezogen. In diesem Schnitt beinhaltet der rechte Lobus einen Anschnitt des Corpus lamellosum (cl). **D**

Anterograde Färbung mit Neurobiotin bei *L. forficatus*. 3D Voltexrendering. Axone und zum Teil langgestreckte olfaktorische Glomeruli sind deutlich gefärbt. Die contralateralen Projektionen (og clc) sind sichtbar. **E** Histologischer Querschnitt durch eine deutocerebrale Hemisphäre von *C. tasmanianus*. Die olfaktorischen Glomeruli (og) sind am dorsomedianen Rand lokalisiert. **F** Horizontaler, optischer Schnitt durch die rechte Hälfte des Gehirns von *Craterostigmus tasmanianus*. Autofluoreszenzpräparation. Langgestreckte, tropfenförmige Glomeruli (og) sind im olfaktorischen Lobus sowie ein Anschnitt des posterior liegenden Corpus lamellosum (cl) erkennbar.

cl Corpus lamellosum, **dc** Deutocerebrum, **na** Nervus antennalis, **og** olfaktorische Glomeruli, **og clc** contralaterale Verbindung der olfaktorischen Glomeruli, **pc** Protocerebrum. Maßstäbe A, C-F = 100 µm, B = 50 µm.

Innerhalb der Crustacea variiert die Form der OG von kugelig bei den Leptostraca (Kenning und Harzsch in Vorb.), Isopoda und Euphausiacea (Johansson und Hallberg 1992, Harzsch et al. 2011) zu keilförmig gestreckt (Schmidt und Ache 1996b, Harzsch und Hansson 2008, Krieger et al. 2010). Bei letzterem Fall sind die keilförmigen OG präzise aneinander und radiär in der Peripherie des olfaktorischen Lobus angeordnet. Einen Sonderfall scheinen die Remipedia darzustellen, bei denen die kugelförmigen OG in mehreren Teilloben angeordnet sind (Fanenbruck und Harzsch 2005).

Bei vielen Taxa der Hexapoda umgeben die radiär angeordneten OG ein grobes zentrales Neuropil (Boeckh und Tolbert 1993, Galizia et al. 1999, Schachtner et al. 2005). Dieses Merkmal wurde bei den Chilopoda nicht nachgewiesen. Zudem treten bei den Hexapoda geschlechtsspezifische Makroglomeruli bei verschiedenen Taxa auf, wie bei Blattaria (Rospars 1988), und Hymenoptera (Galizia et al. 1999). Prinzipiell variiert die Größe der OG innerhalb einzelner Taxa der Chilopoda nur geringfügig (Abbildung 12-14, 20). Die ventral gelegenen, vergrößerten OG bei *Scolopendra oraniensis*, *Cryptops hortensis*, *Haplophilus subterraneus* und *Stigmatogaster dimidiatus* stellen aber eine Ausnahme dar (Abbildung 13 B, 14). Leider konnte eine geschlechtsspezifische Korrelation nicht durchgeführt werden, sodass Macroglomeruli im Sinne der Definition bei den Hexapoda nicht bestätigt werden konnten.

In sämtlichen Präparationen wurden olfaktorische Glomeruli bei den Chilopoden als solide Neuropile ohne Kompartimentierung gefunden. Die Innervierung der OG erfolgt vom distalen Ende aus (Abbildung 7, 9) wobei die Axone parallel zueinander laufen und sich dann im OL zu verzweigen scheinen (Abbildung 7 D, 16). Bei den Tetraconata treten zum Teil Kompartimentierungen auf: bei *Apis mellifera* (Hexapoda: Hymenoptera) haben die OG eine konzentrische Organisation (Pareto 1972, Arnold et al. 1985, Fonta et al. 1993, Galizia et al. 1999, Nishino et al. 2005) wobei sensorische Axone nur die Peripherie innervieren. Projektionsneurone hingegen innervieren das Zentrum und die Peripherie. Longitudinale Kompartimente in „cap", „subcap" und „core" sind bei einigen Decapoda (Anomura, Astacidea) realisiert (Sandeman und Luff 1973, Sandeman und

Sandeman 1994, Langworthy et al. 1997, Schmidt und Ache 1997, Wachowiak et al. 1997, Harzsch und Hansson 2008, Krieger et al. 2010).

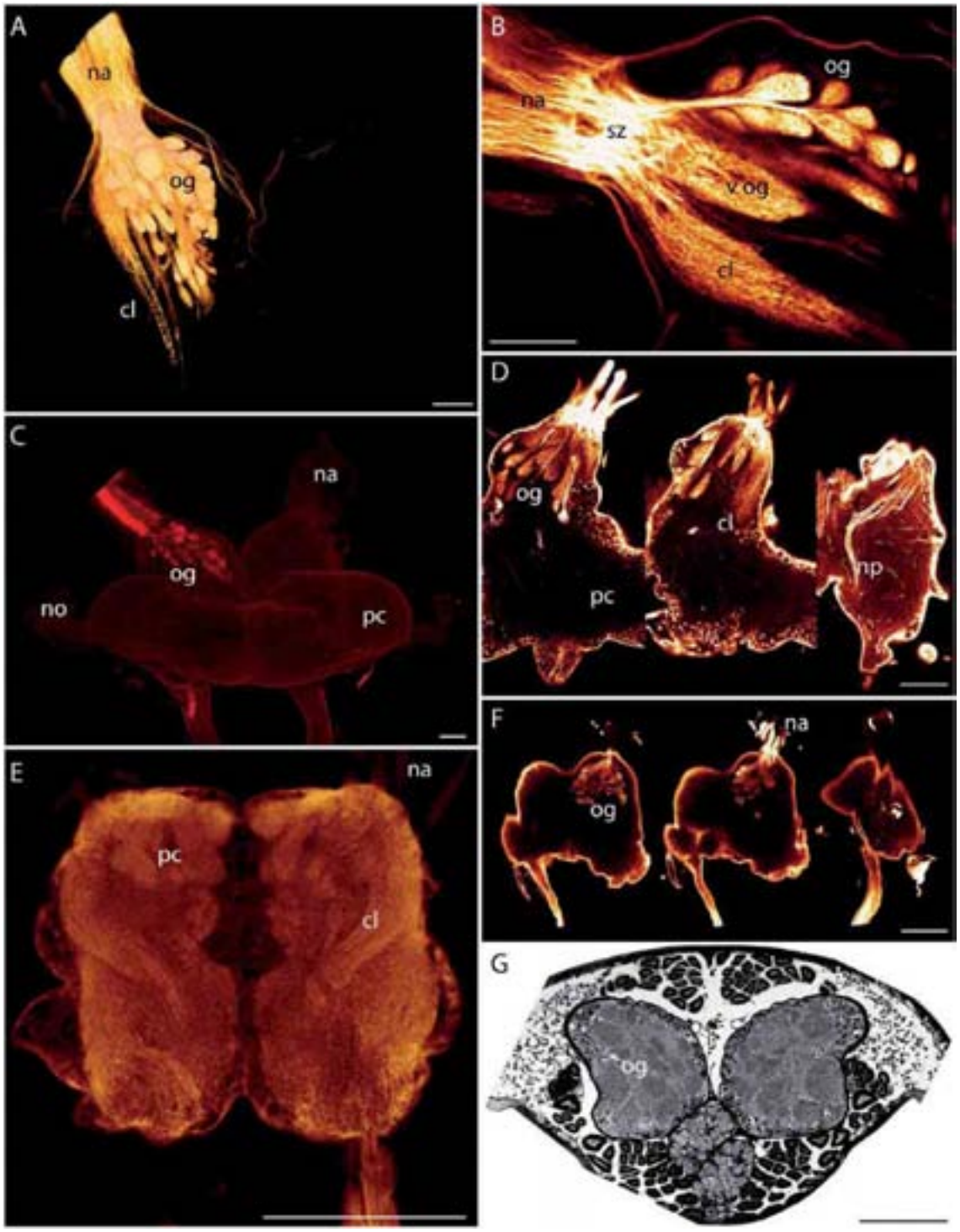

Abbildung 13: Verschiedene Präparationen und Darstellungen der olfaktorischen Glomeruli bei ausgewählten Vertretern der Chilopoda. (Originale). **A** Anterograde Färbung mit Lucifer Yellow des linken Antennalnervs von *Scolopendra oraniensis*. Maximalprojektion. **B** Anterograde Färbung mit Neurobiotin des linken Antennalnervs von *Scolopendra oraniensis*. Dieser optische Schnitt zeigt eine traubenförmige Anordnung von olfaktorischen Glomeruli, den ventralen vergrößerten Glomerulus (v og) und den Corpus lamellosum (cl). In der Sortierungszone (sz) überkreuzen sich die sensorischen Axone in Richtung ihrer Zielstrukturen. **C** Anterograde Färbung mit Neurobiotin des linken Antennalnervs von *S. oraniensis*. Maximalprojektion. Bei dieser Präparation wurde nur eine Subpopulation der sensorischen Axone angefärbt, so dass der Corpus lamellosum sowie Neuritenprojektionen nicht sichtbar sind. **D** Anterograde Färbungen mit Neurobiotin des rechten Antennalnervs von *Cryptops hortensis*. Ausgewählte horizontale optische Schnitte von dorsal nach ventral. Die langgestreckten olfaktorischen Glomeruli, der Corpus lamellosum sowie die Neuritenprojektionen sind sichtbar. **E** Autofluoreszenzpräparation des Gehirns von *Haplophilus subterraneus*. Horizontaler optischer Schnitt mit anterior gelegenen olfaktorischen Glomeruli und dem posterior gelegenen Corpus lamellosum. **F** Anterograde Färbung mit Neurobiotin des rechten Antennalnervs von *Stigmatogaster dimidiatus*. Ausgewählte horizontalem, optische Schnitte. Die kugeligen olfaktorischen Glomeruli, der leicht konkave olfaktorische Lobus sowie Neuritenprojektionen sind sichtbar. **G** Histologischer Querschnitt durch den Kopf von *C. hortensis*. Die olfaktorischen Glomeruli sind am medianen und dorsalen Rand lokalisiert.

cl Corpus lamellosum, **na** Nervus antennalis, **no** Nervi optici, **np** Neuritenprojektion, **og** olfaktorische Glomeruli, **pc** Protocerebrum, **sz** Sortierungszone, **v og** ventraler olfaktorischer Glomerulus. Maßstab = 100 µm.

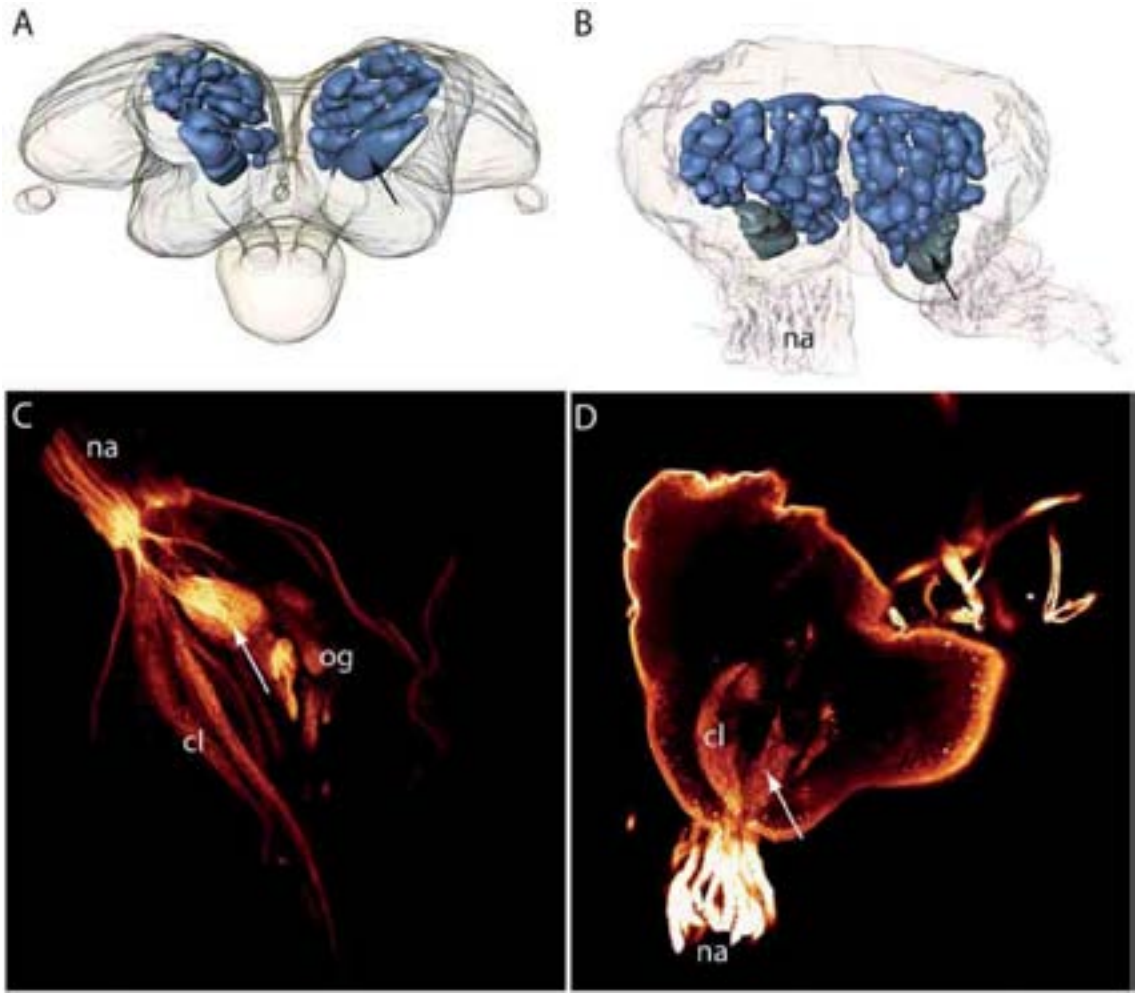

Abbildung 14: Vergrößerte, ventral gelegene olfaktorische Glomeruli bei ausgewählten Chilopoda (Markierungen durch Pfeile). (Originale). **A** 3D Rekonstruktion des Gehirns von *Cryptops hortensis* mit olfaktorischen Glomeruli (blau). Ansicht von anteroventral. **B** 3D Rekonstruktion des Gehirns von *Stigmatogaster dimidiatus* mit olfaktorischen Glomeruli (blau). Ansicht von frontal. **C** Anterograde Färbung mit Neurobiotin des linken Antennalnervs von *Scolopendra oraniensis*. Optischer Schnitt. Ansicht von dorsal. **D** Anterograde Färbung mit Neurobiotin des rechten Antennalnervs von *Stigmatogaster dimidiatus*. Optischer Schnitt. Ansicht von dorsal.

cl Corpus lamellosum, **na** Nervus antennalis, **og** olfaktorische Glomeruli.

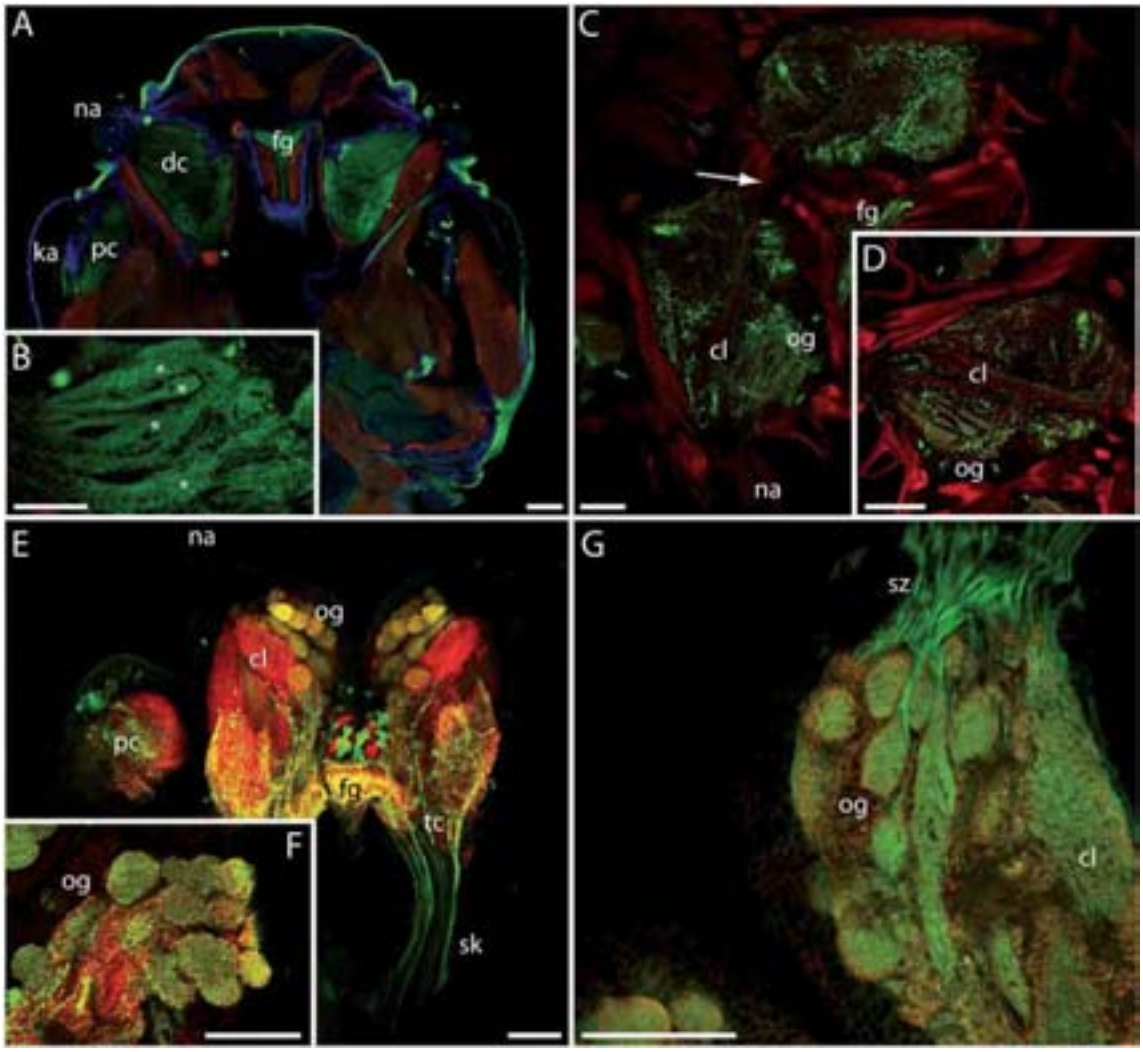

Abbildung 15: A Horizontaler optischer Schnitt durch den Kopf von *Scutigera coleoptrata*. Immunhistochemische Markierungen gegen Synapsin (rot) und Histochemie gegen Phalloidin (rot) sowie Zellkerne (blau). **B** Vergrößerung der olfaktorischen Glomeruli (Sternchen) aus A. **C** Horizontaler optischer Schnitt durch den Kopf von *S. coleoptrata*. Immunhistochemische Markierung gegen Allatostatin (grün). Histochemie gegen Phalloidin (rot). Der Pfeil markiert die contralaterale Verbindung des Corpus lamellosum. **D** Olfaktorische Glomeruli und Corpus lamellosum im rechten Deutocerebrum von *S. coleoptrata* (gleiche Markierung wie in C). **E** Horizontaler optischer Schnitt durch das Gehirn von *Scolopendra subspinipes*. Immunhistochemische Markierung gegen Synapsin (rot) und Allatostatin (grün). **F** Vergrößerung des olfaktorischen Lobus von *S. subspinipes* (selbe Markierung wie in E). **G** Horizontaler optischer Schnitt durch das rechte Deutocerebrum von *S. subspinipes*. Immunhistochemische Markierung gegen Synapsin (rot). Histochemie gegen Phalloidin (grün). (Originale).

cl Corpus lamellosum, **dc** Deutocerebrum, **fg** Frontalganglion, **ka** Komplexaugen, **na** Nervus antennalis, **og** olfaktorische Glomeruli, **pc** Protocerebrum, **sk** Schlundkonnektive, **sz** Sortierungszone, **tc** Tritocerebrum. Maßstäbe = 100 µm.

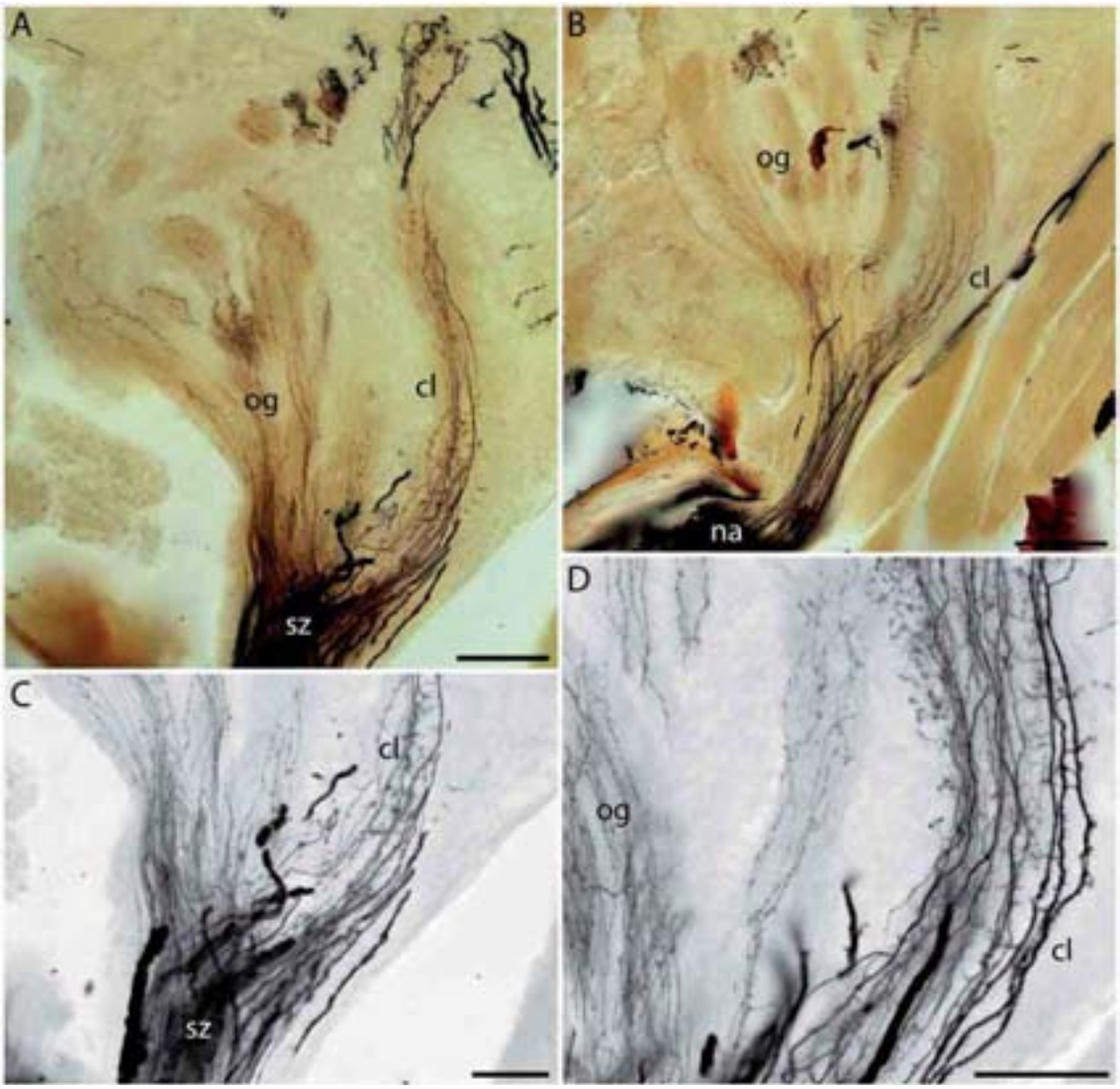

Abbildung 16: Horizontale Schnitte (30 µm) von Golgi-imprägnierten Gehirnen von *Scutigera coleoptrata* (verschiedene Individuen). (Originale). **A, B** und **C** Innervierung der olfaktorischen Glomeruli (links, dünnere Neuriten) und des Corpus lamellosum (rechts, dickere Neuriten) **D** Ausschnitt der Innervierung der olfaktorischen Glomeruli und des Corpus lamellosum.

cl Corpus lamellosum, **na** Nervus antennalis, **og** olfaktorische Glomeruli, **sz** Sortierungszone. Maßbalken: A und B = 100 µm. C und D = 50 µm.

5. Der Corpus lamellosum

Das Deutocerebrum ist aus zwei Neuropilstrukturen aufgebaut: dem anterioren olfaktorischen Lobus und dem posterioren Corpus lamellosum. Zur Zweiteilung des Deutocerebrum schrieb Hörberg (1931):

„Der Gedanke, dass sie Assoziationszentren für verschiedene Sinne bilden, dürfte wohl nicht unmöglich sein.“

In ihrer kurzen Darstellung des Gehirns von *Lithobius variegatus* zeigten Strausfeld et al. (1995), dass ein Teil des Antennalnervs eine Region posterior des olfaktorischen Lobus innerviert und bezeichneten diese in Analogie zum mechanosensorischen Neuropil der Hexapoda als Dorsallobus. Bereits Saint-Rémy (1887) benannte dieses Neuropil als „*masse lamelleuse*“, was von Fahlander (1938) zu Corpus lamellosum (CL) latinisiert wurde. Da diese Bezeichnung die morphologischen Charakteristika dieses Neuropils treffend ausdrückt, wurde dieser Begriff für das posteriore deutocerebrale Neuropil weiterhin übernommen. Wie beschrieben, innerviert ein Teil des Antennalnervs bei *Scutigera coleoptrata* den Corpus lamellosum (CL). Wie bei den olfaktorischen Glomeruli, projizieren sensorische Axone spezifisch in der Sortierungszone zum Corpus lamellosum (Abbildung 13 B, 17 B). Eigene Befunde zeigen, dass sich dieses Neuropil aus sieben bis acht parallelen Lamellen zusammensetzt (Abbildung 17 A, C). Golgi-Imprägnationen konnten zeigen, dass die sensorischen Axone, die den CL innervieren, deutlich dicker sind als diejenigen, die die olfaktorischen Glomeruli innervieren (Abbildung 17 B). Zudem besitzen diese Axone charakteristische, kurze Verzweigungen. Dieser prägnante Unterschied in der axonalen Morphologie ist ebenfalls bei den Tetraconata ausgeprägt (Schmidt und Ache 1992, Nishino et al. 2005). Bei Vertretern der Pleurostigmophora wurde die Golgi-Imprägnation im Rahmen der Dissertation nicht angewendet. Dennoch zeigen anterograde Färbungen des Antennalnervs ähnliche lamellierte Strukturen (Abbildung 6 D; 13 B, D; 17 E, F). Fahlander (1938) diskutierte die Gemeinsamkeiten des posterioren deutocerebralen Neuropils der Chilopoda und Hexapoda. Er homologisierte die „lateralen Lappen“ (Corpus lamellosum) der Chilopoda mit den „parosmetischen Massen“ (Dorsallobus) der Hexapoden.

Aus eigenen Untersuchungen zeigt sich, dass der Corpus lamellosum bei *S. coleoptrata* aus zwei verschiedenen Typen von Lamellen aufgebaut ist: eine äußere Lamelle, die proximal zusammenläuft (und somit eine äußere Schleife bildet; Abbildung 17 A) und innere Lamellen die nach posteriomedian ziehen und sich schließlich contralateral mit dem CL des contralateralen deutocerebralen Lobus verbinden (Abbildung 10, 17 A, C, 18). Diese contralaterale Verbindung stellt die posteriore Deutocerebralkommissur dar (*sensu* Fahlander 1938). Bei den übrigen Chilopoden konnte die für *S. coleoptrata* auffällige äußere Lamelle nicht nachgewiesen werden. Bei *Lithobius forficatus* sind mindestens vier Lamellen vorhanden (Abbildung 12 C; 17 E). Fahlanders (1938) Aussage, dass der CL bei *L. forficatus* nicht aus Lamellen aufgebaut ist, muss somit

widersprochen werden. Weitere eigene Befunde zeigen, dass bei *Craterostigmus tasmanianus* eine Lamellation nur teilweise sichtbar ist (Abbildung 17 D). Bei den Vertretern der Scolopendromorpha und Geophilomorpha ist der CL aus mehreren, dicht aneinander gerückten Lamellen aufgebaut (Abbildung 6 D; 13 B-E; 17 F), die zum Teil nur an der Basis des Neuropils erkennbar sind. Es ist denkbar, dass die Sichtbarkeit einzelner Lamellen mit einer dichteren Packung des CL in Zusammenhang steht.

Der Corpus lamellosum ist bei *Scutigera coleoptrata* und *Scolopendra subspinipes* durch immunhistochemische Experimente durch eine starke Synapsin-Immunreaktivität gekennzeichnet (Abbildung 15 E, G, 17 C). Die Lokalisation von tyrosiniertem Tubulin (Microtubuli-Marker) bei *S. coleoptrata* zeigt, dass neben der Muskulatur und Cytoskelettbestandteilen der CL eine hohe Immunreaktivität besitzt (Abbildung 15 C, D). Ähnliche Ergebnisse lassen sich durch eine histochemische Färbung gegen f-Actin mit Phallotoxinen bei *S. coleoptrata* und *S. subspinipes* erlangen (Abbildung 15 G, 17 C). Phalloidin-Histochemie hebt bei *S. coleoptrata* die einzelnen Lamellen des CL deutlich hervor (Abbildung 17 C). Bei *S. subspinipes* konnte die spezifische Morphologie des CL mit Phalloidin nur sehr schwach nachgewiesen werden (Abbildung 15 G). Bei *S. coleoptrata* wurde im CL keine RFamid-Immunreaktivität nachgewiesen (Sombke et al. 2011a). Allatostatin zeigt eine sehr schwache Immunreaktivität innerhalb des CL bei *S. coleoptrata* und *S. subspinipes* (Abbildungen 15 C-E).

Bei den Pterygota (Hexapoda) innervieren sensorische Neuriten der beiden basalen Antennomere das mechanosensorische Neuropil (Rospars 1988), welches Dorsallobus oder AMMC (antennal mechanosensory and motor center) genannt wird (Homberg et al. 1989). Bei den Tetraconata ist das mechanosensorische Neuropil im posterioren Bereich (bezogen auf die Neurachse) des Deutocerebrums lokalisiert. Mechanosensorische Neuropile mit einer gestreiften oder palisadenartigen Struktur sind bei apterygoten Hexapoden und einigen Crustacea bekannt (Strausfeld 1998, Tautz und Müller-Tautz 1983). Bei *Thermobia* sp. (Hexapoda: Zygentoma) ist das mechanosensorische Neuropil säulenartig organisiert, was laut Strausfeld (1998) dem antennalen mechanosensorischen Neuropil der Crustacea ähnlich ist. Mechanosensorische und gustatorische Afferenzen innervieren das posteriore Deutocerebrum, sowie das anteriore Unterschlundganglion unter anderem bei *Periplaneta americana* (Burdohan und Comer 1996, Nishino et al. 2005), *Apis mellifera* (Kloppenburg 1995), *Gryllus bimaculatus* (Staudacher 1998, Staudacher und Klingenberger 1999) und *Aedes aegypti* (Ignell und Hansson 2005, Ignell et al. 2005). In diesen Taxa besitzen die antennalen Afferenzen lange, dicke Neuriten mit mehreren kürzeren Ästen, die lateral orientiert sind und bilden somit ein mehrfach geschichtetes Arrangement im Dorsallobus (Nishino et al. 2005).

Auch bei den malakostraken Crustacea sind die Afferenzen, die das LAN innervieren, durch dicke Axone mit charakteristischen Seitenästen gekennzeichnet (Schmidt und Ache 1993). Diese Architektur bei Hexapoda und Crustacea zeigt damit

Übereinstimmungen mit dem Muster, das die sensorischen Neuriten im Corpus lamellosum von *Scutigera coleoptrata* bilden.

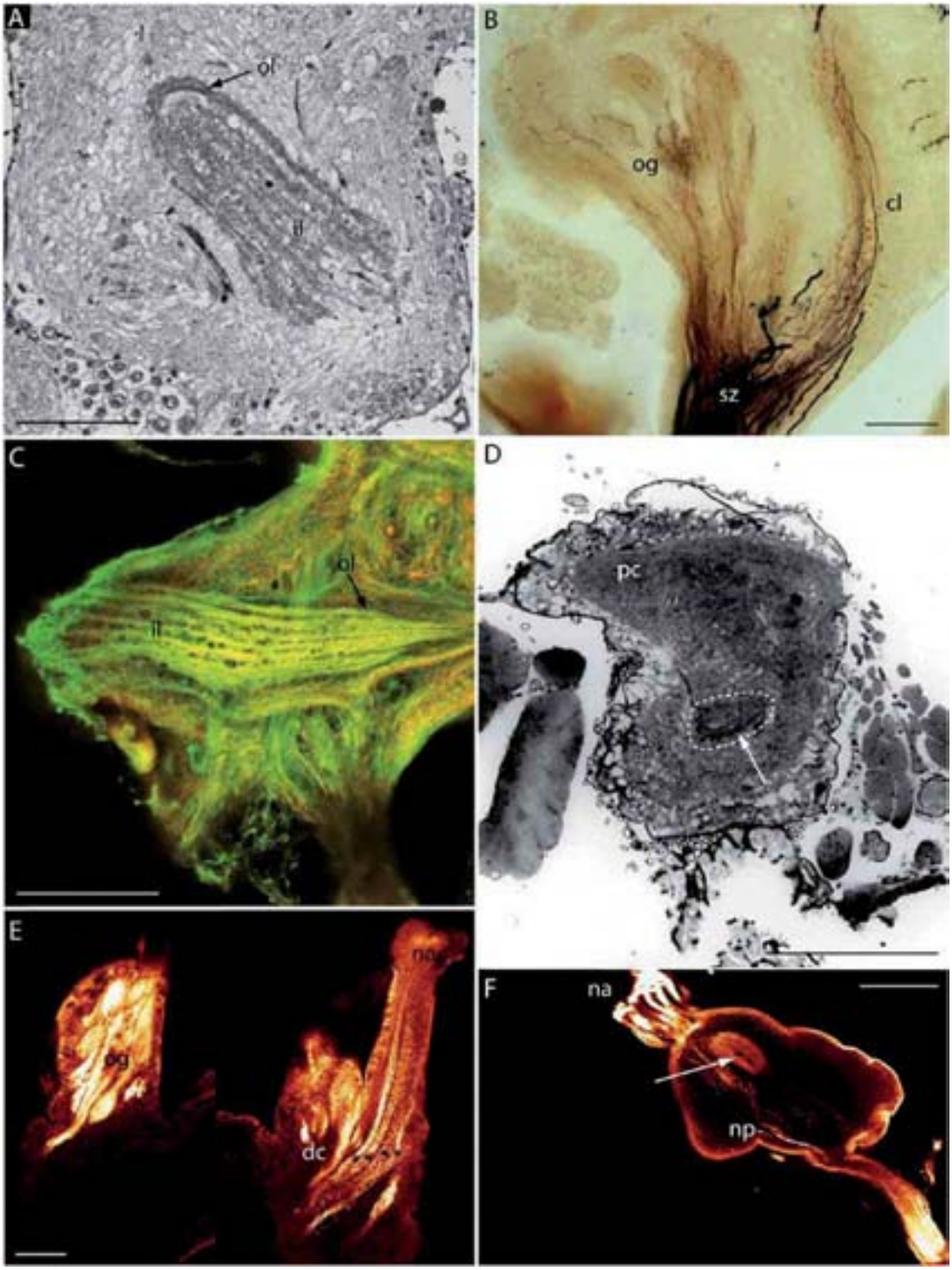

Abbildung 17: Verschiedene Präparationen und Darstellungen des Corpus lamellosum bei ausgewählten Vertretern der Chilopoda. (Originale). **A** Histologischer Querschnitt durch das linke Deutocerebrum von *Scutigera coleoptrata*. Äußere geschlossene Lamelle (ol) und innere Lamellen (il) sind sichtbar. **B** Horizontalschnitt einer Golgi-Imprägnation des Deutocerebrums von *Scutigera coleoptrata*. Dünne afferente Axone mit vermutlich synaptischen Anschwellungen innervieren anterior einzelne olfaktorische Glomeruli, dickere Axone innervieren posterior den Corpus lamellosum. **C** Maximalprojektion eines confokalen Bildstapels eines Corpus lamellosum von *S. coleoptrata*. Synapsin Immunreaktivität (rot) und Phalloidin Markierung (grün). **D** Histologischer Querschnitt durch das Gehirn (rechte Hemisphäre) von *Craterostigmus tasmanianus*. Der Corpus lamellosum ist durch die gestrichelte Linie markiert. Die Lammellierung ist schwach zu erkennen. **E** Einzelne horizontale optische Schnitte (Neurobiotin Anfärbung) des rechten Antennalnervs von *Lithobius forficatus*. Links: dorsaler Schnitt mit einzelnen olfaktorischen Glomeruli (og). Rechts: ventraler Schnitt, in dem die einzelnen Lamellen des Corpus lamellosum sichtbar sind (Sternchen). **F** Sagittaler optischer Schnitt (Neurobiotin Anfärbung) des rechten Antennalnervs von *Stigmatogaster dimidiatus*. Eine Kompartimentierung des Corpus lamellosum ist zu erkennen (Pfeil). Neben den Antennalnerven sind projizierende Neuriten angefärbt (np).

cl Corpus lamellosum, **dc** Deutocerebrum, **il** innere Lamelle, **na** Nervus antennalis, **np** Neuritenprojektion, **ol** äußere Lamelle, **og** olfaktorische Glomeruli, **pc** Protocerebrum, **sz** Sortierungszone. Alle Maßstäbe = 100 µm.

6. Neuritenprojektionen

Durch Backfill-Experimente konnten bei allen untersuchten Vertretern der Chilopoda Projektionen antennaler Afferenzen nachgewiesen werden (Abbildung 6 D, 11 A, 12 A, 13 D, 17 F, 18). Diese sind durch folgende Merkmale charakterisiert: (1) sie projizieren ipsilateral aus dem Antennalnerv in das Deutocerebrum, (2) sie innervieren kein Neuropil im Deutocerebrum und (3) sie verlaufen bei allen untersuchten Vertretern in das Unterschlundganglion. Bei *Scutigera coleoptrata* treten zudem weitere Projektionen in das ventrolaterale Protocerebrum auf (Sombke et al. 2011a). In beiden Fällen (Projektionen in das Protocerebrum bei *S. coleoptrata* und in das Unterschlundganglion bei den Chilopoda) ist der Zielort unklar. Es ist denkbar, dass die nach dorsal ziehende Projektion bei *Scutigera coleoptrata* das Postantennalorgan innerviert. Dieses Sinnesorgan wurde bei *Thereuonema hilgendorfi* (Scutigeromorpha) als CO_2-rezeptives Organ identifiziert (Yamana et al. 1986). Da bei *Lithobius forficatus* diese Projektion nach eigenen Untersuchungen nicht nachgewiesen wurde, bleibt diese Annahme unsicher. Als mögliche Funktion ist eine Integration antennaler Sinneseingänge in einem zum Postantennalorgan zugehörigen Neuropil denkbar. Dieses wurde allerdings bis jetzt nicht detailliert untersucht.

Fahlander (1938) erkannte bereits die Projektion in das Unterschlundganglion bei *Thereuopoda clunifera* (Scutigeromorpha). Er war allerdings unsicher, ob diese „Fibrillenbündel" aus dem Unterschlundganglion in die Antennen projizieren oder aus den Antennen stammen. Durch die antennalen Backfills mittels Dextran-Biotin bei *S. coleoptrata*, kann hier nun eindeutig von einer afferenten Projektion aus dem Antennalnerv ausgegangen werden. Die Projektion in das Unterschlundganglion könnte bei den Chilopoda ein gustatorisches oder motorisches Zentrum innervieren. Bei den Hexapoda ziehen bestimmte Neuriten von Kopf- und antennalen Sensillen in den Dorsallobus und bis in das Unterschlundganglion sowie die Thorakalganglien (Bräunig et al. 1983, Homberg 1994, Nishino et al. 2005). Bei *Periplaneta americana* (Hexapoda) innervieren primäre antennale, mechanosensorische und gustatorische Afferenzen den Dorsallobus und die anteriore Region des Unterschlundganglions (Nishino et al. 2005). Bei *Rhodnius prolixus* (Hexapoda: Heteroptera) projizieren antennale, nicht-olfaktorische Afferenzen in den Dorsallobus, das Unterschlundganglion sowie in die Thorakalganglien (Barrozo et al. 2009). Selbige Autoren postulieren, dass diese charakteristischen Neuriten mit dickerem Durchmesser eine schnellere neurale Übertragung von sensorischem Input zu speziellen Zentren der Motorkontrolle ermöglichen und zudem für physiologische Prozesse wichtig sind.

Bei den Crustacea wurden antennale Afferenzen, die in das Unterschlundganglion projizieren, bisher nicht detailliert beschrieben.

7. Phylogenetische Betrachtungen

7.1 Deutocerebrale Neuropile innerhalb der Chilopoda

Das Deutocerebrum der Chilopoda wird von antennalen Neuriten innerviert und beinhaltet zwei Neuropilbereiche: ein anterior gelegener olfaktorischer Lobus, der sich aus diskreten olfaktorischen Glomeruli (OG) zusammensetzt und dem posterior gelegenen Corpus lamellosum (CL), der wahrscheinlich ein mechanosensorisches Neuropil darstellt (Abbildung 18-20). Beide Neuropilbereiche sind primäre Verarbeitungszentren sensorischer Informationen. Die olfaktorischen Glomeruli treten bilateralsymmetrisch und in einer taxonspezifischen Anzahl auf. Bei drei der untersuchten Taxa der Chilopoda wurden contralaterale Verbindungen der olfaktorischen Loben nachgewiesen. Zudem sind bei allen Chilopoda afferente Neuritenbündel vorhanden, die das Deutocerebrum durchqueren und über die circumösophagealen Konnektive in das Unterschlundganglion projizieren. Die Form der olfaktorischen Glomeruli variiert taxonspezifisch, wodurch auch die spezifische Form des olfaktorischen Lobus bedingt wird. Die OG erscheinen unkompartimentiert und der olfaktorische Lobus weist ebenfalls keine Kompartimente auf. Der Corpus lamellosum stellt sich als strukturiertes Neuropil dar, welches aus diskreten Lamellen aufgebaut ist, die zumindest bei *Scutigera coleoptrata*, durch dickere Neuriten gekennzeichnet sind und sich somit deutlich von den Rezeptorneuriten, die die OG innervieren, abgrenzen lassen. Da die lamellierte Struktur des Corpus lamellosum der übrigen untersuchten Chilopoda der bei *S. coleoptrata* ähnelt, wird die morphologische Divergenz der Neuriten für alle Chilopoden durch die Befunde dieser Dissertation angenommen. Projektionen zu protocerebralen Neuropilen (Pilzkörper bzw. Corpora pedunculata) sind nach Strausfeld und Andrew (2011) bei den Chilopoda vorhanden.

Zusammenfassend lässt sich das Deutocerebrum der Chilopoden als Merkmalskomplex aus folgenden Komponenten deuten:

1. Innervierung durch antennale sensorische Neuriten, welche sich in zwei morphologische Typen (dünnere und dickere mit Seitenästen) unterteilen lassen.

2. Der anteriore olfaktorische Lobus setzt sich aus zahlreichen, diskreten und unkompartimentierten olfaktorischen Glomeruli zusammen.

3. Der posteriore Corpus lamellosum ist ein strukturiertes Neuropil und stellt höchstwahrscheinlich ein mechanosensorisches Neuropil dar.

4. Afferente Neuriten aus dem Antennalnerv projizieren in das Unterschlundganglion.

5. Verbindungen zwischen olfaktorischen Loben und protocerebralen Neuropilen sind vorhanden.

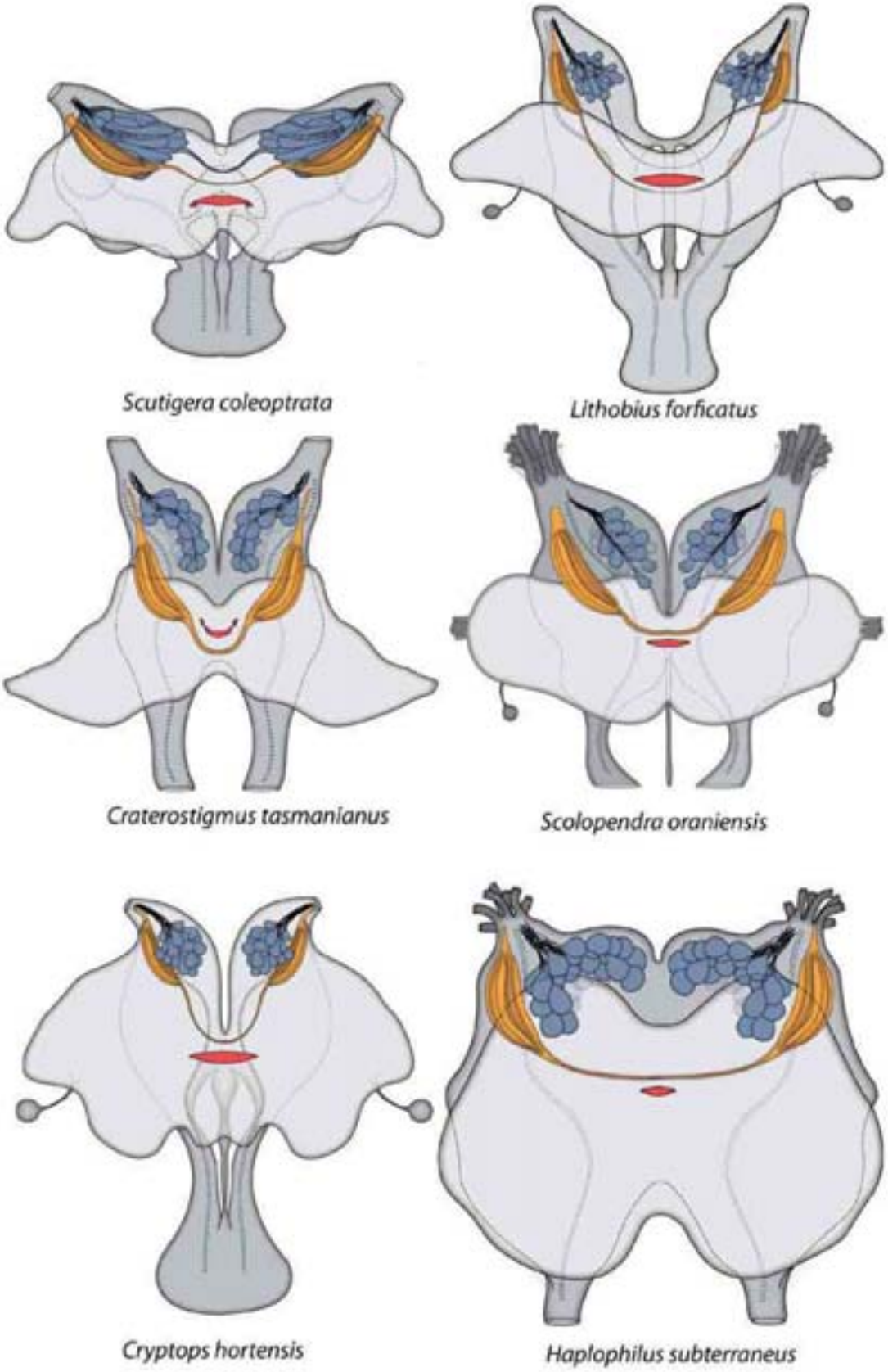
Scutigera coleoptrata
Lithobius forficatus
Craterostigmus tasmanianus
Scolopendra oraniensis
Cryptops hortensis
Haplophilus subterraneus

Abbildung 18: Schematische Rekonstruktion der Gehirne, der deutocerebralen Neuropile inklusive der contralateralen Verbindungen, des Zentralkörpers und Neuritenprojektionen bei verschiedenen Vertretern der Chilopoda. Protocerebraldrüsen sind nicht für alle Taxa dargestellt. Ansichten von dorsal ohne Skalierung. Olfaktorische Glomeruli (blau), Corpus lamellosum (gelb) und Zentralkörper (rot), gestrichelte Linien = Neuritenprojektionen. (Original).

Betrachtet man die Formenvielfalt der olfaktorischen Glomeruli innerhalb der Chilopoden, so lassen sich langestreckte, tropfenförmige und kugelige Form erkennen. Prinzipiell lassen sich aus diesem Merkmal zwei phylogenetische Szenarien postulieren: (1) Im Grundmuster der Chilopoda tritt ein olfaktorischer Lobus mit langgestreckten olfaktorischen Glomeruli auf, die sich bei den abgeleiteten Gruppen zu einer kugeligen Form transformieren (Pleurostigmophora-Konzept), oder (2) Im Grundmuster der Chilopoda tritt ein olfaktorischer Lobus mit kugeligen olfaktorischen Glomeruli auf, die sich bei abgeleiteten Gruppen hin zu einer langgestreckten Form transformieren (Heteroterga-Konzept). Das Merkmal der Form kann hier also nicht dazu dienen, eine dieser beiden Verwandtschaftskonzepte zu untermauern. Auch nach einem Außengruppenvergleich mit Vertretern der Diplopoda (siehe Kapitel 7.2) kann nicht ohne Zweifel darauf geschlossen werden, welche Form der OG bei den Chilopoda den ursprünglichen Typus darstellt. In aktuellen phylogenetischen Untersuchungen wird das Pleurostigmophora-Konzept favorisiert (siehe Kapitel 1.1). Daher scheint das erste Szenario wahrscheinlicher. Zusammenfassend stellt Abbildung 19 die interne Phylogenie der Chilopoda nach Edgecombe (2011) mit einer schematischen Darstellung deutocerebraler Neuropile bei untersuchten Vertretern dar.

Abbildung 20 zeigt die deutocerebralen Neuropile ausgewählter Chilopoden in einer gleichen Skalierung, durch welche die Veränderung von Form und Größe deutlich sichtbar wird. Die Transformation zu kugeligen OG könnte daher auch mit der Größe des olfaktorischen Lobus in Zusammenhang stehen. Dieser ist bei *Haplophilus subterraneus* beispielsweise ca. 5fach kleiner als bei *Scutigera coleoptrata* (vergleiche Abbildungen 16A und 13 E). Daher ist es denkbar, dass eine räumliche Limitierung im anterioren Deutocerebrum in einer spezifischen Form der OG resultiert. Im Umkehrschluss kann dies auch bedeuten, dass langgestreckte OG bei den Scutigeromorpha einen abgeleiteten Zustand darstellen.

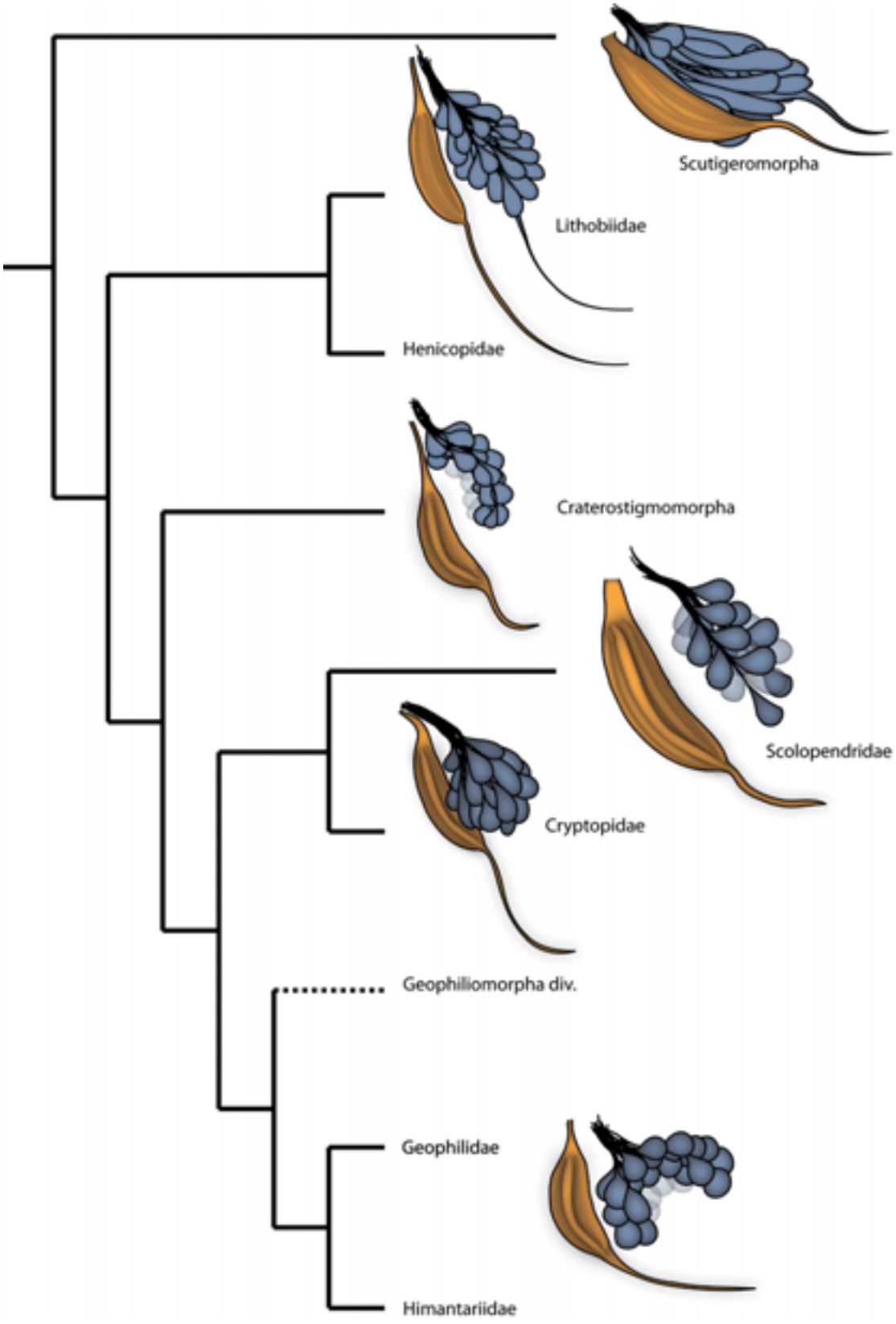

Abbildung 19: Verwandtschaftsziehungen innerhalb der Chilopoda nach dem Pleurostigmophora-Konzept und Darstellung der Neuropile einer Hemisphäre des Deutocerebrum ausgewählter Taxa. Ansichten von dorsal ohne Skalierung. Olfaktorische Glomeruli (blau) und Corpus lamellosum (gelb). Verändert nach Edgecombe (2011).

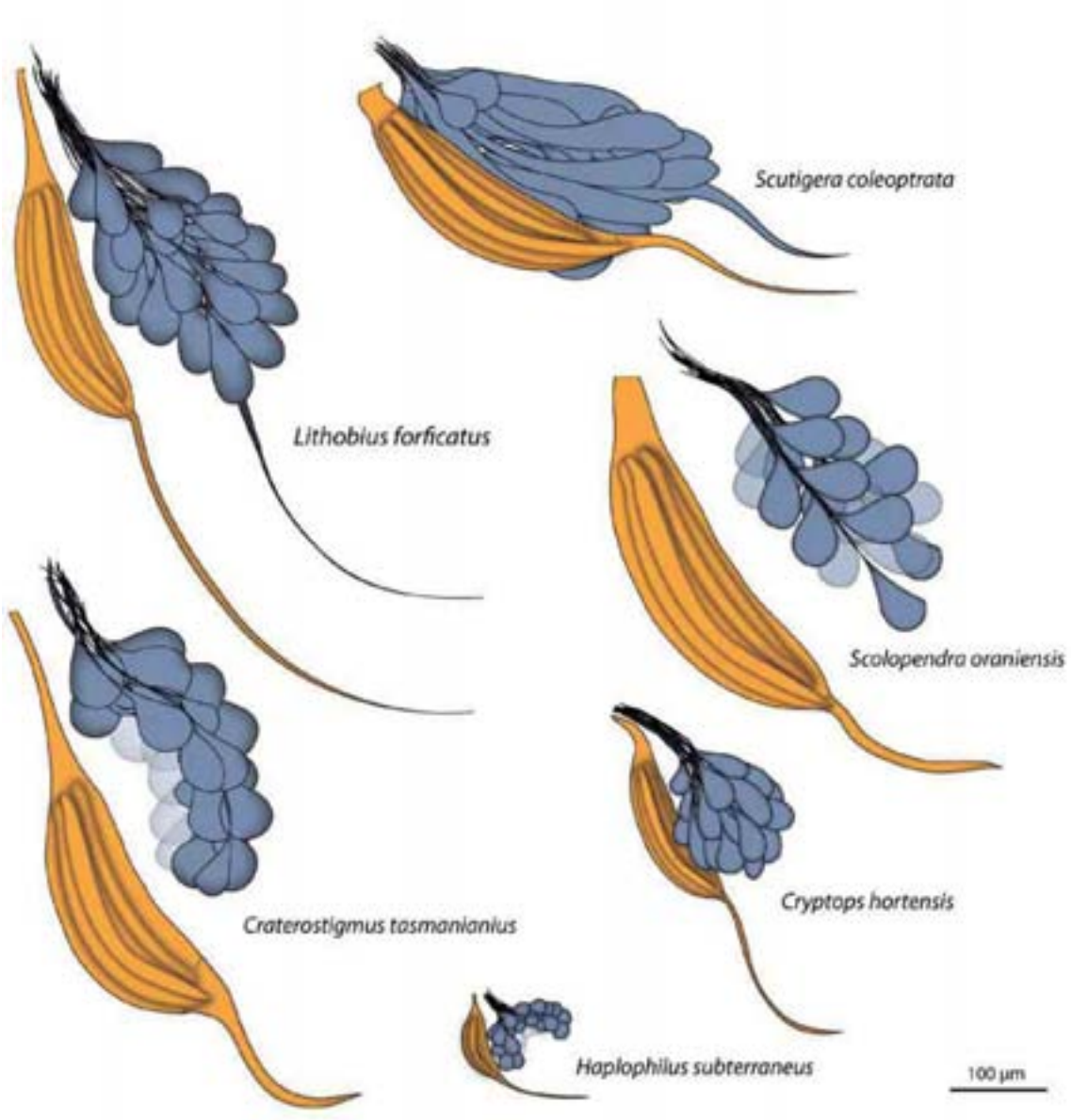

Abbildung 20: Schematische Darstellung der Neuropile inklusive contralateraler Verbindungen einer deutocerebralen Hemisphäre verschiedener Chilopoda. Ansichten von dorsal in gleicher Skalierung. Olfaktorische Glomeruli (blau) und Corpus lamellosum (gelb).

7.2 Deutocerebrale Neuropile innerhalb der Myriapoda

Detaillierte Daten zu den Symphyla und Pauropoda liegen nicht vor. Bei den Symphyla wird das Deutocerebrum von zwei separaten antennalen Nerven (ein sensorischer und ein motorischer) innerviert (zusammengefasst in Szucsich und Scheller 2011). Hier scheinen die olfaktorischen Loben aus einer „hohen Anzahl" von glomerulären Kondensationen zu bestehen (Szucsich und Scheller 2011). Bei den Pauropoda bildet das Deutocerebrum zwei laterale Vergrößerungen, die sich in Richtung des Postantennalorgans ausbreiten (Scheller 2011).

Die Organisation deutocerebraler Neuropile bei den Progoneata (siehe Abbildung 2) ist ähnlich wie bei den Chilopoda nur ansatzweise untersucht. Bereits Saint-Rémy (1890) beschrieb olfaktorische Glomeruli bei Diplopoda, wenngleich eine genaue Lokalisierung nicht angegeben wurde. Nachfolgend beschrieben Holmgren (1916) und Hanström (1928) antennale Glomeruli im Deutocerebrum der Diplopoda. Die einzige detaillierte Arbeit zu deutocerebralen Neuropilen der Diplopoda liegt für *Cylindroiulus punctatus* vor (Nguyen Duy-Jacquemin und Arnold 1991). Bei dieser Art wird das Deutocerebrum von Neuriten antennaler Rezeptorzellen innerviert und teilt sich in vier Bündel (Projektionen) auf. Zwei dieser Projektionen innervieren die 18-19 kugeligen bis langgetreckten olfaktorischen Glomeruli, der dritte Trakt zieht in Richtung des Unterschlundganglions und der vierte projiziert contralateral (Nguyen Duy-Jacquemin und Arnold 1991). Zudem wiesen die Autoren ein nichtglomeruläres (unstrukturiertes) Neuropil im ventralen Bereich des Deutocerebrums nach. Auf den Abbildungen dieser Arbeit wird aber nicht ersichtlich, ob es sich bei dem ventral gelegenen, unstrukturierten Neuropil oder dem vierten (contralateral ziehenden) Trakt um eine dem Corpus lamellosum der Chilopoda homologe Struktur handelt. Nach eigenen, vorläufigen Untersuchungen bei Polydesmida (Diplopoda) ist ein contralateral verbundenes posteriores Neuropil vorhanden, welches dem Corpus lamellosum der Chilopoda entspricht (Seefluth und Sombke in Vorb.). Der dritte Trakt kann daher mit den in das Unterschlundganglion ziehenden antennalen Neuritenprojektionen homologisiert werden.

Trotz des Fehlens detaillierter vergleichend-morphologischer Untersuchungen bei den Progoneata, lassen sich mit Hilfe der Beobachtungen von Nguyen Duy-Jacquemin und Arnold (1991) Vergleiche zu den Chilopoda ziehen. Bei Chilopoda und Diplopoda wird das Deutocerebrum durch antennale sensorische Neuriten innerviert. Der olfaktorische Lobus besteht aus zahlreichen, diskreten olfaktorischen Glomeruli. Zudem treten antennale Neuritenprojektionen auf, die in das Unterschlundganglion projizieren. Verbindungen zwischen den olfaktorischen Loben und protocerebralen Neuropilen liegen nach Holmgren (1916) vor. Bestätigt sich der homologe Aufbau des Corpus lamellosum inklusive einer contralateralen Verbindung bei den Diplopoda, treffen die in Kapitel 7.1 für die Chilopoda formulierten Merkmale des Deutocerebrum ebenso für die

Diplopoda zu und können daher für das Grundmuster der Myriapoda postuliert werden (Abbildung 22).

Durch die Befunde für die Diplopoda lässt sich somit ein Außengruppenvergleich zu den Chilopoda durchführen und folglich können kugelige bis langgestreckte olfaktorische Glomeruli für das Grundmuster der Myriapoda postuliert werden. Die charakteristische Form der OG bei *Scutigera coleoptrata* wäre damit, wie bereits erwähnt, ein abgeleiteter Zustand. Abschließend kann dies aber nur durch eine detaillierte Untersuchung der deutocerebralen Neuropile bei weiteren Vertretern der Diplopoda, Symphyla und Pauropoda geklärt werden.

7.3 Deutocerebrale Neuropile innerhalb der Tetraconata

Die Charakteristika des Deutocerebrums der Crustacea und Hexapoda im Vergleich zu denen der Chilopoda wurden zum Teil bereits in den Kapiteln 4, 5 und 6 dargelegt.

Das Deutocerebrum der Tetraconata wird durch die erste Antenne innerviert. Nach Scholtz und Edgecombe (2006) sind die deutocerebrale Antenne und das Deutocerebrum innerhalb der Mandibulata einander homolog (siehe dazu weiter Kapitel 7.4).

Die olfaktorischen Loben der Malacostraca und der Hexapoda werden von sensorischen Neuriten der ersten Antenne innerviert (Strausfeld 2009). Nach Strausfeld (1998) sind die olfaktorischen Glomeruli der Tetraconata nicht homolog, weil bei den malakostraken Crustacea die OG aus geschichteten synaptischen Lagen aufgebaut sind, während sie bei den Hexapoda eine diffuse oder konzentrische Organisation besitzen. Ein weiterer Unterschied innerhalb der Tetraconata ist das vermeintliche Fehlen uniglomerulärer Projektionsneurone (von protocerebralen Neuropilen) bei den Crustacea (Schmidt und Ache 1997). Interessanterweise stellen neuerdings Strausfeld und Andrew (2011) die Tetraconata basierend auf Nervensystemmerkmalen (inklusive der OG) als monophyletische Gruppe dar und widersprechen damit eigenen vormals geäußerten Vorstellungen (vergl. Strausfeld 1998).

Grundsätzlich sind bei den Hexapoda kugelförmige OG realisiert, wobei die Archaeognatha mit langgestreckten OG eine Ausnahme bilden (Mißbach et al. 2011). Das Vorhandensein eines weiteren deutocerebralen Neuropils bei den Archaeognatha (ventral deutocerebral neuropil *sensu* Mißbach et al. 2001), scheint eine Neubildung oder Modifikation zu sein und kann ohne weitere Untersuchungen nicht eindeutig mit dem olfaktorischen Lobus oder dem mechanosensorischen Neuropil homologisiert werden (Abbildung 22). Bei verschiedenen Gruppen der Malacostraca findet sich ebenso eine kugelige Form der OG, wie zum Beispiel bei Stomatopoda (Abele 1991), Leptostraca (Kenning und Harzsch in Vorb.), Euphausiacea (Johannson und Hallberg

1992) und Isopoda (Harzsch et al. 2011). Die Remipedia, welche als Schwestergruppe der Hexapoda (Regier et al. 2010), der Malacostraca (Fanenbruck und Harzsch 2005) oder auch als basale Crustacea (Strausfeld und Andrew 2011) diskutiert wurden, besitzen ebenfalls kugelförmige OG (Fanenbruck und Harzsch 2005). Die komplexeste Architektur der olfaktorischen Loben tritt bei den dekapoden Crustacea auf (Schachtner et al. 2005). Diese lässt sich ohne weiteres von der Architektur bei basaleren Gruppen, wie Leptostraca oder Stomatopoda, ableiten. Wie bereits im Kapitel 7.1 für die Chilopoda besprochen, könnte die Form der OG mit der räumlichen Limitierung des olfaktorischen Lobus in Zusammenhang stehen. Dies wird vor allem bei den Decapoda deutlich, deren OG keilförmig aneinandergereiht sind und somit ein geordnetes Muster bilden. Wie in Kapitel 4 dargelegt, scheint die Anzahl der OG bei den Hexapoda taxonspezifisch konstant zu sein. Bei den Crustacea ist dies fraglich, wenngleich hierzu nur wenige Daten vorliegen. Eine Kompartimentierung der OG tritt nur innerhalb der Pterygota und Decapoda auf (siehe Kapitel 4).

Schachter et al. (2005) stellten basierend auf dem Vergleich der olfaktorischen Loben der dekapoden Crustacea und der neopteren Hexapoda folgende Apomorphien der Tetraconata auf:

1. Aufbau der primären olfaktorischen Zentren aus glomerulären Modulen.

2. Cholinerge olfaktorische Rezeptorneuriten (ORN) mit uniglomerulären Endigungen penetrieren die olfaktorischen Loben aus der Peripherie.

3. Inhibitorische lokale Interneurone (GABAerg oder Histaminerg, beinhalten Neuropeptide als Co-Transmitter).

4. Olfaktorische Loben, die von einem prominenten, serotonergen Neuron multiglomerulär innerviert werden.

5. Verbindungen von olfaktorischen Loben und protocerebralen Neuropilen (laterales Horn, Pilzkörper, Hemielipsoidkörper) über Projektionsneurone.

Das Deutocerebrum der Tetraconata ist durch den Besitz (mindestens) eines posterior (bezogen auf die Neurachse) gelegenen Neuropils charakterisiert, welches ein mechanosensorisches und motorisches Zentrum darstellt und wie die OG von primären sensorischen Neuriten der ersten Antennen innerviert wird (Dorsallobus oder AMMC bei den Hexapoda, LAN und MAN bei den Crustacea). Wie dargelegt können die Neuriten aufgrund ihrer charakteristischen Morphologie eindeutig identifiziert werden (dickere Neuriten mit Seitenästen). Nach Strausfeld (1998) sind mechanosensorische Kopfanhänge und ein damit assoziiertes gestreiftes/ pallisadenartiges, mechanosensorisches Neuropil eine Apomorphie der Mandibulata. Generell sind die deutocerebralen mechanosensorischen Neuropile bei den Tetraconata nicht in dem Maße untersucht, wie die olfaktorischen Loben.

Bei den Eumalacostraca und den Remipedia sind paarige LAN und ein unpaares MAN vorhanden (Fanenbruck und Harzsch 2005). Zwischen den Loben des LAN treten zum Teil contralaterale Verbindungen auf. Das Vorhandensein eines distinkten MAN ist bei den Leptostraca unsicher, ein LAN ist in dieser Gruppe hingegen vorhanden (Kenning und Harzsch in Vorb.). Innerhalb der Branchiopoda, „Maxillopoda" und Cephalocarida scheinen die mechanosensorischen Neuropile bei untersuchten Vertretern nicht vorhanden zu sein (Fanenbruck und Harzsch 2005, Stegner und Richter 2011).

Als weiteres Merkmal lassen sich bei Vertretern der Hexapoda (z.B. *Periplaneta americana* [Nishino et al. 2005], *Rhodnius prolixus* [Barrozo et al. 2009], *Lepismachilis y-signata* [Mißbach et al. 2001]) antennale Neuriten finden, die ipsilateral in das Unterschlundganglion projizieren und kein Neuropil im Deutocerebrum innervieren. Bei den Crustacea wurden diese Projektionen bis jetzt nicht beschrieben bzw. dargestellt.

7.4 Deutocerebrum und olfaktorische Loben außerhalb der Mandibulata

Das Deutocerebrum

Ein Vergleich von *Hox*-gen Expressionsmustern ermöglicht Rückschlüsse auf die Homologien der Neuromere des Nervensystems der Euarthropoda (Urbach und Technau 2007). Tatsächlich unterstützen diese Muster die Homologie des Deuto- und Tritocerebrums sowie die der drei Neuromere des fusionierten Unterschlundganglions innerhalb der Mandibulata. Demnach sind bei Chelicerata und Mandibulata das Cheliceren-Segment dem ersten Antennalsegment (Deutocerebrum) sowie das Pedipalpen-Segment dem Intercalarsegment (Tritocerebrum) homolog (Damen et al. 1998, Telford und Thomas 1998) (vergleiche Abbildung 21). Diese Homologiehypothese wird ebenso durch morphologische Befunde gestützt (Mittmann und Scholtz 2003). Die Transformation tritocerebraler Antennen zu Cheliceren (*sensu* Cotton und Braddy 2004) ist dagegen weniger wahrscheinlich. Es ist eher anzunehmen, dass in der Evolution der Euarthropoda ein deutocerebraler Anhang zu Cheliceren transformiert wurde (Chen et al. 2004, Waloszek et al. 2005). Nach Scholtz und Edgecombe (2006) ist die sensorische deutocerebrale Antenne ein apomorphes Merkmal der Mandibulata. Das schließt ebenso die paläozoischen Trilobita mit ein, die von Scholtz und Edgecombe (2006) in die Stammlinie der Mandibulata eingeordnet werden.

Olfaktorische Loben

Der Besitz von olfaktorischen Loben, welche aus Glomeruli aufgebaut sind, beschränkt sich nicht nur auf die Mandibulata (Abbildung 22). Sie sind auch bei Mollusca (Wertz et al. 2006), Annelida (Heuer und Loesel 2009), Onychophora (Strausfeld et al. 2006), einigen Chelicerata (Brownell 1989) sowie bei Vertebrata (Strotmann 2001) ausgebildet.

Alle genannten Taxa verfügen über bipolare, olfaktorische Rezeptorneuriten (ORN) (Eisthen 2002). Der Zielort der ORN wird in diesen Taxa Glomerulus genannt. Im Detail treten neben Gemeinsamkeiten aber auch Unterschiede auf, was den Besitz von Odorant-Bindeproteinen, G-Protein gekoppelte Odorantrezeptoren, die zwei Stufen Signaltransduktion und die Organisation der glomerulären Neuropile betrifft (zusammengefasst in Eisthen 2002).

Die deutocerebrale Antenne der Mandibulata ist konvergent zur protocerebralen Antenne der Onychophora entstanden (Eriksson und Budd 2000, Scholz und Edgecombe 2005, 2006; Mayer und Koch 2005). Hier innervieren ORN olfaktorische Loben, die aus diskreten olfaktorischen Glomeruli aufgebaut sind (Strausfeld et al. 2006). Ein mechanosensorisches Neuropil ist bei den Onychophora nicht beschrieben (Abbildung 22).

Innerhalb der Chelicerata sind olfaktorische Loben mit glomerulären Neuropilen bei Solifugae und Amblypygi (Strausfeld und Reisenman 2009), Scorpiones (Wolf 2008) sowie bei Acari (Szlendak und Oliver 1992, van Wijk et al. 2006a, b) ausgebildet (Abbildung 22). Die Lokalisation chemosensorischer Organe und deren afferente Projektion in ein spezifisches Neuromer variiert allerdings bei den Chelicerata.

Bei den Ixodidae (Acari) ist das erste Laufbeinpaar (viertes Neuromer) funktionell chemorezeptiv (Szlendak und Oliver 1992). Hier wurden olfaktorische Glomeruli im Pedalganglion nachgewiesen. Auch bei den Phytoseiidae (Acari) innervieren Rezeptorneuriten olfaktorischer Sensillen des ersten Laufbeinpaares einen OL der sich im Synganglion (erstes Pedalganglion) befindet (van Wijk et al. 2006a, b). Die OL sind hier contralateral verbunden. Interessanterweise innervieren die 13 bis 25 Rezeptorneuriten des Pedalnervs individuell die ca. 14 bis 21 OG. Höchstwahrscheinlich innerviert also jedes Rezeptoraxon „seinen eigenen" Glomerulus. Ein mechanosensorisches Neuropil wurde nicht nachgewiesen. Allerdings gibt es eine Reihe von angefärbten Neuriten, die das Pedalganglion aus dem ersten Beinpaar innervieren (van Wijk et al. 2006b: Abbildung 2 d), aber anscheinend nicht in weitere Neuromere projizieren. Bei den Amblypygi und Solifugae dienen die Pedipalpen (drittes Neuromer) als funktionelle chemorezeptive Anhänge (Brownell 1989, Strausfeld und Reisenmann 2009). Auch hier werden olfaktorische Loben mit diskreten OG innerviert. Eine ähnliche Innervierung durch das Malleoli-tragende vierte Beinpaar der Solifugae wurde bis jetzt nicht nachgewiesen. Ein mechanosensorisches Neuropil wurde nicht gefunden.

Bei den Scorpiones sind die Pectines (neuntes Neuromer, posterior des Laufbeins) funktionell betrachtet chemo- und mechanorezeptive Organe (Wolf 2008). Eine Vielzahl von neuroanatomischen Merkmalen wurden von Wolf (2008) im Zusammenhang mit den Pectines bei den Scorpiones beschrieben: Rezeptorneuriten innervieren ein posteriores und ein anteriores Neuropil im Unterschlundganglion, welche sich caudal bis in das 7. (posteriores Neuropil) bzw. 6. (anteriores Neuropil) Neuromer ausbreiten.

Zudem existiert ein dorsolateraler, nach anterior verlaufener Trakt, der die beiden genannten Neuropile nicht innerviert. Das posteriore Neuropil ist aus unregelmäßigen Glomeruli aufgebaut, welche in mehreren Schichten organisiert sind und von Wolf (2008) Lobuli genannt werden. Aufgrund der sehr dünnen Axone wurde eine Verarbeitung chemosensorischer Stimuli vermutet. Eine Subpopulation der Lobuli ist GABAerg. Die Innervation scheint multiglomerulär zu sein. Das anteriore Neuropil erscheint unstrukturiert. Ein mechanosensorisches Neuropil scheint nicht vorhanden zu sein (Wolf pers. Mitt.). Eine contralaterale Verbindung scheint ebenso nicht zu bestehen. Verbindungen zu sekundären Zentren (Pilzkörper im weiteren Sinne) wurden nicht untersucht.

7.5 Zusammenfassende Interpretationen

Nach den vorliegenden Befunden scheint bei den Arthropoda die Ausbildung von olfaktorischen Glomeruli (OG) an die Evolution von spezialisierten chemosensorischen Strukturen (Anhängen) gekoppelt zu sein. Demnach handelt es sich bei den Glomeruli um eine neuronale Architektur für die Verschaltung primärer Rezeptorneuriten, die wegen ihrer weiten, lückenhaften Verbreitung innerhalb der Arthropoda stark konvergenzverdächtig sind. Dennoch besteht die Möglichkeit, dass dieses Konstruktionsprinzip ein plesiomorphes Merkmal der Bilateria darstellen könnte und Glomeruli damit eine phylogenetische Relevanz besitzen.

Bei den Myriapoda, Crustacea und Hexapoda sind die OG im Deutocerebrum lokalisiert, da dieses durch die erste Antenne innerviert wird (Abbildung 21, 22). Nach Scholtz und Edgecombe (2006) sind die deutocerebrale Antenne und das Deutocerebrum innerhalb der Mandibulata einander homolog (siehe Kapitel 7.4). Bei den Chelicerata und Onychophora sind die OG (mit Ausnahme der Scorpiones) in dem Segment lokalisiert, das durch sensorische Anhänge innerviert wird (Abbildung 21); nicht aber im Deutocerebrum.

Ein distinktes mechanosensorisches Zentrum ist bei Chelicerata nicht mit den sensorischen Anhängen assoziiert (Abbildung 22). Eine charakteristische, morphologische Divergenz der Rezeptorneuriten, die das anteriore und posteriore Neuropil bei den Scorpiones innervieren, wurde nicht festgestellt (Wolf pers. Mitt.). Projektionen aus der sensorischen Extremität, die kein distinktes Neuropil im zugehörigen Neuromer innervieren, konnten bei den Scorpiones festgestellt werden (Abbildung 22). Rezeptorneuriten scheinen uniglomerulär zu sein. Eine Subpopulation der OG bei Scorpiones ist GABAerg. Nach Strausfeld und Andrew (2011) besitzen die Chelicerata Projektionstrakte zwischen OL und Pilzkörpern.

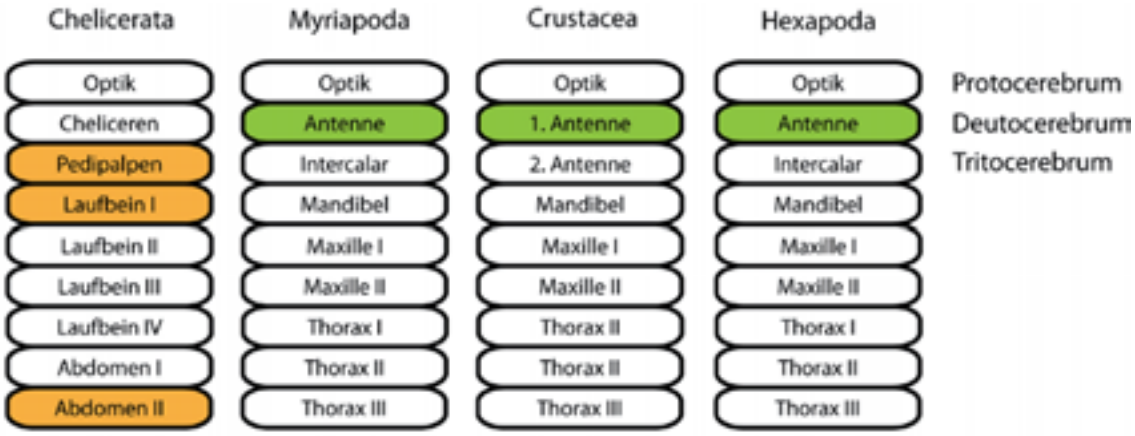

Abbildung 21: Schematische Darstellung der Neuromere bzw. Körpersegmente und der zugehörigen Extremitäten bzw. Segmentalanhänge bei Chelicerata, Myriapoda, Crustacea und Hexapoda (verändert nach Damen et al. 1998). Die Markierung zeigt das Auftreten von olfaktorischen Glomeruli in Zusammenhang mit einer Innervierung durch chemosensorische Anhänge. **Grün**: Deutocerebrum, **Orange**: anderes Neuromer.

Aus den in den Kapiteln 7.1 bis 7.4 dargelegten Merkmalen lässt sich die Schlussfolgerung ziehen, dass das Deutocerebrum der Chilopoda und Diplopoda homolog organisiert ist. Resultierend aus dem Befund, dass bei *S. coleoptrata*, malakostraken Crustacea und Hexapoda distinkte Neuropile für die Verarbeitung chemo- und mechanosensorischer Stimuli ausgebildet sind, wurde vorgeschlagen, dass der Besitz dieser Zentren ein gemeinsames Architekturprinzip der Mandibulata darstellen könnte (Sombke et al. 2009, 2011a). Die regionale Differenzierung und Bifunktionalität des Deutocerebrum wird durch die axonale Morphologie (Dicke und Verzweigung) untermauert (Sombke et al. 2011a). Neuroanatomische Daten zeigen, dass ein Schwestergruppenverhältnis zwischen Myriapoda und Chelicerata höchst unwahrscheinlich ist, da das durch sensorische Anhänge innervierte Neuromer bei den Chelicerata nicht durch ein mechanosensorisches Neuropil charakterisiert ist.

Das Deutocerebrum der Mandibulata als Merkmalskomplex

Nach Remanes (1956) Kriterium der Lage sind die im Deutocerebrum anterior gelegenen olfaktorischen Loben und das posterior gelegene mechanosensorische Neuropil im Deutocerebrum der Mandibulata einander homolog. Dies wird durch die charakteristische axonale Morphologie (Dicke und Verzweigungsmuster) der innervierenden Rezeptoraxone untermauert. Nach Remanes (1956) Stetigkeitskriterium lassen sich die olfaktorischen Glomeruli innerhalb der Mandibulata homologisieren. Dies trifft allerdings ebenso für die olfaktorischen Glomeruli innerhalb der Chelicerata

zu. Die mechanosensorischen Neuropile treten nur innerhalb der Mandibulata auf (Abbildung 22). Auch wenn diese Neuropile teilweise unterschiedlich konstruiert sind (lamelliert bei Vertretern der Myriapoda, homogen und zum Teil mehrteilig bei Crustacea und Hexapoda), ist eine Homologie postulierbar. Nach Remanes (1956) Kriterium der speziellen Qualität der Strukturen ist das Deutocerebrum der Mandibulata als Merkmalskomplex homolog, da es aus einer Reihe von apomorphen Merkmalen besteht, die bei den Chelicerata nicht ausgeprägt sind (Abbildung 22).

Daraus ergibt sich als Apomorphie der Mandibulata, dass der sensorische Eingang der homologen ersten Antenne im Deutocerebrum zwei distinkte Neuropilbereiche innerviert: anterior gelegene olfaktorische Glomeruli sowie ein posterior gelegenes mechanosensorisches Neuropil. Die sensorischen Neuriten lassen sich morphologisch unterscheiden.

Als sparsamste Erklärung einer Evolution dieser kombinierten primären Verarbeitungszentren erscheint die Annahme, dass sich die Bifunktionalität des Deutocerebrum in Verbindung mit der Ausprägung von zwei spezifischen Neuropilbereichen für die Verarbeitung chemo- und mechanosensorischer Sinnesmodalitäten im Grundmuster der Mandibulata evolviert hat.

Form und Struktur der olfaktorischen Glomeruli der Mandibulata

Betrachtet man ausschließlich die Form der olfaktorischen Glomeruli von *Scutigera coleoptrata* im Vergleich zu den Tetraconata, ergibt sich ein offensichtlicher Unterschied (Sombke et al. 2009, 2011a). Daraus resultierend wurde abgeleitet, dass die Myriapoda ihren eigenen Pfad in der Evolution eines olfaktorischen Systems bei der Erschließung des terrestrischen Lebensraums eingeschlagen haben (Sombke et al. 2009). Da die Form der olfaktorischen Glomeruli innerhalb der Chilopoda von langgestreckt bis kugelförmig variiert, muss diese Ansicht allerdings revidiert werden. Ähnlich wie bei den Chilopoda und Diplopoda sind auch bei den Archaeognatha die olfaktorischen Glomeruli (OG) nicht um ein lockeres, zentrales Neuropil angeordnet. Dieses Merkmal scheint konvergent in diesen drei Teiltaxa der Mandibulata ausgebildet zu sein. Die Subkompartimentierung der OG bei Vertretern der Tetraconata kann als Apomorphie oder als Konvergenz der Malacostraca und Hexapoda interpretiert werden. Bei den Chilopoda, Archaeognatha und Vetretern der Chelicerata tritt dieses Merkmal nicht auf. Wie in Kapitel 4 dargelegt, scheint die Anzahl der OG bei den Chilopoda und Hexapoda taxonspezifisch konstant zu sein. Im Grundmuster der Tetraconata sind nach Schachtner et al. (2005) kugelige OG in den olfaktorischen Loben vorhanden. Diese Architektur ist nach der vorliegenden Datenlage auch innerhalb der Myriapoda verbreitet. Entgegen der Interpretationen von Strausfeld (1998) und Fanenbruck et al. (2004) kann der Besitz von olfaktorischen Loben aus diskreten olfaktorischen Glomeruli im Deutocerebrum als Plesiomorphie der Tetraconata im Rückgriff auf das Stammartmuster der Mandibulata

angenommen werden. In der Konsequenz bedeutet dies, dass das Fehlen von olfaktorischen Loben bei verschiedenen Gruppen der Crustacea (Branchiopoda und einige „Maxillopoda“) und Hexapoda (Odonata, einige Hemiptera und Coleoptera) (zusammengefasst in Schachtner et al. 2005) das Resultat einer Reduktion darstellt (Sombke et al. 2011a).

Bei den Chilopoda konnten die von Schachtner et al. (2005) aufgestellten Synapomorphien bezüglich der olfaktorischen Glomeruli der neopteren Hexapoda und dekapoden Crustacea teilweise nachgewiesen werden: (1) der olfaktorische Lobus ist aus glomerulären Modulen (OG) aufgebaut, (2) die olfaktorische Rezeptorneuriten (ORN) penetrieren die olfaktorischen Loben aus der Peripherie (Abbildung 9). Diese sind wahrscheinlich uniglomerulär (Abbildung 7, 16) und (3) die Neuropeptide RFamid und Allatostatin wurden zumindest für *S. coleoptrata* und *S. subspinipes* in den OG nachgewiesen (vergl. Sombke et al. 2011a). (4) Verbindungen zwischen den olfaktorischen Loben (OL) und protocerebralen Zentren sind vorhanden. Cholinerge Innervierungen, GABA- bzw. histaminerge Neuriten und serotonerge, multiglomeruläre Neurone wurden bis jetzt nicht nachgewiesen.

Mechanosensorische Neuropile der Mandibulata

Das posteriore Deutocerebrum der Mandibulata ist durch den Besitz von (mindestens) einem Neuropil charakterisiert, welches ein mechanosensorisches Zentrum darstellt (Abbildung 22). Unter der Annahme, dass diese Struktur in den einigen Taxa der Crustacea reduziert bzw. teilweise reduziert (MAN bei den Leptostraca) wurde und Strausfelds (1998) Annahme korrekt ist, handelt es sich hierbei um eine Apomorphie der Mandibulata. Bei den Chilopoda, Diplopoda und Decapoda (Crustacea) sind zusätzlich contralaterale Verbindungen (clc) der mechanosensorischen Neuropile vorhanden (Sombke et al. 2011a). Eine Homologie dieser clc bei Myriapoda und Decapoda ist nach bisheriger Datenlage nicht überzeugend zu vertreten.

Der Besitz einer zweiten Antenne wurde für das Grundmuster der Mandibulata vorgeschlagen (zusammengefasst in Wägele 1993). Bei den malakostraken Crustacea innerviert diese hauptsächlich mechanosensorische Antenne II (Antenna) das tritocerebrale Antenne 2 Neuropil (AnN) (Sandeman und Luff 1973, Blaustein et al. 1988, Sandeman et al. 1992, Schmidt und Ache 1992, 1996a, Mellon und Alones 1993). Ähnlich dem Corpus lamellosum der Chilopoda ist das AnN seriell strukturiert; allerdings in Form einer transversalen Streifung.

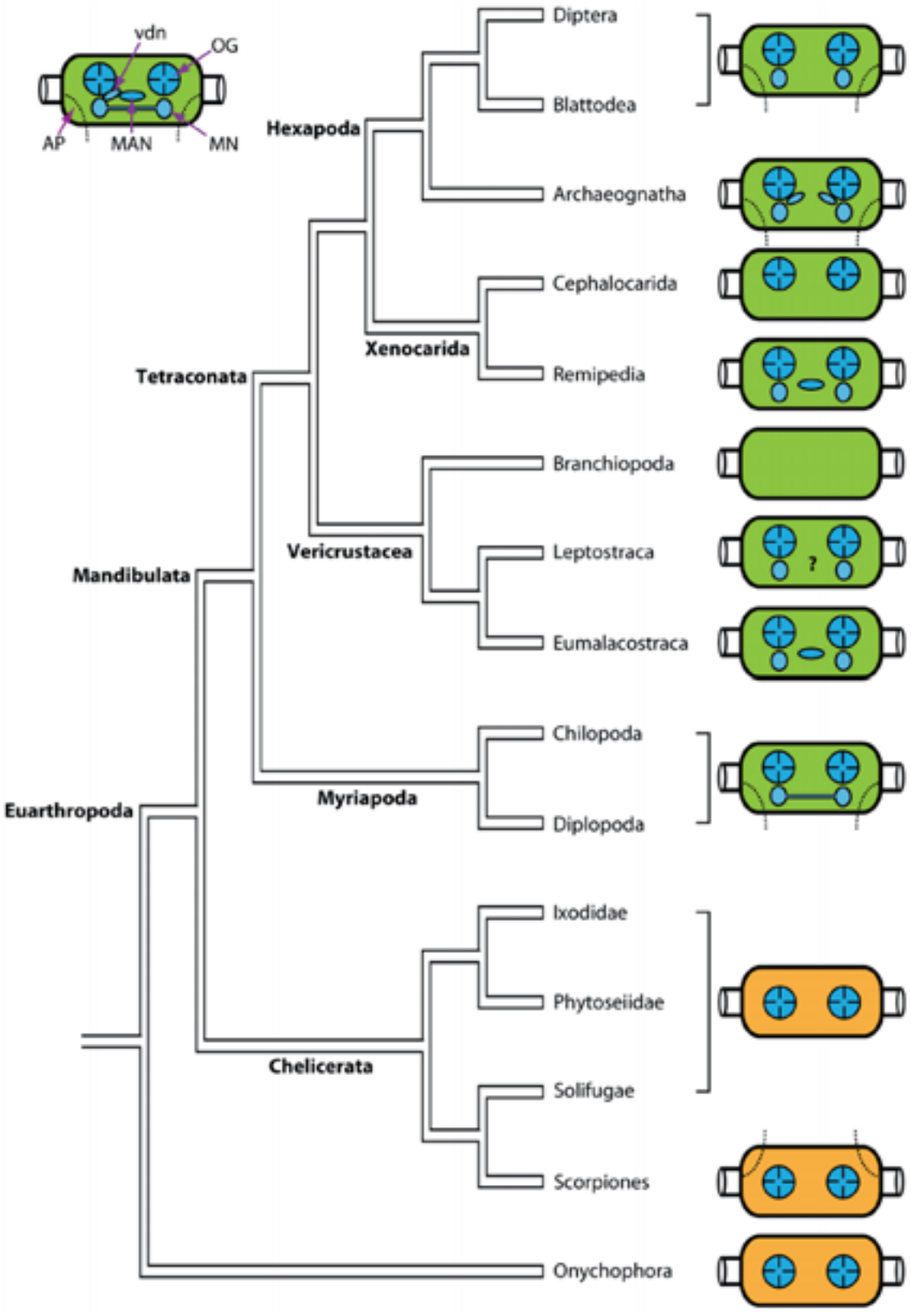

Abbildung 22: Phylogenetische Verwandtschaftshypothese der Arthropoda verändert nach Regier et al. (2010) mit Darstellung der in der Zusammenfassung besprochenen Taxa. **Grün**: Deutocerebrum. **Orange**: Neuromer mit olfaktorischen Glomeruli (nicht Deutocerebrum). **Gestrichelte Line**: Neuritenprojektionen. **NP** Neuritenprojektion, **MAN** medianes Antenne 1 Neuropil, **MN** mechanosensorisches Neuropil (Corpus lamellosum, Dorsallobus, laterales Antenne 1 Neuropil), **OG** olfaktorische Glomeruli, **vdn** ventral deutocerebral neuropil (Archaeognatha).

Antennale Neuritenprojektionen der Mandibulata

Als weiteres, phylogenetisch potenziell interessantes Merkmal lassen sich bei den Chilopoda, Diplopoda (Seefluth und Sombke in Vorb.) und Hexapoda antennale Neuriten diskutieren. Diese projizieren ipsilateral in das Unterschlundganglion und innervieren kein Neuropil im Deutocerebrum. Sollten diese antennalen Projektionen bei den Crustacea gänzlich fehlen, dann wäre dies als Reduktion in der Stammlinie der Crustacea anzusehen. Sensorische Projektionen sind ebenfalls bei den Scorpiones beschrieben (siehe Kapitel 7.4) (Abbildung 22). Ob es sich hierbei um eine homologe Struktur handelt, bleibt ohne weitere Untersuchungen unklar.

Protocerebrale Verbindungen der Mandibulata

Als einzige Übereinstimmung der deutocerebralen Loben bei Chilopoda und Hexapoda nennen Strausfeld und Andrew (2011) die ipsilaterale Verbindung der olfaktorischen Glomeruli zu protocerebralen Neuropilen. Diese Projektionen sollen aber im Gegensatz zu den gut ausgebildeten antennocerebralen Trakten der Hexapoda bei den Chilopoda nicht als Bündel ausgebildet sein (Strausfeld und Andrew 2011). Die Projektionen zu sekundären protocerebralen Zentren der Chilopoda (Corpora pedunculata) standen nicht im Fokus dieser Arbeit. Gleichwohl sind die Corpora pedunculata in allen untersuchten Taxa der Chilopoda vorhanden (z.B. Fahlander 1938, Sombke et al. 2001a, b: Fig. 7 A). Ein Projektionstrakt zwischen olfaktorischen Glomeruli zu protocerebralen Neuropilen (Tractus olfactorio-globularis) wurde bislang wenigstens für Vertreter der Julidae (Diplopoda) von Holmgren (1916) beschrieben. Aktuell ist unklar, ob die Corpora pedunculata der Chilopoda eine homologe Struktur zu den Pilzkörpern der Hexapoda darstellen. Darüber hinaus besteht immer noch Dissens, ob diese protocerebralen Zentren (laterales Horn und/oder Pilzkörper der Hexapoda und Hemielipsoidkörper der Crustacea) bei den Tetraconata homolog sind. Sollte es sich bei protocerebralen Zielstrukturen und den Trakten um homologe Strukturen handeln, wäre ein ungekreuzter Projektionstrakt zwischen dem olfaktorischen Lobus und protocerebralen Neuropilen in das Grundmuster der Mandibulata zu stellen.

Interne Phylogenie der Mandibulata

Über die interne Phylogenie der Mandibulata lassen sich nach den dargelegten Merkmalen des Deutocerebrum nur wenige Aussagen treffen. Abbildung 22 zeigt das auf molekularen Sequenzdaten basierende phylogenetische Verwandtschaftsmodell der Arthropoda nach Regier et al. (2011). Die Myriapoda bilden hier die Schwestergruppe zu den Tetraconata. Basierend auf den im Rahmen dieser Dissertation konzeptionalisierten Merkmalen und dem Mandibulata-Modell ist nunmehr davon

auszugehen, dass der letzte gemeinsame Vorfahre der Myriapoda über eine neuropiläre, contralaterale Verbindung der mechanosensorischen Neuropile verfügte (Abbildung 22). Bezogen auf die Charakteristika des Deutocerebrum ist dies prinzipiell das einzige Grundmustermerkmal, das gegen das Taxon Tracheata spricht. Bei den Crustacea liegt diese Verbindung in Form von Neuriten vor; bei Hexapoda scheint diese Verbindung zu fehlen.

Die Position der Remipedia bzw. Xenocarida (Remipedia + Cephalocarida) ist nach den Merkmalen des Deutocerebrum schwierig zu interpretieren. Der Besitz eines distinkten medianen Antenne 1 Neuropil (MAN) bei den Remipedia spricht eher für eine engere Verwandtschaft zu den Eumalacostraca (alterative Hypothese *sensu* Fanenbruck und Harzsch 2005) als zu den Hexapoda (*sensu* Regier et al. 2010). Im Detail widersprechen die neuroanatomischen Daten dieser Dissertation allerdings der neurophylogenetischen Verwandtschaftshypothese von Fanenbruck und Harzsch (2005), denn die Autoren postulieren den Besitz distinkter deutocerebraler Neuropile für chemo- und mechanosensorische Qualitäten für das Grundmuster einer hypothetischen Stammart der Remipedia, Malacostraca und Hexapoda. Sollten die Remipedia die Schwestergruppe eines Taxons Branchiopoda + (Malacostraca + Hexapoda) innerhalb der Crustacea sein (*sensu* Strausfeld und Andrew 2011), dann wäre das MAN als apomorphes Merkmal der Crustacea anzusehen und implizit in den meisten Gruppen wieder reduziert worden.

Merkmale der olfaktorischen Glomeruli bei den Euarthropoda

Durch den Vergleich der olfaktorischen Glomeruli bei Chelicerata und Mandibulata lassen sich gemeinsame Merkmale finden, die zum Teil von Schachtner et al. (2005) für die Tetraconata als Apomorphien genannt wurden: Die olfaktorischen Glomeruli werden aus der Peripherie innerviert und Rezeptorneuriten besitzen uniglomeruläre Endigungen. Die OG sind mit lokalen Interneuronen assoziiert, die Neuropeptide als Co-Transmitter beinhalten und sind über Projektionstrakte mit protocerebralen Neuropilen verbunden. Sensorische Projektionen sind wenigsten für die Scorpiones beschrieben worden.

Die Evolution von olfaktorischen Glomeruli soll laut Strausfeld (1998) dreimal unabhängig bei den Pterygota, Malacostraca und Chilopoda (*Lithobius variegatus*) stattgefunden haben. Die neuroanatomischen Befunde aus dieser Dissertation unterstützen, aber relativieren auch das neurophylogenetische Konzept von Schachtner et al. (2005) (siehe Kapitel 7.3).

8. Antennale Sensillen der Chilopoda

Die Cuticularsensillen der Arthropoden sind Sinnesorgane die aus drei strukturellen Elementen bestehen: Sinneszellen, Hüllzellen und einer cuticulären Struktur (Hallberg und Hansson 1999). Aufgrund der morphologischen Diversität sind verschiedene Modalitäten realisiert, die zum Teil aus der äußeren Struktur, endgültig aber nur aus der inneren Organisation sowie Physiologie erkannt wird. Mechanorezeptive Sensillen sind durch den Besitz eines Tubularkörpers charakterisiert, der eine Auslenkung des Sensillums registriert. Sensillen bei denen dendritische Fortsätze der Sinneszellen bis zu einem terminalen Porus des Haarschafts ziehen, wird eine gustatorische Funktion zugeschrieben. Olfaktorische Sensillen sind im Allgemeinen durch Wandporen im Sensillenschaft, an welche dendritische Endigungen heranreichen, charakterisiert. Diese Poren können von der Epicuticula überzogen sein, so dass kein direkter Kontakt zwischen Sensilleninnenraum und Außenmedium besteht. Thermo- und Hygrorezeptive Sensillen sind meist in der Cuticula versenkt.

Sensillen kommen überall im Integument der Chilopoda vor (Rosenberg 2009). Frühe Untersuchungen der antennalen Sensillen der Chilopoden gehen auf das 19. und frühe 20. Jahrhundert zurück. Mittels lichtmikroskopischer Methoden wurden sie als Riechzapfen, Riechhaare oder Riechstäbchen bezeichnet (Leydig 1860, Sazepin 1884, Fuhrmann 1922, Verhoeff 1902-1925). Mit dem Beginn der elektronenmikroskopischen Forschung in der zweiten Hälfte des 20. Jahrhunderts wurden viele der lichtmikroskopischen Untersuchungen weitergeführt und um raster- sowie transmissionselektronenmikroskopische Methoden erweitert. So wurden die antennalen Sensillen einer Reihe von Chilopoden untersucht: *Scutigera coleoptrata* (Sombke et al. im Druck), *Lithobius forficatus* (Keil 1975, 1976), *Craterostigmus tasmanianus* (Ernst et al. 2006, Müller et al. 2011), *Cryptops hortensis* (Ernst et al. 2009), *Scolopendra oraniensis* (Ernst et al. in Vorb.) und *Geophilus flavus* (Ernst 1976, 1979, 1981, 1983, 1996, 1997, 1999, 2000). Durch die Untersuchung der antennalen Sensillen von *S. coleoptrata* konnte ein Vergleich der antennalen Sensillenausstattung der Chilopoda durchgeführt werden (Sombke et al. im Druck).

Die auffälligste Eigenschaft der Antennen von *S. coleoptrata* ist ihre Länge. Mit über 500 Antennomeren ist sie in etwa so lang wie der Körper. Die Antennen der Pleurostigmophora sind wesentlich kürzer mit 17 bis ca. 100 bei den Lithobiomorpha, 18 bei den Craterostigmomorpha, 17 bis 27 bei den Scolopendromorpha und 14 bei den Geophilomorpha (Lewis 1981, Rosenberg 2009, Sombke et al. im Druck).

Eine umfassende Darstellung des Feinbaus antennaler Sensillen der Chilopoda wurde jüngst von Müller et al. (2011) (Appendix II) angefertigt. Diese Arbeit stellt zehn verschiedene Typen von Cuticularsensillen vor und fasst darüber hinaus die sogenannten „sensory cones" und das Schaftorgan der Scutigeromorpha als stark modifizierte Varianten von Cuticularsensillen auf.

	Scutigera coleoptrata	*Lithobius forficatus*	*Craterostigmus tasmanianus*	*Cryptops hortensis*	*Geophilus flavus*
S. trichodeum	95-105	2,000	~ 1,100	~ 4,000	620-660
S. microtrichodeum	-	240	wenige	~ 100	80-114
Beak-like sensillum	~ 2,200	-	-	-	-
S. coeloconicum	-	-	5-8	-	wenige
S. brachyconicum	3	80	-	20-26	7
S. basiconicum	-	80	1	61-114	36-53
Bottle-like sensillum	-	-	4-16	-	-
Club-shaped sensillum	-	-	-	2-4	-
Hat-like sensillum	-	-	-	2-6	-
Tube-like sensillum	-	-	6-13	-	-
Sensory cones	+	-	-	-	-
Shaft organ	+	-	-	-	-

Abbildung 23: Typologie und Anzahl der antennalen Cuticularsensillen und Vorkommen von "sensory cones" und des Schaftorgans bei Vertretern der Chilopoda. + vorhanden, - nicht vorhanden.

Bei den Chilopoda und ebenso bei den Diplopoda sind Sensilla trichodea (Abbildung 24 A) vorhanden (Nguyen Duy-Jacquemin 2004, Chung und Moon 2006, Müller et al. 2011). Auch bei den Hexapoda sind trichoide Sensillen bekannt (z.B. Steinbrecht 1970). Aufgrund von feinstrukturellen Untersuchungen bei *Geophilus flavus* (Ernst 1976, 1996, 2000) und *Lithobius forficatus* (Keil 1975, 1976) kann eine Kombination chemo- und mechanorezeptiver Modalitäten angenommen werden. Propriorezeptive microtrichoide Sensillen (Abbildung 24 B) (kleiner als S. trichodea, weniger Sinneszellen, nur an der Basis der Antennomere lokalisiert) sind bis jetzt nur bei den Pleurostigmophora beschrieben worden (Keil 1975, Ernst 1983, Ernst et al. 2006, 2009). Ernst et al. (2009) diskutieren, ob es strukturelle Gemeinsamkeiten mit den propriorezeptiven Sensillen bei *Apis mellifera* (Hexapoda) gibt. Eine bimodale Funktion als Kontaktchemorezeptor wird ebenfalls angenommen. Coeloconische Sensillen sind nur bei den Craterostigmomorpha und Geophilomorpha bekannt (Ernst 2000, Ernst et al. 2009). Dieser Typ ist zudem bei allen Chilopoden auf den Maxillipeden sowie bei den Diplopoda auf den Antennen von *Polyxenus lagurus* und vielen Hexapoda vorhanden (zusammengefasst in Ernst und Rosenberg 2003). Die typischen multiporösen, olfaktorischen Sensilla basiconica (Abbildung 24 D) sind innerhalb der Chilopoda ausschließlich bei den Pleurostigmophora vorhanden. Sie sind auch bei den Diplopoda und den Hexapoda ausgeprägt (z.B. Steinbrecht 1970, Nguyen Duy-Jacquemnin 2004, Chung und Moon 2006).

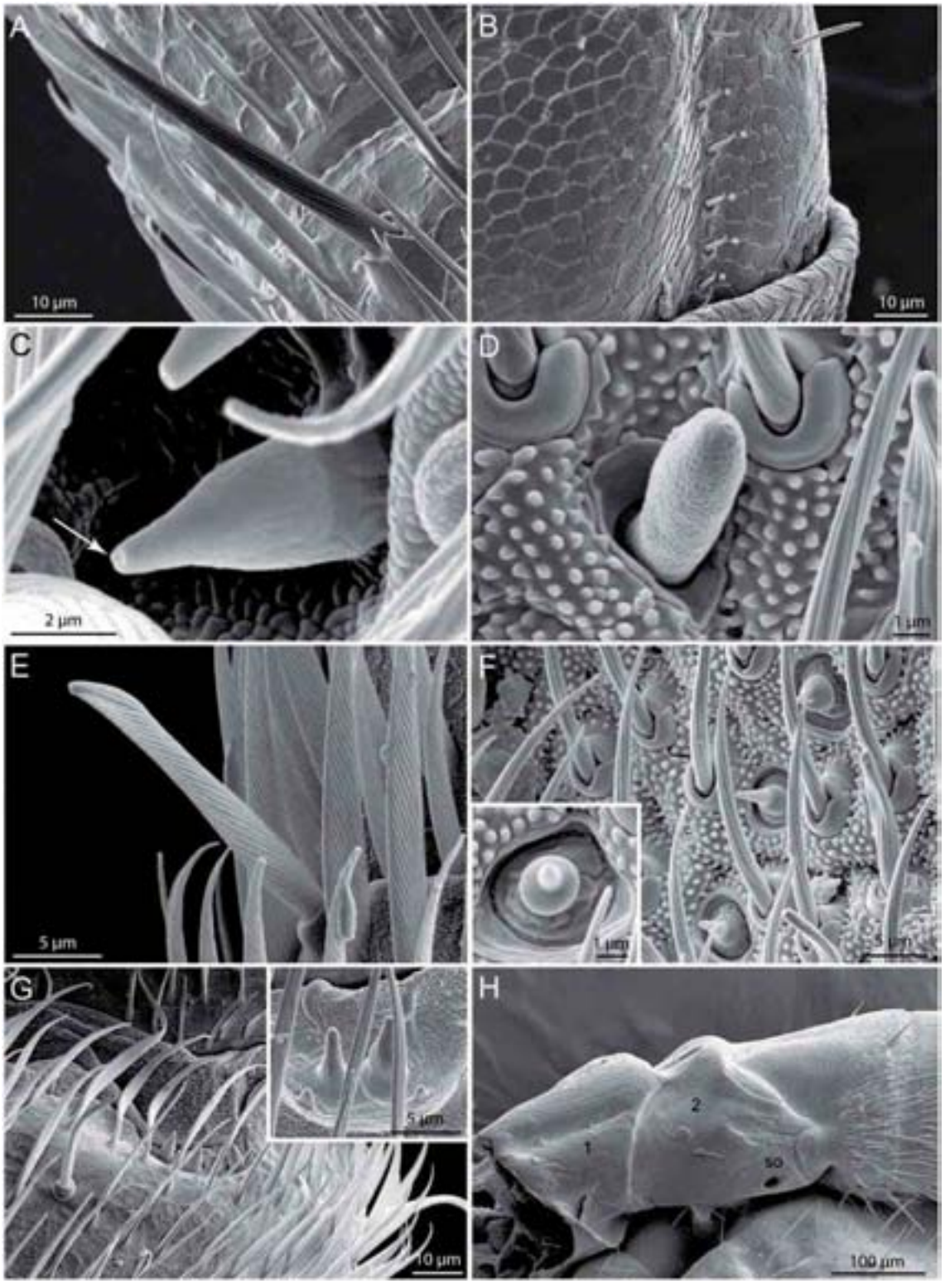
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Abbildung 24: Rasterelektronenmikroskopische Aufnahmen verschiedener antennaler Sensillentypen von *Scutigera coleoptrata* und *Scolopendra oraniensis*. (Sombke et al im Druck, Ernst et al. subm.) **A** Sensillum trichodeum mit charakteristischer basaler Schuppe *bei S. coleoptrata*. **B** Sensilla microtrichodea (Sternchen) bei *S. oraniensis* an der Basis eines Antennomers. **C** Sensillum brachyconicum am terminalen Antennomer von *S. coleoptrata*. Der Pfeil markiert die terminale Pore. **D** Sensillum basiconicum bei *S. oraniensis* mit charakteristischen Poren. **E** Beak-like Sensillum von *S. coleoptrata*. **F** Hat-like Sensilla bei *S. oraniensis*. Diese Sensillum ist durch eine dickere Basis und eine konische Spitze charakterisiert. **G** „Sensory cones" an einem antennalen Nodus bei *S. coleoptrata*. Die Vergrößerung zeigt größere und kleinere Morphen des gleichen Typs. **H** Die Antennenbasis von *S. coleoptrata* mit dem charakteristischen, zweigeteilten Schaft und dem Schaftorgan (so).

Sensilla brachyconica (Abbildung 24 C) sind nur von bei Chilopoda beschrieben worden (mit Ausnahme von *Craterostigmus tasmanianus*). Da bei *S. coleoptrata* eine Terminalpore vorhanden zu sein scheint (Abbildung 24 C, Pfeil), wird eine kontaktchemorezeptive Funktion angenommen (Sombke et al. im Druck). Club-shaped und Hat-like Sensilla (Abbildung 24 F) sind bisher nur bei *Cryptops hortensis* nachgewiesen worden (Ernst et al. 2009). Für beide wird eine chemorezeptive Funktion vermutet. Zusammengesetzte Sensillen (Bottle-like und Tube-like sensillum) wurden bisher nur auf den Antennen von *Craterostigmus tasmanianus* und *Scolopocryptops* sp. nachgewiesen (Ernst et al. 2006, Edgecombe und Giribet 2004).

Bei *S. coleoptrata* konnte zudem ein neuer Sensillentyp beschrieben werden: das Beak-like Sensillum (Abbildung 24 E) (Sombke et al. im Druck). Dieses scheint auch bei anderen Vertretern der Scutigeromorpha vorhanden zu sein (Edgecombe und Barrow 2007, Edgecombe und Giribet 2006). Bei *S. coleoptrata* konnten ca. 2.200 dieser Sensillen pro Antenne nachgewiesen werden. Es gibt einige Übereinstimmungen mit dem Sensillum trichodeum, aber auch Unterschiede, wie das spindelförmige Querschnittprofil des Haarschaftes, die gebogene Spitze und die Form der cuticulären Basis. Basierend auf rasterelektronenmikroskopischen Befunden, wurde eine chemo- und mechanorezeptive Funktion vermutet (Sombke et al. im Druck). Feinstrukturelle Untersuchungen (Müller, Lipke und Sombke in Vorb.) zeigen allerdings, dass 12 bis 18 äußere Dendritenglieder in den Schaft projizieren, welcher deutliche Poren aufweist. Dies lässt eindeutig auf eine olfaktorische Funktion schließen. In Kombination mit mechanorezeptiven Elementen (Tubularkörper) und beweglicher Haarschaftgelenkung sowie Dendritenaußengliedern, die zur terminalen Pore ziehen, kann darüber hinaus eine Trimodalität des Beak-like Sensillum angenommen werden. Diese Kombination verschiedener Sinnesmodalitäten in einem Sensillum ist für die Myriapoda bislang nicht beschrieben und findet auch kein strukturelles Äquivalent bei den Chelicerata, Crustacea und Hexapoda. An den distalen Enden der antennalen Noden, die die Antenne in vier Abschnitte teilen, wurden bei *S. coleoptrata* „sensory cones" nachgewiesen (Abbildung 24 G). Aufgrund der Lokalisation kann, ähnlich wie den S. microtrichodea, eine propriorezeptive Funktion angenommen werden. Eine weiteres Merkmal der Antenne der Scutigeromorpha ist der zweigliedrige Schaft (basale

Antennomere), welcher das Schaftorgan trägt (Abbildung 24 H). In dieser grubenartigen Vertiefung befinden sich mehrere conusförmige Sensillen, für die eine chemorezeptive Funktion angenommen wird (Sombke et al. im Druck).

Auf Basis rasterelektronenmikroskopischer Studien ist es möglich die antennalen Sensillen der Chilopoden zu klassifizieren und zu vergleichen. Innerhalb der Chilopoda lassen sich für die Scutigeromorpha einzigartige antennale Strukturen feststellen: (1) der Besitz von antennalen Noden, die „sensory cones" tragen, (2) der Besitz eines zweigliedrigen Schaftes, der das Schaftorgan trägt und (3) der Besitz des Beak-like Sensillums. Alle anderen Sensillentypen sind bei den Chilopoda vorhanden und können auch bei den Diplopoda bzw. zum Teil bei den Hexapoda gefunden werden. Eine einheitliche Nomenklatur der Sensillen liegt für die Arthropoda nicht vor. Ein Vergleich zwischen Chilopoda und Diplopoda ist möglich, auch wenn uneinheitliche Terminologien bei beiden Taxa Homologievergleiche erschweren. Zum Bespiel ist es unklar, ob die Apical cone Sensilla der Diplopoda den terminalen Sensilla brachyconica der Chilopoda entsprechen. Spezialisierte Typen wie Beak-like Sensilla, Sensilla brachyconica sowie Hat-, Tube-, Bottle-like und Club-shaped Sensilla stellen höchstwahrscheinlich Transformationen kontaktchemorezeptiver Sensillen dar. Es ist ebenso unklar, ob das Schaftorgan der Scutigeromorpha ähnlich aufgebaut ist, wie die tarsalen Porenorgane der Ricinulei (Chelicerata) (Talarico et al. 2005). Bei den Crustacea scheint die morphologische Diversität antennaler Sensillen noch viel größer als bei Hexapoda und Myriapoda zu sein (Hallberg et al. 1997). Generell teilen Sensillen zu viele gemeinsame Merkmale (strukturelle und funktionelle Elemente) und können daher wahrscheinlich nicht zur phylogenetischen Analyse der Arthropoda beitragen (Hallberg et al. 1997).

9. Neuroethologie

Perzeption

Aus den rasterelektronenmikroskopischen Studien bei *Scutigera coleoptrata* geht hervor, dass die antennalen Sensillen mechano- und kontaktchemorezeptive Funktionen ausüben. Die Perzeption taktiler Stimuli durch die Laufbeine wurde als wichtiger Faktor beim Beutefang von *S. coleoptrata* vorgeschlagen (Rosenberg 2009). Da *S. coleoptrata* hauptsächlich dämmerungs- und nachtaktiv ist scheint es möglich, dass zudem die Fähigkeit zur Olfaktion durch Sensillen ausgebildet ist, die einen Beutefang effektiver machen. Für das Beak-like Sensillum wird aufgrund des Vorhandenseins von Wandporen (Müller, Lipke und Sombke in Vorb.) unter anderem auch eine olfaktorische Funktion vermutet. Typische olfaktorische Sensilla basiconica sind bei *S. coleoptrata* nicht ausgebildet, so dass ohne feinstrukturelle Befunde zu den Beak-like Sensilla vermutet wurde, dass die Perzeption olfaktorischer Reize über die terminalen Poren der Beak-like Sensilla und Sensilla trichodea möglich wäre (Sombke et al. im Druck). Olfaktion über die Terminalporen der Sensilla trichodea ist bei Hexapoda beschrieben (z.B. Steinbrecht 1970). Die Sensilla brachyconica am terminalen Antennomer von *S. coleoptrata* sind höchstwahrscheinlich Kontaktchemorezeptoren oder Thermo- und Hygrorezeptoren, da die Antennenspitze zur Untersuchung der Umgebung genutzt wird. Generell benutzt *S. coleoptrata* die Antenne aber nicht ausschließlich, um die Umgebung abzutasten, da sie selten kompletten Kontakt zum Boden haben. Sie werden hauptsächlich parallel zum Boden gehalten, wobei der distale Nodus die Antennenspitze vertikal auslenken kann (Abbildung 25).

Verhaltensbiologische Untersuchungen

Bereits zu Beginn des 20. Jahrhunderts wurden ethologische Experimente durchgeführt, um die sensorischen Fähigkeiten diverser Chilopoda zu testen. Hennings (1904) und Friedel (1928) präsentierten Individuen von *Lithobius forficatus* „Fleischsaft" und kamen zu dem Ergebnis, dass dieser Stimulus in einer erhöhten Antennenbewegung resultiert. Antennenamputierte Individuen zeigten keine Reaktionen. Allerdings kam Scharmer (1935) mit dem gleichen experimentellen Aufbau zu gegensätzlichen Ergebnissen. Simon (1960) berichtete, dass die Schlüsselstimuli bei *L. forficatus* taktil und gustatorisch (Kontaktchemorezeption) sind. Schließlich untersuchte Meske (1961) die Fähigkeit zur Olfaktion bei *L. forficatus* und zeigte, dass die Putzfrequenz der Antennen bei erhöhten Konzentrationen von Buttersäure und „Regenwurmsaft" deutlich erhöht wurde. Daraus schloss er, dass *L. forficatus* olfaktorische Stimuli wahrnehmen kann.

Abbildung 25: *Scutigera coleoptrata* mit typischer Haltung der Antenne. Der proximale Teil der Antenne wird für gewöhnlich nach oben gehalten, der erste Nodus (**n**) biegt den Rest der Antenne in eine parallele Haltung zum Untergrund. Das distale Ende der Antenne (Pfeil in B) ist charakteristisch nach oben oder unten gebogen. (Originale).

Vergleichbare Experimente bei *Scutigera coleoptrata* wurden von Klingel (1960) durchgeführt. Er konnte keine Reaktionen auf olfaktorische und optische Reize feststellen. So hielt er lebende Fliegen in die Nähe der Tiere, was zu keiner Reaktion führte. Erst wenn er die Beine oder Antennen mit den Fliegen berührte, „sprang Scutigera regelmäßig zu". Dies deckt sich nicht mit eigenen Beobachtungen, bei denen *S. coleoptrata* regelmäßig juvenile Heimchen, die in einem Abstand von 1 bis 2 cm gehalten wurden, aus einer Pinzette ergriff.

Den einzigen Nachweis einer intraspezifischen Kommunikation über Pheromone bei Chilopoda konnten Littlewood und Blower (1987) für *Lithobius forficatus* darlegen. Bei

dieser Spezies wird in den Coxalporen das sogenannte Coxalpheromon produziert, welches ein „arrestment-behaviour" (Anlockung) auslöst. Zudem steigt die Putzfrequenz der Antenne.

Eigene intensive ethologische Untersuchungen konnten die Beobachtungen von Klingel (1960) erweitern und zum Teil widerlegen. Ungefütterte Individuen wurden nachts (während ihrer aktiven Periode) für einen längeren Zeitraum (maximal zehn Stunden) in einer Arena untersucht (Sombke et al. 2011a). Diese bestand aus einer Plastikschale (25 x 16 x 9 cm) in der zwei Löcher (1 cm Durchmesser) den Zugang zu zwei Behältern (8 x 5 cm) ermöglichte. In jeweils einem dieser Behälter wurde ein Stimulus bestehend aus fünf lebenden Heimchen oder einem chemischen Ganzkörperextrakt, der fünf Heimchen entsprach (zur Methode siehe Sombke et al. 2011a) dargeboten (Abbildung 26 A). Ein Versuch wurde jeweils nur für ein Individuum durchgeführt. Als positive Wahl wurde gewertet, wenn sich am folgenden Morgen ein Individuum in einen der Behälter aufhielt (eine Rückkehr in die Plastikschale war nicht möglich). Wenn eine Kontrolle gegen lebende Heimchen getestet wurde, entschieden sich die getesteten Individuen signifikant für den Stimulus (36:12, Binominaltest $p<0,001$). Wenn eine Kontrolle gegen den Ganzkörperextrakt getestet wurde, entschieden sich die getesteten Individuen ebenfalls signifikant für den Stimulus (33:14, Binominaltest $p<0,005$) (Abbildung 26 A).

In einem Freilandexperiment unter kontrollierten Bedingungen konnte ebenfalls eine eindeutige Reaktion auf den Ganzkörperextrakt nachgewiesen werden. Über drei Nächte wurden fünf Individuen in einem Terrarium im Freiland getestet (Stimuli: Extrakt und Wasser sowie eine Kontrolle) und mit einer Infrarotvideokamera beobachtet. Zwei verschiedene Verhaltensmuster wurden unterschieden: ungerichtetes Laufen und ein „arrestment-behavior" (Anlockung). Unter Anlockung wurde ein Verhalten definiert, bei dem Individuen direkt auf eines der drei Filterpapiere zulaufen und dieses für mindestens drei Sekunden untersuchen. Ungerichtetes Laufen wurde an allen drei Filterpapieren festgestellt. Das Anlockungsverhalten wurde mit 19 Kontakten einzig beim Stimulus festgestellt (Abbildung 26 B). Die Kontakte am Stimulus waren signifikant höher als an der Kontrolle und am wassergetränkten Filterpapier (Kruskal-Wallis Test $p<0,001$). Beide Ergebnisse weisen auf eine olfaktorische Perzeption aus der Distanz und eine gustatorische Perzeption (Untersuchung der Nahrungsquelle) in unmittelbarer Nähe hin (Sombke et al. 2011a).

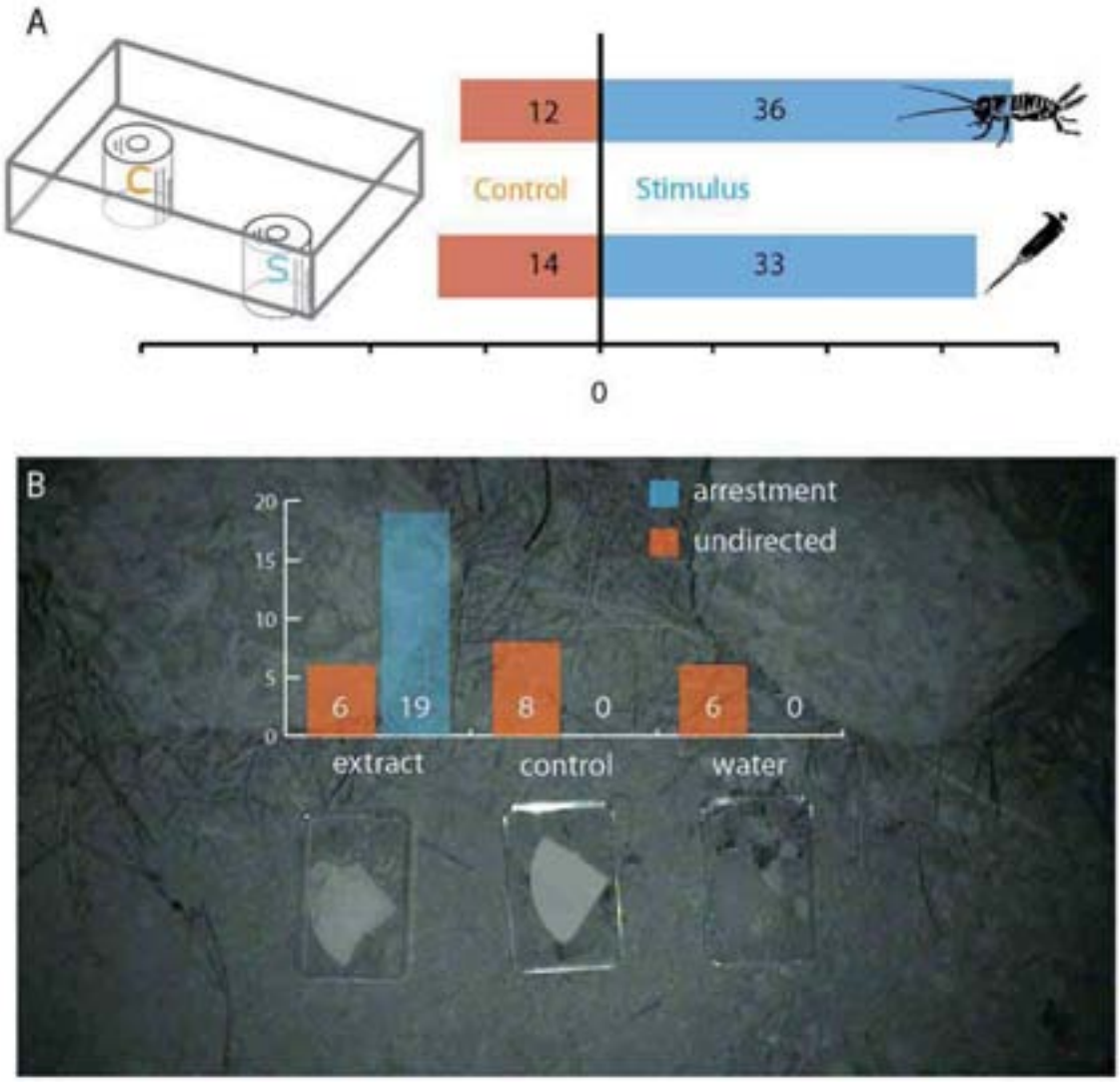

Abbildung 26: A Versuchsaufbau der ethologisches Experimente mit *Scutigera coeloptrata*. C = Kontrolle, S = Stimulus. Rote Balken zeigen die Anzahl der Individuen an, die sich für die Kontrolle entschieden; blaue Balken für die jeweiligen Stimuli. **B** Standbild einen Infrarot-Videos, das den experimentellen Aufbau des kontrollierten Freilandversuchs darstellt. In den Plastikschalen befinden sich drei Filterpapiere, die mit einem Extrakt (links) bzw. Wasser (rechts) getränkt wurden. Das mittlere Filterpapier dient als unbehandelte Kontrolle. Das Diagramm zeigt die Kontakte an den Filterpapieren. Rot = ungerichtetes Laufen, blau = Anlockung. (aus Sombke et al. 2011a).

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11. Selbständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, daß ich diese Arbeit selbständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

Andy Sombke

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13. Appendix

I The Nervous system of Chilopoda

Eigener Beitrag: Recherche (50%), Abbildungen und Bildtafeln, Textverfassung (60%)

II The sense organs of Chilopoda

Eigener Beitrag: Recherche (50%), Abbildungen und Bildtafeln, Textverfassung (40%)

III Antennal sensilla of *Scutigera coleoptrata*

Eigener Beitrag: Materialbeschaffung, Bildtafeln, Textverfassung (60%)

IV Anatomy of the deutocerebum of *Scutigera coleoptrata*

Eigener Beitrag: Experimente, Abbildungen und Bildtafeln, Textverfassung (90%)

V Deutocerebral organization and olfactory behavior in *Scutigera coleoptrata*

Eigener Beitrag: Experimente, Abbildungen und Bildtafeln, Textverfassung (90%)

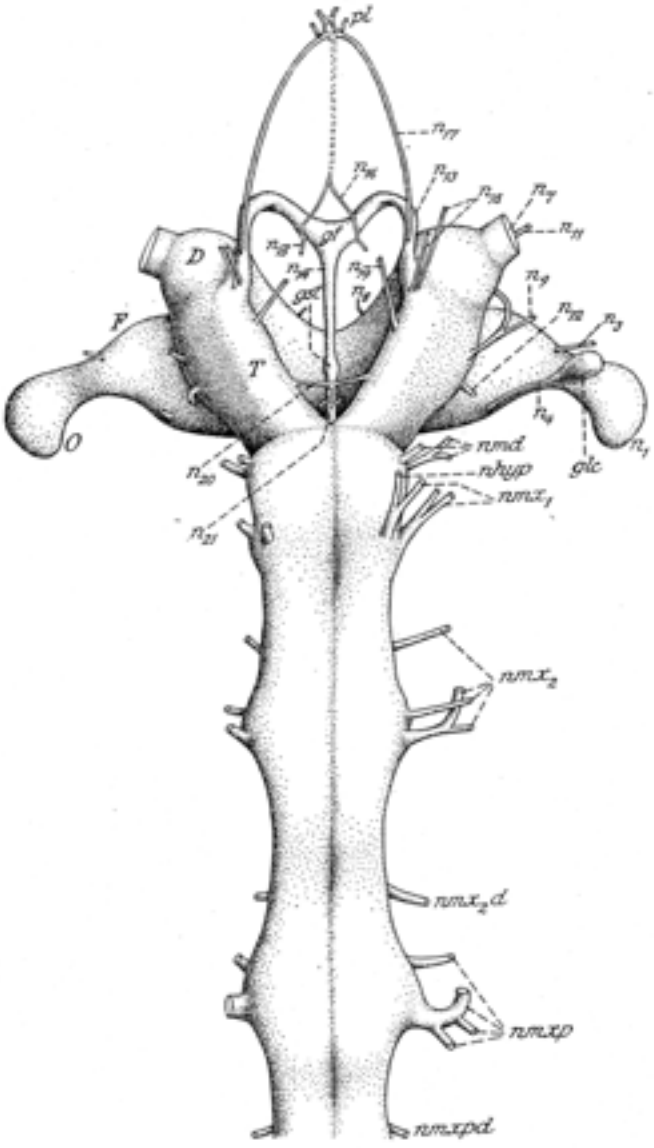
VI Deutocerebral organization in Chilopoda

Eigener Beitrag: Experimente (80%), Abbildungen und Bildtafeln, Textverfassung (90%)

I

The nervous system of Chilopoda

Sombke A, Rosenberg J, Hilken G. 2011. Chilopoda – The Nervous System. In: Minelli A (Ed.). Treatise on Zoology – Anatomy, Taxonomy, Biology – Myriapoda I. Brill Leiden. S. 217-234.



Chapter 11

CHILOPODA – THE NERVOUS SYSTEM

Andy Sombke, Jörg Rosenberg & Gero Hilken

The central nervous system

The nervous system of chilopods is divided into the central nervous system, composed of the brain and ventral nerve cord, and the peripheral nervous system with its projecting nerves. The central nervous system (brain or syncerebrum) is divided into the proto-, deuto-, and tritocerebrum. The peripheral nervous system is linked by nerves from the brain or ventral nerve cord to sense- and locomotion organs.

The protocerebrum

The protocerebrum is the largest part of the brain. It is well developed in Lithobiomorpha, Scolopendromorpha, and, especially, in Scutigeromorpha. In analogy to the Hexapoda, the posterodorsal region is called pars intercerebralis (Joly and Descamps, 1987). The protocerebrum extends laterally and includes the optic lobes. Geophilomorpha and eyeless Scolopendromorpha have reduced protocerebral lobes. The optic lobes in Scutigeromorpha (Fig. 11.3 A) comprise two neuropils: (1) the lamina, which supplies uncrossed axons to the optic tectum (Melzer et al., 1996; Strausfeld, 2005), the latter consisting of a thin plate-like neuropil extending out from the lateral edge of the protocerebrum; (2) the optic or visual tectum (formerly called medulla) is equipped with large relay neurons that send their axons into the brain. After Strausfeld (2005) the second neuropil matches the characteristics of the hexapod lobula plate. Therefore, it should be named optic tectum to avoid the assumption of a homology between the medulla of Hexapoda and malacostracan Crustacea and the second optic neuropil of Chilopoda. In *Lithobius forficatus*, long axons branch within the optic tectum and possess long collaterals that project to different regions of the neuropil. Large interneurons connect the optic lobes with the dorsal and ventrolateral protocerebrum. The neurons possess large neurites projecting from the optic tectum region medially and dorsally (Melzer et al., 1996). In Scutigeromorpha, the lamina has a simple organization; for each ommatidium, there are two relay neurons (monopolar cells) but no evidence of

local interneurons. The second optic neuropil is equipped with large tangential neurons that send their axons from its inner edge into the protocerebrum, where they terminate at the dendrites of descending neurons (Strausfeld, 2005). According to Strausfeld, the cell arrangements in the optic tectum are identical to the organization of neurons in optic neuropils of hexapods exemplified by the lobula plate of dipterous insects.

Laterally, above the protocerebrum, a large cluster comprising many small globuli cells (characterized by a minute amount of perikaryal cytoplasm and by nuclei with condensed chromatin) provide a system of parallel fibers that provide two substantial lobate neuropils, one lateral and one close to the brain's midline. Together these structures have been termed the mushroom bodies or corpora pedunculata or globuli (Joly and Descamps, 1987). However, homology with structures having the same name in hexapods is unclear. In Scutigeromorpha, the pedunculus is divided into two strands. In Lithobiomorpha and in Scolopendromorpha, only undivided pedunculi are present, whereas they are lacking in Geophilomorpha.

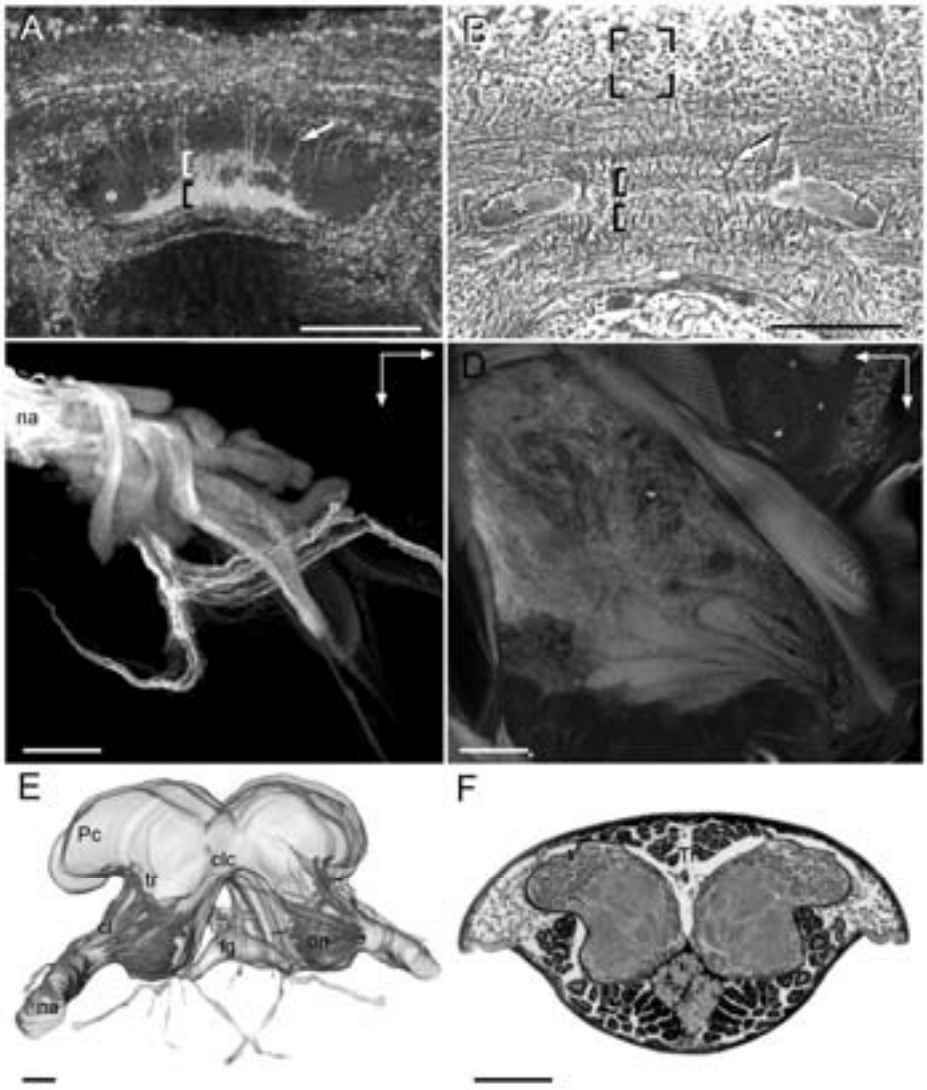
The midline neuropil (or central complex) in *Scolopendra* spp. has been described by Loesel et al. (2002). In the investigated species, the central complex is roughly hemi-ellipsoid in shape and situated between the proximal tips of the medial lobes of the corpora pedunculata (Fig. 11.1 A, B). The central complex neuropil consists of at least three horizontal layers: a ventral, a medial, and a dorsal. In *Scolopendra* spp., the midline neuropil is innervated by fine fibers that are arranged in a more or less columnar manner and show an immunoreactivity against the neurotransmitter allatostatin The medial lobes of the corpora pedunculata are immunonegative. Many delineated lateral brain regions, as well as commissural tracts, are immunoreactive to allatostatin and tachykinine-related peptide. In addition, Bodian staining of *Scolopendra polymorpha* revealed a system

Fig. 11.1 A *Scolopendra polymorpha*, columnar array of allatostatin-like immunoreactive fibres that innervate the unique midline neuropil from its upper surface. The arrow indicates columnar fibres. The asterisk indicates the tip of the medial lobe of the mushroom body (corpora pedunculata). **B** *S. polymorpha*, Bodian staining shows an additional system of interwoven fibres. Square brackets show corresponding layers of the midline neuropil in A and B. The open bracket indicates cluster of large cell bodies. **C** Dextranbiotin backfill of the left deutocerebral lobe of *Scutigera coleoptrata*. **D** Immunolocalization of synaptic proteins and phalloidin labeling in the left deutocerebral lobe of *Scutigera coleoptrata* (horizontal section). **E** 3D-reconstruction of the brain of *Scutigera coleoptrata*. corpus lamellosum (posterior) and olfactory neuropils (anterior). **F** Cross section of the head of *Cryptops hortensis*. All scale bars 100 µm. A and B after Loesel et al. (2002), C-E modified after Sombke et al. (2009), F original A. Sombke.

Cl Corpus lamellosum; **clc** contralateral connection; **d** dorsal; **Dc** deutocerebrum; **f** frontal; **Fg** frontal ganglion; **m** median; **Na** nervus antennalis; **On** olfactory neuropil; **Pc** protocerebrum; **Tr** afferent tracts

of interweaving fibers in the midline neuropil. The central body is well developed in Scutigeromorpha, whereas in Geophilomorpha it is vestigial.

The cerebral nerves were first described by Holmgren (1916) and Fahlander (1938) and later compared by Seifert (1967b) (see Table 11.2). Rilling (1968) and Joshi et al. (1977) described the cerebral nerves from *Lithobius forficatus* and *Scolopendra morsitans*. In *Lithobius forficatus*, the nervus Tömösváryi (only known in Scutigeromorpha and Lithobiomorpha),



which originates in the proximal region of the postantennal organ (= temporal or Tömösváry organ; see p. 000), projects posteriorly and medially to the cell body rind of the optic lobes (Petykó et al., 1996). Here, the nerve runs medially near the anterior surface of the brain. Adjacent to the optic tectum, its fibers pass through the cell body layer and enter the neuropil proper. They terminate in the neighborhood of the ipsilateral globuli cell cluster and pedunculus of the corpora pedunculata. The paired cerebral gland is connected with the protocerebrum over the nervus glandulae cerebralis. Only in Lithobiomorpha is this nerve paired. According to Rilling (1968), the pathways of these nerves are comparable to that of the allatocardiac nerves in hexapods.

The deutocerebrum

The deutocerebrum is the most anterior part of the brain with regard to the body axis, but is caudal to the protocerebrum with respect to the neuraxis. A visible transition between the proto- and deutocerebrum is, however, not obvious. The deutocerebrum receives the antennal nerves at its frontolateral edges. The deutocerebrum is densely packed with both hemispherical and elongated partitions called olfactory neuropils (Sombke et al., 2009). These extend anteriorly through the deutocerebrum. In contrast to the other Chilopoda, Geophilomorpha show medially fused deutocerebral lobes.

In *Scutigera coleoptrata*, the deutocerebral lobes contain dense neuropils that have the shape of elongated cylinders (Fig. 11.1 C, D). In analogy to hexapods, the sum of these neuropils is called the antennal lobe. In *S. coleoptrata* one neuropil provides a contralateral connection. In hexapods, antennal lobe neuropils are organized as numerous and approximately spherical glomeruli. In crustaceans, the olfactory lobes are divided into

Table 11.1 Stomatogastric nerves. Number of positive signs equals the pairs of nerves. After Seifert (1967a).

	Scutigeromorpha	Lithobiomorpha	Scolopendromorpha	Geophilomorpha
Stomodeal bridge	-	+	+	+
Frontal connectives	+	-	-	-
Nervus labralis	+	++	+	+
Nervus recurrens	+	+	+	+
Nervus connectivus I	+	+	++	+
Nervus connectivus II	-	+	-	-

elongated columnar subunits (reviewed in Schachtner et al., 2005). Elongated to spherical olfactory neuropils are also present in Lithobiomorpha, Scolopendromorpha (Fig. 11.1 F), and Geophilomorpha.

In *S. coleoptrata*, the antennal nerve divides into two branches: (1) the dorsofrontal part innervates the olfactory neuropils (as mentioned above), and (2) the ventrocaudal part innervates the corpus lamellosum (Fig. 11.1 C, E). Seifert (1967a) argued that the second antennal nerve serves to process mechanosensory signals. Therefore, the corpus lamellosum, which is also present in Scutigeromorpha, Lithobiomorpha and Scolopendromorpha, may represent a specialized mechanosensory neuropil.

The tritocerebrum and stomatogastric nervous system

The tritocerebrum is the smallest part of the brain and is located ventrally on both sides of the esophagus. The tritocerebral lobes presumably are linked by two commissures: (1) a supra-oesophageal commissure or stomatogastric/stomodeal bridge, and (2) a subesophageal commissure or tritocerebral commissure.

The stomatogastric nervous system innervates the mouth- and the preoral region as well as the frontal part of the gut and carries both motor and sensory axons. There is a stomatogastric bridge in Scutigeromorpha, Lithobiomorpha, Scolopendromorpha and Geophilomorpha. Only in Scutigeromorpha is it substituted by long frontal connectives linking the tritocerebral lobes with the frontal ganglion. All centipedes have a nervus recurrens, which leaves the frontal ganglion ventrocaudally. Through the dorsal dilators of the foregut, the nerve enters the musculature of the pharynx. The n. recurrens then extends caudally between the outer ring musculature layer and the inner longitudinal musculature of the epithelium of the gut. A nervus recurrens dorsalis is present exclusively in Geophilomorpha (Fig. 11.5 D, Nrd). In all taxa a hypocerebral ganglion is formed in the course of the n. recurrens (Figs. 11.4, 11.5; Hcg). Although Fahlander (1938) described a nervus frontalis in Scutigeromorpha and Lithobiomorpha (Fig. 11.4 A, B; Nf) the existence of this nerve was not verified and Seifert (1967a) thought it to be an artifact. In Lithobiomorpha, a connective nerve (nervus connectivus II, Table 11.1) is present, from which branches a thin nerve between the frontal ganglion and the brain.

This is the dorsal cardiac nerve (Fig. 11.4 D; Ncd) that projects to the roof of the cephalic aorta and runs almost the entire length of the dorsal vessel. Labral nerves also have their origin in the tritocerebrum. In Scutigeromorpha, Geophilomorpha and Scolopendromorpha, there is one pair of labral nerves whereas in Lithobiomorpha there are two pairs. A pair of nerves (nervus connectivus I; Figs. 11.4 C, D; 11.5 C; Nc) leaves the

esophageal connectives ventrally. In Scolopendromorpha, a second pair of connective nerves can be found (Fig. 11.5A, Nc). They were misinterpreted as a free tritocerebral commissure (Seifert, 1967a). In contrast to diplopods where a free tritocerebral commissure occurs, in chilopods the tritocerebral commissure takes its course within the subesophageal ganglion.

Subesophageal ganglia and the ventral nerve cord

The ventral nervous system is segmentally organized, with a pair of fused ganglia serving each segment. In the Geophilomorpha and Lithobiomorpha, the ventral nerve cord innervates the heart via segmental heart nerves (not studied in other taxa) (Scheffel, 1961; Ernst, 1971).

In *Lithobius forficatus*, the ganglia associated with the mandibles and both maxillae are fused as a single mass. This is caudally linked by paired connectives to a sequence of sixteen well-separated ganglia that innervate the trunk limbs. A terminal ganglion - possibly a fusion product of several neuromeres - succeeds the sixteenth trunk ganglion (Harzsch, 2004).

In *Geophilus flavus* the pregenital and genital ganglia are linked via short connectives (Ernst, 1971).

n *L. forficatus*, eight pairs of nerves arise from the ganglia (Fig. 11.2 A). Three are purely motor nerves innervating musculature, while the others also contain sensory axons. Exclusively sensory nerves have not been described (Rilling, 1968). The innervation pattern of dorsolateral motor neurons and interneurons differs considerably from that seen in hexapods, although in all euarthropods the motor neurons supplying an appendage derive from two populations belonging to two adjacent ganglia (Kutsch & Breidbach, 1994; Kutsch & Heckmann, 1995; Heckmann & Kutsch, 1995). All axons exit via the posterior nerve of the anterior ganglion. The fibers from the posterior ganglion ascend via the lateral region of the connective. All axons exit via an intersegmental nerve. The fibers that stem from the anterior and posterior neuron group run through the lateral region of the connective (Heckmann & Kutsch, 1995).

Somata of excitatory leg motor neurons in Euarthropoda in general seem to be arranged in three clusters in the ventral ganglionic cell cortex. These clusters are subdivided into smaller soma groups. Single cells appear to innervate the walking appendages (arrows in Fig. 11.2 B). In *L. forficatus*, there is a contralateral cluster composed of up to five cell bodies, which apparently correspond to the contralateral clusters observed in scorpions, crustaceans, and hexapods (Fig. 11.2B). At least one of

these cell bodies is GABA-immunoreactive (arrow with asterisks in Fig. 11.2 B) (Harzsch et al., 2005). Three median pairs of serotonin immunoreactive neurons and one pair on the lateral border of the ganglion are identifiable (Harzsch, 2004).

The extremely rapid escape responses of centipedes suggest that the ventral nerve cord contains fast through-conducting pathways. Babu (1964) studied the giant fibers in the ventral nerve cord in Scolopendromorpha. According to their conducting speeds, three groups of giant fibers can be distinguished: (1) 3–4.5 m/s, (2) 3–3.5 m/s, and (3) 2–3 m/s. Giant fibers with slower conducting speed are responsible for the leg movement. In *Scolopendra morsitans*, the giant fibers are arranged in three groups: (1) dorso-lateral, (2) dorso-intermediate, and (3) dorso-medial. The diameters of giant fibers range between 28–58 µm. The dorso-medial and dorso-intermediate groups correspond to the ascending pathway of crustacean and hexapods, the dorso-lateral group to the descending pathway (Varma, 1971).

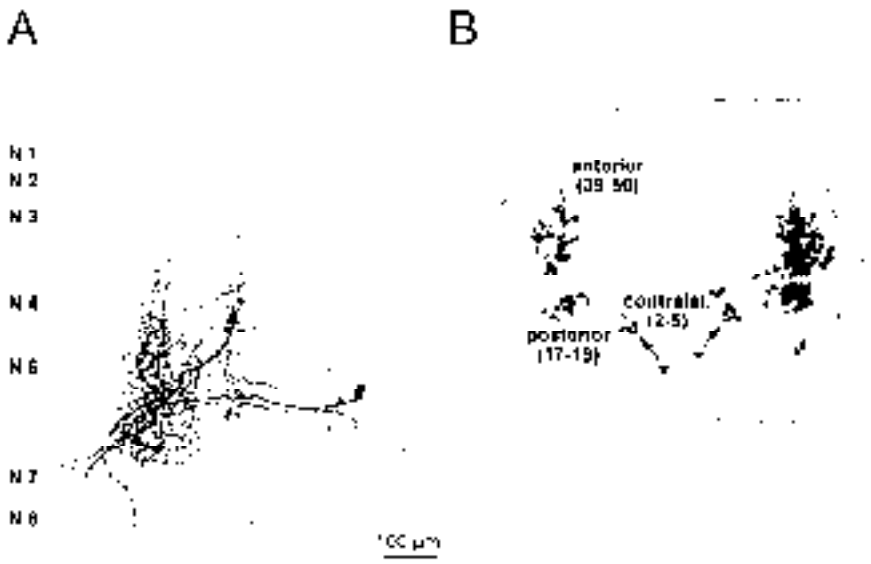


Fig. 11.2 A Selective drawing of some of the neurites belonging to the anterior set supplying the dorsal longitudinal muscles in ganglion 8 in *Lithobius forficatus*. There are large contralateral and two small midline somata. **N 1-4, 6-8** Lateral nerve roots. **B** Distribution of walking leg motor neuron somata in the ventral soma complex in *Lithobius forficatus*. The anterior cluster contains the largest number of somata. Putative inhibitors are marked by arrows with asterisks. Only the ipsilateral portions of the ganglia are shown; broken lines indicate ganglion midlines. A modified after Heckmann & Kutsch (1995); B modified after Harzsch et al. (2005).

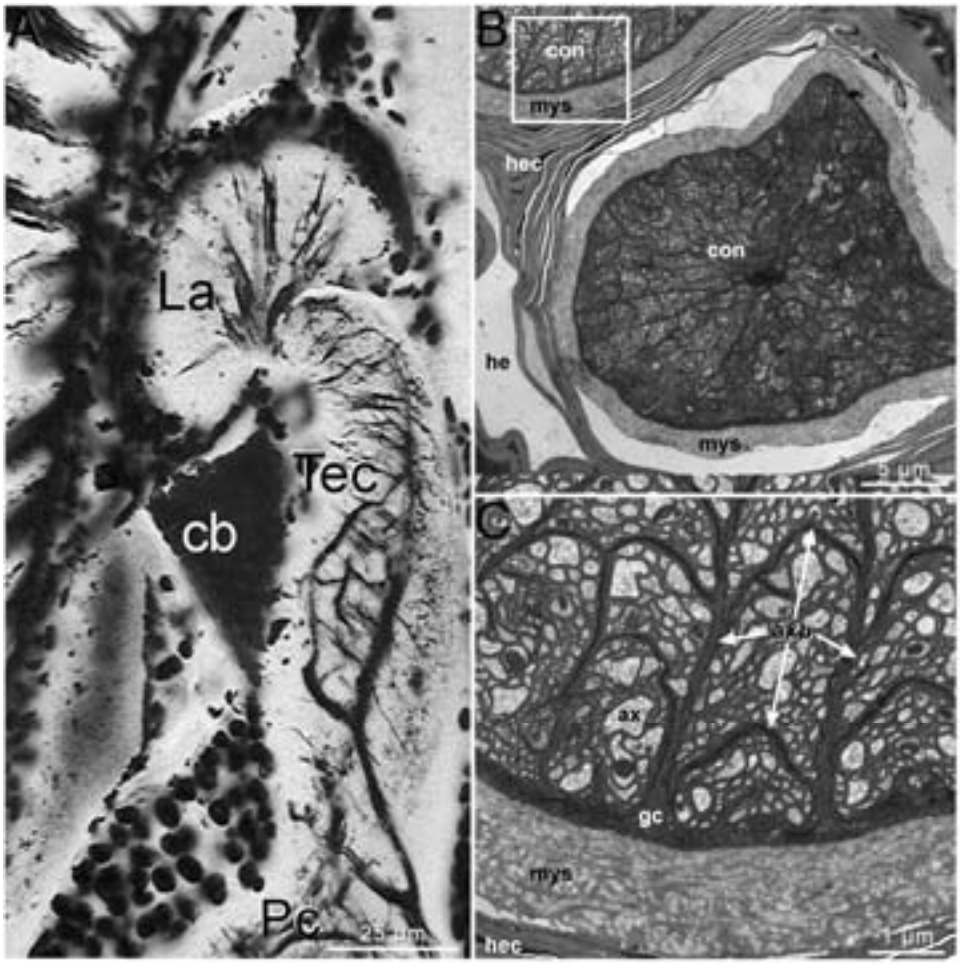


Fig. 11.3 **A** Bodian staining of optic neuropils in *Scutigera coleoptrata*. The passage of axons is constricted midway between the lamina and visual tectum. The two neuropils are connected by uncrossed axons. **B** Cross section of a connective of the ventral nerve cord with myelin sheath. **C** Magnification of connective and myelin sheath. A modified after Strausfeld (2005); B, C original C. H. G. Müller.

ax axon; **axb** axon bundles; **cb** cell bodies of neurons associated with the tectum (location behind the tectum's inner surface); **con** connective; **gc** glial cells **he** hemolymph; **hec** hemolymphatic channel; **La** lamina; **mys** myelin sheath; **Pc** protocerebrum; **Tec** visual tectum

The nervous system of Scutigeromorpha

Due to their roundish head shape, the appearance of parts of the central nervous sys-

tem in representatives of the Scutigeromorpha differs from that of other chilopod taxa (Fig. 11.4 A, C). The protocerebrum extends as a huge mass dorsally from the deutocerebrum. The elongated optic lobes, which extend contiguously from the lateral protocerebrum, are well developed compared to those of other chilopods in that they serve a sophisticated compound retina and are composed of orderly organized neurons. The cerebral glands (endocrine organs) are connected with the lateral protocerebrum by the nervus glandulae cerebralis (Fig. 11.4 A, Ngc).

The deutocerebrum, which is the second neuromere of the brain, actually extends forward to receive the robust antennal nerves. Within the deutocerebrum, the olfactory neuropils and the corpus lamellosum (mechanosensory neuropil) are visible as separate structures (Fig. 11.1 C, D, E) (Sombke et al., 2009). Like in hexapods, the deutocerebrum is partitioned into many discrete neuropils receiving what are thought to be olfactory receptor axons. However, these subunits have a cylindrical form instead of the spherical glomeruli that typify many insect olfactory lobes. Saint-Remy (1887, 1889) and Hörberg (1931) described these cylinders as irregular, convoluted ribbons. Dextranbiotin backfills reveal that these neuropils are first order processing units in the deutocerebrum (Fig. 11.1 C). In *Scutigera coleoptrata*, thirty-four distinct and uniquely identifiable subunits have been identified that are invariant between individuals. At least one subunit is typically linked to its contralateral counterpart. There is no evidence of further partitions within the cylindrical subunits, in contrast to the layering that hallmarks columns in the olfactory lobe of malacostracan crustaceans (Sandeman et al., 1992; Harzsch and Hansson, 2008). The ventrocaudal part of the antennal nerve innervates the presumed mechanosensory neuropil of the corpus lamellosum, which is composed of approximately eight lamellae divisible into two classes: (1) the outer two lamellae that form a 180° loop, (2) the inner lamellae that project further on dorsomedially (Fig. 11.1 E) (Sombke et al., 2009). In the Scutigeromorpha and Scolopendromorpha, these neuropils are connected contralaterally by the posterior antennal commissure (Fahlander, 1938). By analogy with the Hexapoda, a chemosensory function for the olfactory neuropils and a mechanosensory function for the corpus lamellosum have been suggested (Sombke et al., 2009).

A demarcation between the tritocerebrum and deutocerebrum is not apparent although the nervi frontales are supposed to indicate the anterior margin of the tritocerebrum, just posteroventral to the antennal lobe. The frontal connectives of the tritocerebrum project slightly caudad and converge medially at the frontal ganglion. They are distinguished from the stomodeal bridge by the absence of a layer of tritocerebral gangliar cells (see also Table 11.1). The nervus recurrens projects dorsally

from the frontal ganglion on top of the pharynx and esophagus. The nerve descends into the hypocerebral ganglion and its axons disperse over the foregut epithelium and musculature of the pharynx. Fahlander (1938) described a nervus frontalis (Fig. 11.4 A, B Nf) in *Thereuopoda clunifera* and *L. forficatus* but this was disputed by Seifert (1967a), who suggested that most likely Fahlander described a trachea. A pair of labral nerves emerges in the front of the tritocerebrum. These nerves converge and innervate the musculature of the clypeolabral region and the labral gland (buccal gland) (Fig. 11.4 A, C Nl). Paired connective nerves leave the tritocerebrum posteriorly and merge with the subesophageal ganglion. From the subesophageal ganglion an unpaired nerve projects between the pharynx and the musculus tentorio-pharyngealis medialis. In *T. clunifera*, Fahlander (1938) described a free tritocerebral commissure (Fig. 11.4 A, Tcc). According to Seifert (1967a), in *S. coleoptrata* this commissure lies within the subesophageal ganglion (Fig, 11.4 C, Tcc).

The nervous system of Lithobiomorpha

In *Lithobius forficatu*s, the brain (Fig. 11.4 B) is located in the frontal part of the head above and in front of the pharynx (Fig. 11.4 D). The nerve to the cerebral glands is paired. A transition between the deutocerebrum to the large tritocerebrum is not visible.
The tritocerebral lobes are connected to the frontal ganglion over a short stomodeal bridge.

A short unpaired nerve projects from the frontal ganglion to the pharyngeal musculature that connects to the ventral side of the protocerebrum (Fig. 11.4 D, Nc II). According to Seifert (1967a), this nerve projects as the nervus connectivus II (see Table 1) into the medial part of the brain. Rilling (1968), however, was unsure about the destination of this nerve. Herbst (1891) first discovered the dorsal heart nerve (Fig. 11.4 D, Ncd), which projects to the ending of the aorta cephalica and follows it thereon (Seifert, 1967b). The nervus recurrens projects caudally from the frontal ganglion and supplies the frontal part of the alimentary tract dilators of the pharynx. Ganglionic swellings mark the hypocerebral ganglion.

A nerve projects to the hypopharynx from each connective of the tritocerebrum. The two nerves merge after entering the hypopharynx.

According to Rilling (1968) this is the free tritocerebral commissure, which innervates the musculature of the hypopharynx. However, Seifert (1967a) claims that a free tritocerebral commissure does not exist and that the tritocerebral commissure continues within the subesophageal ganglion.

The nervous system of Scolopendromorpha

In representatives of the Scolopendromorpha the head is flattened and the shape of the brain reflects this shape (Fig. 11.5 C). The protocerebrum is located dorsally and caudal to the deutocerebrum. It provides short optic lobes that receive four visual nerves. Eyeless species, like *Cryptops* spp., have vestigial optic lobes (Fig. 11.1 F). In all Scolopendromorpha, the nervus glandulae cerebralis projects from the ventral border of the protocerebrum to the protocerebral glands (Fig. 11.5 B). Postantennal organs and nervi Tömösváryi are not present.

The deutocerebrum, although the second neuromere, extends forward from the protocerebrum. The dorsal antennal flexor muscle is innervated by a nerve that arises caudally from the antennal basis. A second nerve arises caudally in the ventrolateral part of the deutocerebrum. This carries the axons of motor neurons to antennal muscles and the axons of sensory neurons from receptors on the antenna. The deutocerebrum merges with the tritocerebrum without any noticeable border. The tritocerebral lobes are connected to the frontal ganglion by a short stomodeal bridge.

The nervus recurrens projects dorsally from the frontal ganglion and enters the outer and inner ring musculature of the pharynx. Caudally, the nerve swells into a hypocerebral ganglion equipped with a few neurons (Seifert, 1967a; Joshi et al., 1977). Two nerves arise from the tritocerebral ganglia in the vicinity of the frontal ganglion and split up into three branches (Fig. 11.5 A, C, Nl). According to Seifert (1967a) these nerves are the basis of the nervus labralis. Joshi et al. (1977) claimed that yet another nerve arises from the tritocerebrum. Fahlander (1938) and Joshi et al. (1977) described a free tritocerebral commissure which, according to Seifert (1967a), is covered with a joint neural lamella inside the subesophageal ganglion. The caudal connective nerves arising laterally from the esophageal connectives project into the mandibles. Varma (1971) described the dorsal heart nerve of *S. morsitans* that extends from the head up to the segment 21.

The nervous system of Geophilomorpha

The brain of the Geophilomorpha, all of which are eyeless, is relatively flattened and is also the most modified in Chilopoda (Fig. 11.5 B, D). Correlated with the absence of a visual system, the protocerebrum is reduced. Ventrally, the paired nervus glandulae cerebralis emerge and project to the cerebral gland. This nerve was misleadingly described as nervus tömösváry by Saint-Remy (1887). However, a postantennal organ is missing in Geophilomorpha.

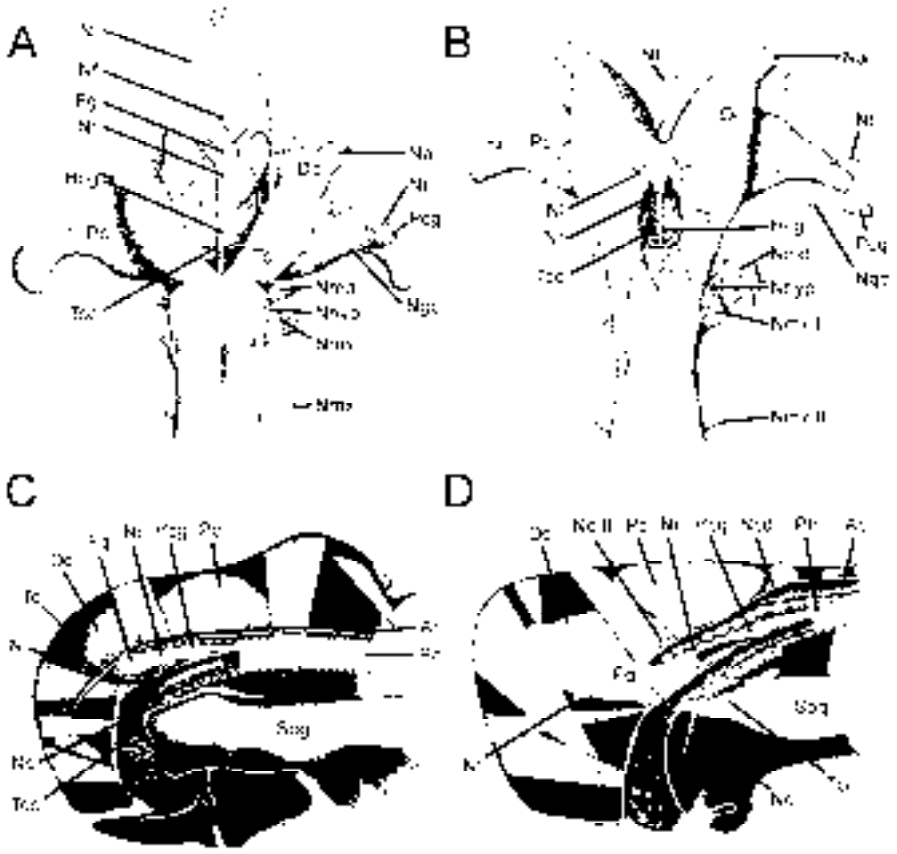


Fig. 11.4 **A** Nervous system of *Thereuopoda clunifera* (Scutigeromorpha). Ventral view. **B** Nervous system of *Lithobius forficatus* (Lithobiomorpha). Ventral view. Only one nervus glandulae cerebralis is shown. Nervus connectivus not shown. **C** Mediosagittal section through the head of *Scutigera coleoptrata* (Scutigeromorpha). **D** Mediosagittal section through the head of *Lithobius forficatus* (Lithobiomorpha). A–B modified after Fahlander (1938); C–D modified after Seifert (1967a).

Ac Aorta cephalica; **Dc** Deutocerebrum; **Fg** Frontal ganglion; **Hcg** Hypocerebral ganglion; **Na** nervus antennalis; **Nc** nervus connectivus 1; **Nc II** nervus connectivus 2; **Ncd** nervus cephalicus dorsalis; **Nf** nervus frontalis (existence unclear after Seifert); **Ngc** nervus glandulae cerebralis; **Nhyp** nervus hypopharyngealis; **Nl** nervus labralis; **Nmd** nervus mandibularis; **Nmx I** nervus maxillaris 1; **Nmx II** nervus maxillaris 2; **Nr** nervus recurrens; **Nt** nervus tömösváry; **Pc** Protocerebrum; **Pcg** Protocerebral gland; **Ph** Pharynx; **Sog** Subesophageal ganglion; **Tc** Tritocerebrum; **Tcc** Tritocerebral commissure

Table 11.2 Areas innervated by nerves of the brain and the subesophageal ganglion of *L. forficatus*; nerve numbers after Fahlander (1938) (first column) and Rilling (1968) (second column).

	Protocerebrum	
N1	N1	ocelli
N3	N2	organ of Tömösváry
	N3	cerebral gland
N4	N4	cerebral gland
N6 (Dc)	N5	ocellar region, epicranium
	N6	ocellar region, epicranium
N2	N7	ocellar region, epicranium
	N8	?
	Deutocerebrum	
N7	N9	antennal nerve (motor/sensory)
	N10	antennal musculature-sensory organ
	N11	free nerve endings on antennifer/antenna articulation
	N12	?
N10	N13	basal antennal musculature
N12	N14	basal antennal musculature
N11	N15	basal antennal musculature
	Tritocerebum	
N13	N16	stomodeal bridge/frontal ganglion
N15	N17	pharynx dilator 122/N. connectivus
N14	N18	nervus recurrens
	N19	epipharyngeal muscle, labral muscles
	N20	clypeo-labral and clypeo-tentorial musculature
N18	N21	lateral preoral cavity
N19+N20	N22	hypopharyngeal muscle and wall
	N23	epicranial tentorial muscles
	Subesophageal ganglion	
Nhyp + Nmx 1	Nmd	mandible
	N hph & Nmx I	hypopharynx, first maxillae
	Nmx II, N 1 – 5	second maxillae
	Forcipular ganglion	
	Mxp N 1 – 4	forcipules

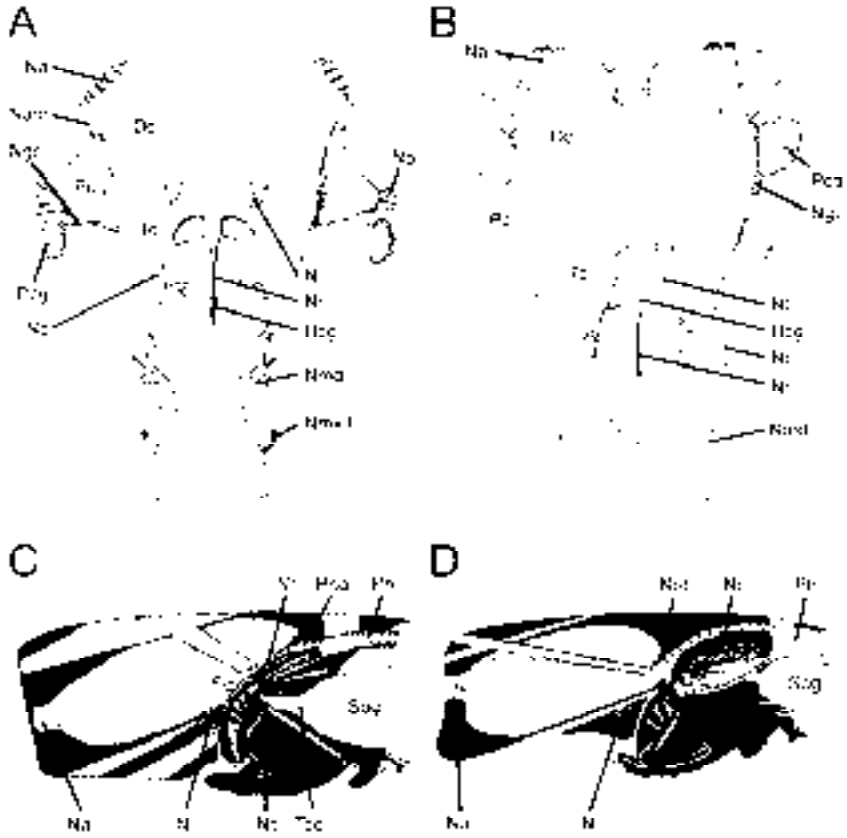


Fig. 11.5 **A** Nervous system of *Scolopendra cingulata* (Scolopendromorpha), ventral view. **B** Nervous system of *Geophilus* sp. (Geophilomorpha)., ventral view. **C** Mediosagittal section through the head of *Cryptops hortensis* (Scolopendromorpha). **D** Mediosagittal section through the head of *Geophilus flavus* (Geophilomorpha). All modified after Seifert (1967a).

Dc Deutocerebrum; **Fg** Frontal ganglion; **Hcg** Hypocerebral ganglion; **Na** nervus antennalis; **Nam** nervus antennalis (motor); **Nc** nervus connectivus; **Ngc** nervus glandulae cerebralis; **Nl** nervus labralis; **Nmd** nervus mandibularis; **Nmx I** nervus maxillaris 1; **No** nervus opticus; **Nr** nervus recurrens; **Nrd** nervus recurrens dorsalis; **Pc** Protocerebrum; **Pcg** Protocerebral gland; **Ph** Pharynx; **Sog** Subesophageal ganglion; **Tc** Tritocerebrum; **Tcc** Tritocerebral commissure

The largest section of the brain is the deutocerebrum. Again, a clear transition between the proto- and deutocerebrum is not noticeable. The antennal nerves contain

10-15 bundles of sensory neurons and three motor nerves (Lorenzo, 1960) that innervate the ventral flexor muscles of the antenna. According to Lorenzo (1960), another motor nerve arises from the protocerebrum and innervates the sensory organs in the lateral clypeus region.

The tritocerebrum is little developed and is contiguous with deutocerebral lobes without any visible border in between. The lobes of the tritocerebrum are connected by a short stomodeal bridge. The nervus recurrens projects from the frontal ganglion to the epithelia and musculature of the pharynx. A nervus recurrens dorsalis is present only in Geophilomorpha (Fig. 11.5 D, Nrd). Ventrally, the n. recurrens swells to a small hypocerebral ganglion and splits into two branches in the vicinity of the second maxillae. According to Lorenzo (1960), the labral nerve originates near the n. recurrens and innervates the frontodorsal dilators and the lateral areas of the labrum. Seifert (1967a) instead stated that the labral nerve emerges from the frontal ganglion (Fig. 11.5 D, Nl). Fahlander (1938) described a free tritocerebral commissure that has not been confirmed by Lorenzo (1960) or Seifert (1967a).

Histology and ultrastructure

As in other arthropods, the brain of *Lithobius forficatus* is enclosed within a neurilemma that serves as a barrier between it and the hemolymph. The neurilemma is divided into two components. (1) The external neural lamella is composed of collagen fibers (maximal 0.3 µm) (Füller, 1964). (2) The more internal discontinuous perilemma (or perineurium) is composed of glial cells (Jamault-Navarro and Joly, 1977; Jamault-Navarro, 1981). Both act as a blood-brain barrier. Along with septate junctions, glial cells are connected over linker junctions, which are apparently characteristic for chilopods (Lane, 1989, 1991). The neuropil of the brain lies within a rind composed of neuronal somata. Three types of these can be found: (1) small perikarya (10-15 µm in diameter) with a round and voluminous nucleus and reduced cytoplasm, (2) larger neurons (about 20 µm diameter) with occasionally lobate nuclei and voluminous cytoplasm, and (3) large neurosecretory cells filled with neurosecretory granules (NCSs). NSCs are located in paired symmetrical groups in the anterolateral area of the frontal lobes and in the ventral nerve cord (Gabe, 1966; Jamault-Navarro & Joly, 1977).

The nerves of the peripheral nervous system are coated by a distinctive double-layered neural lamella. The inner layer is composed of mucopolysaccharides with a specific alignment of collagen fibers. The outer lamella is composed of connective tissue (Füller, 1964; Füller & Ude, 1969). In different species of Geophilomorpha, ganglia, connectives

and peripheral nerves of the ventral nerve cord are coated by a convoluted multicellular myelin-like sheath (Fig. 11.3 B, C) (Rosenberg & Seifert, 1978). The number of cells and convolutions is variable. Sheath cells contain lentiform nuclei and only little cytoplasm.

Mitochondria, cytosomes, granular endoplasmatic reticulum, ribosomes, and Golgi complexes can be found. No cytoplasm is visible within the myelinated areas. Cell membranes are coated with a broad polysaccharide matrix. Hemocoel clefts are visible between the myelinated cells. Filaments of cells overlap and are connected via septate desmosomes. The main function of the myelin-like sheath is thought to be a substantial barrier to the hemolymph (Rosenberg & Seifert, 1978).

Neuroactive substances

In *Lithobius forficatus*, acetylcholin, GABA, serotonin (5-hydroxytryptamine), noradrenalin (norepinephrine), and dopamine have been documented as neuroactive substances (Descamps & Lasalle, 1983, 1986). The effect of dopamine differs according to seasons and is thus thought to play a role in the control of seasonal rhythms. Serotonin and noradrenalin have been localized in the frontal lobes and the lateral areas of the deuto- and tritocerebrum, as well as in the ventral nerve cord (Descamps et al., 1985). Its effect on the growth of spermatocytes has been shown. Gamma aminobutyric acid (GABA) has been suggested as the inhibitory transmitter whereas serotonin is thought to play a role in the maturation of sperm with dopamine having an inhibitory effect (Beniouri, 1983).

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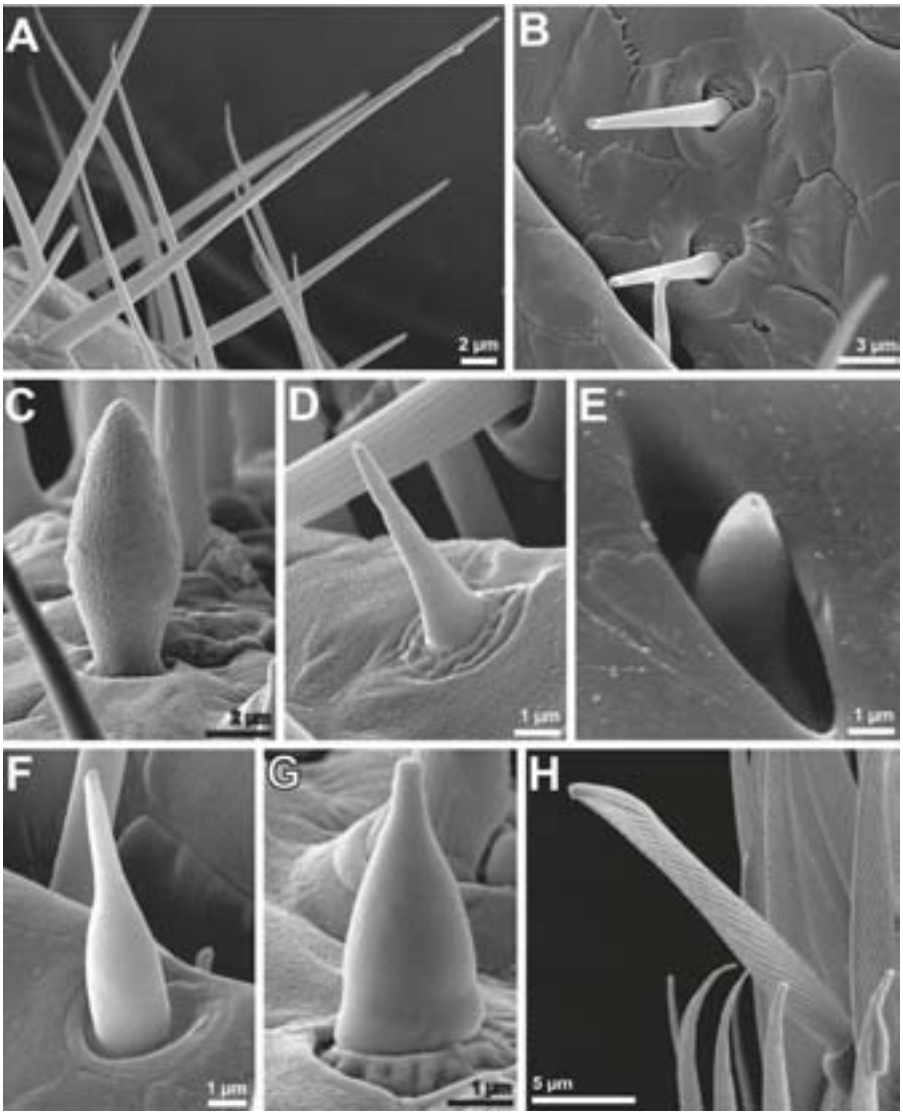
II

The sense organs of Chilopoda

Müller CHG, Sombke A, Hilken G, Rosenberg J. 2011. Chilopoda - Sense organs. In: Minelli A (Ed.) Treatise on Zoology – Anatomy, Taxonomy, Biology – Myriapoda I. Brill Leiden. pp. 217-234.



Chapter 12

CHILOPODA – SENSE ORGANS

Carsten H. G. Müller, Andy Sombke, Gero Hilken & Jörg Rosenberg

Photoreceptor organs

Based on their recently published electron microscopic examinations, Müller and Meyer-Rochow (2006a) defined two types of lateral eyes in Chilopoda according to the presence or absence of a crystalline cone:

- compound eyes constructed of ommatidia possessing a crystalline cone formed by four cone cells (Scutigeromorpha; Müller et al., 2003b) (Fig. 12.1A-B)
- lateral ocelli without a crystalline cone, but with a unicorneal lens (Lithobiomorpha: Müller and Rosenberg, 2006; Scolopendromorpha: Müller and Meyer-Rochow, 20006a; Craterostigmomorpha: Müller and Meyer-Rochow, 2006b) (Fig. 12.1E-L)

The compound eyes of Scutigeromorpha

Because their fine structure did not seem to correspond to that described in the Mandibulata, scutigeromorph ommatidia were generally believed to form a "pseudo-compound eye" or "pseudo–facetted eye", that is, not being homologous with other arthropod compound eyes. However, Paulus (1979) considered the eye of Scutigera a secondary construct built by modified ommatidia putatively emerged from a dissolution of a primary facetted eye. Recently, a comprehensive examination of the fine structural organisation of the compound eye of S. coleoptrata has been provided by Müller et al. (2003b), forming the basis for the following description.

General organization of a notostigmophoran ommatidium

The triangular compound eye of adult *S. coleoptrata* usually contains about 150 ommatidia (Fig. 12.1A). Higher numbers, up to 600 ommatidia, are reached in the larger species *Thereuopoda clunifera* and *Thereuonema tuberculata* (Hanström, 1934). Each cuticular lens displays an approximately biconvex profile, particularly curved along its distal surface. In the apical region of the eye, the ommatidia are of a hexagonal shape with average diameters of 50 µm (Fig. 12.1B). Pentagonally shaped facets are found at the margins of the eye. The total number of cells contributing to an ommatidium varies

within the eye region. Each ommatidium contains a dioptric apparatus, a photoreceptive dual-type retinula and accessory pigment cells. Interommatidial exocrine glands are located in the interspace between the ommatidia (Müller et al. 2003a,b) (Fig. 12.1B).

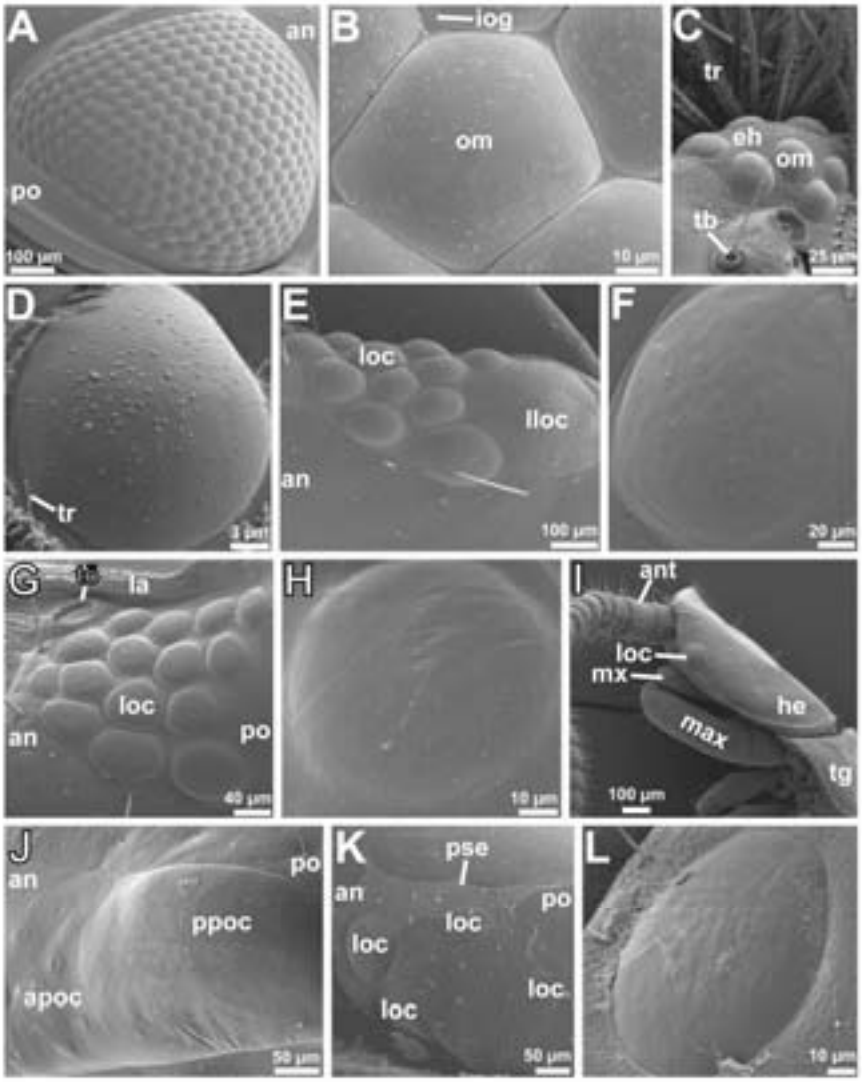
Dioptric apparatus

The dioptric apparatus includes a biconvex corneal facet, made up by 8-10 corneagenous cells, and a multipartite crystalline cone formed by four cells (Fig. 12.2B,D). The corneagenous cells are arranged in a circle around the distalmost part of the crystalline cone and directly underly the corneal facet (Fig. 12.2B). The cytoplasm is filled with numerous pigment granules showing various degrees of osmiophily. Some pigment granules have diameters (0.5-1.2 µm) slightly higher than in the retinula cells (Fig. 12.3B).

Corneagenous cells show a similar appearance and cytoplasmic composition to the primary pigment cells in hexapod ommatidia, in which the same cell type secretes the cornea and produces a primary pigment shield around the crystalline cone. In crustaceans, however, the corneagenous cells are always free of pigment granules (Paulus, 1979, 2000). Nevertheless, the occurrence of electron-dense pigment granules and the widely accepted homology of the crustacean and hexapod ommatidia (cf. Paulus,

Fig. 12.1. Various eye types of Chilopoda (A-B, E-L) and penicillate Diplopoda (C-D) observed by scanning electron microscopy. **A-B** *Scutigera coleoptrata*: **A** Right compound eye. **B** One ommatidium and its surrounding interommatidial spaces housing pore openings of interommatidial glands. **C-D** *Phryssonotus platycephalus* (Penicillata): **C** Eye hill bearing 9-12 modified ommatidia, **D** Detailed view of an ommatidium with streak-like sculpturation on the corneal surface. **E-F** *Eupolybothrus fasciatus*: **E** Dorsal view of right lateral ocellar field with far distanced posterior ocellus pointing towards lateroposterior direction, **F** Detailed view of a lateral ocellus in the middle of lateral ocellar field. **G-H** *Lithobius dentatus*: **G.** Dorsal view of right lateral ocellar field with subjacent organ of Tömösváry, **H** Detailed view of a lateral ocellus placed medioposteriorly in the cluster. **I** *Lamyctes emarginatus*: Lateral view of the left half of the head and first trunk segment. Note the occurrence of one lateral ocellus on the side of the head capsule. **J** *Craterostigmus tasmanianus*: Detailed view of the left lateral ocellus. **K-L** *Scolopendra oraniensis*: **K** Left ocellar field with four ocelli in decussate formation. **L** Anterior ocellus in the same ocellar field as in K, higher magnification. A-B originals Pohl/Müller, C-D after Müller et al. (2007), E, G after Müller and Rosenberg (2006), F,H-I originals Müller, J. Müller and Meyer-Rochow (2006b), K-L. after Müller and Meyer-Rochow (2006a).

an anterior direction; **ant** antenna; **apoc** anterior part of the ocellus not covered by the corneal vault; **eh** eye hill; **he** head capsule; **iog** opening of the interommatidial exocrine gland; **la** lateral direction (lateral end of the head capsule); **lloc** large ocellus at the posterior end of the cluster; **loc** lateral ocellus; **max** forcipule (maxillipede); **mx** maxilla; **om** ommatidium; **po** posterior direction; **ppoc** posterior; regular part of the ocellus; **pse** secretion plaque on the cuticle and corneal lens; **tb** trichobothrium; **tg** tergit of first trunk segment; **tr** (feathered) trichome; **tö** organ of Tömösvary



1979, 2000; Dohle, 2001; Richter, 2002) may support a common origin of scutigeromorph and tetraconate corneagenous cells. The only argument against this assumption would be the differing number of corneagenous cells.

Each spheroidal crystalline cone houses up to eight compartments, unequally distributed across the cone corpus (Figs. 12.2A-C, 12.3A,C,E). These cone compartments are mostly produced by four eucone cells with their somata displaced to the proximal

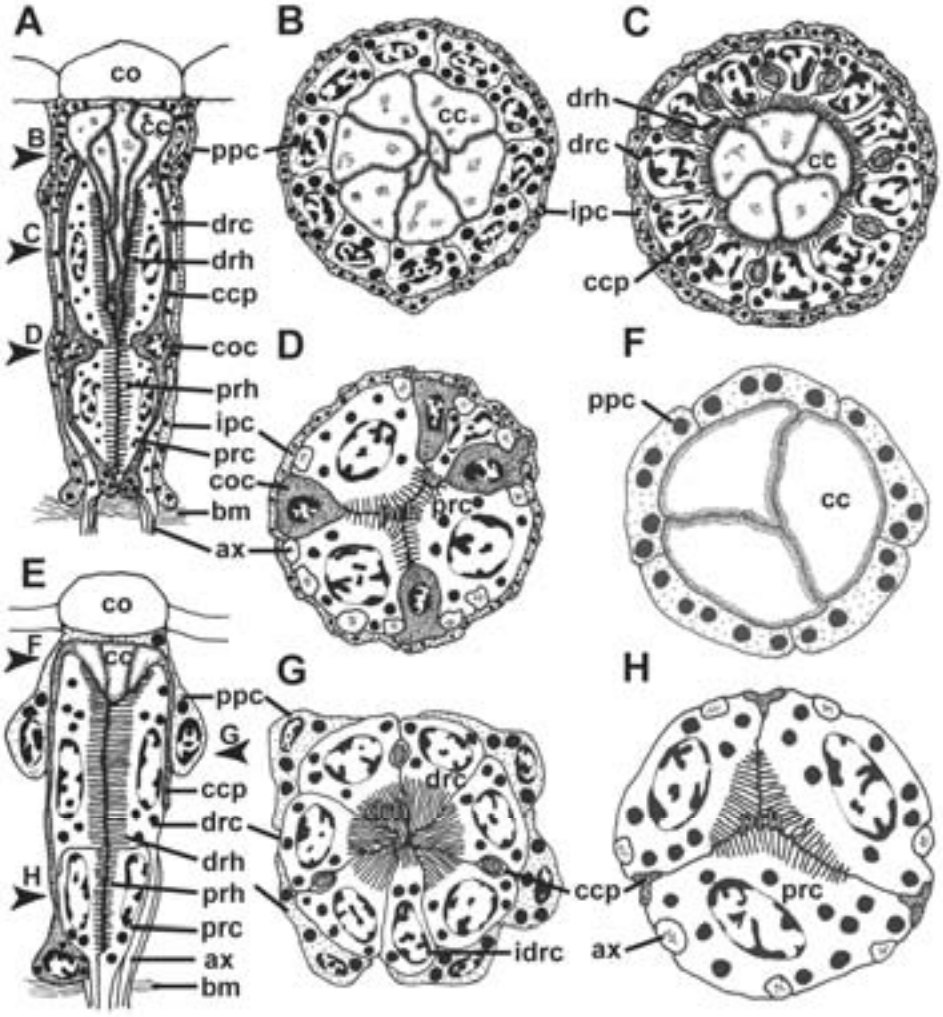
region of the ommatidium. The nuclear zone of the cone cells is located between the four proximal retinula cells (Figs. 12.2D, 12.3F). The cone cell somata are linked to either one or two cone segment(s) by cytoplasmic strands (Figs. 12.2A,C, 12.3A-B,E-F). Directly above the rhabdomeres of the distal retinula cells, each cytoplasmic process unites with one crystalline cone compartment (e.g. Figs. 12.2A, 12.3B). It is, however, impossible to propose a general distribution pattern of the distal processes of the cone cells in the sense of Melzer et al. (1997) or Dohle (2001) because the number of distal retinula cells as well as infraretinular spaces is not fixed. The cone cell somata emit four proximal cytoplasmic processes running towards the basal matrix where they widen to sac-like terminals containing numerous pigment granules (Figs. 12.2A, 12.3G). Most basally, the terminals come into contact and surround the proximal tip of the proximal rhabdome (Figs. 12,2A, 12.3H).

Retinula cells and rhabdom

The retinula of scutigeromorph ommatidia is arranged in two stacked horizontal coronae of retinula cells of distinct typology (Figs. 12.2A, 12.3A). In the distal retinula, one can count 9-13 cells forming a ring-like rhabdome around the middle and proximal third of the crystalline cone (Figs 12,2A,C, 12.3E).

Fig. 12.2 Schematic reconstructions of scutigeromorph and penicillata ommatidia based on light microscopic and transmission electron microscopic studies. **A-D** *Scutigera coleoptrata* (Chilopoda): **A** Mediosagittal section through an ommatidium containing a dual type retinula comprising a single corona of distal and proximal retinula cells. **B-D** Transverse sections through different regions of an ommatidium (section planes indicated by black arrowheads in A): **B** Mediodistal region of the crystalline cone surrounded by primary pigment cells. **C** Medioproximal region of the crystalline cone surrounded by distal retinula cells, **D** Nuclear level of proximal retinula cells and intermediate cone cells surrounded by interommatidial pigment cells. **E-H** *Phryssonotus platycephalus* (Diplopoda): **E** Mediosagittal section through miniaturized ommatidium containing a dual type retinula (distributed among a distal and proximal layer of retinula cells). **F-H** Transverse sections through different regions of an ommatidium (section planes indicated by black arrowheads in E): **F** Distomedian region of the tripartite crystalline cone (cut directly beneath the corneal lens) surrounded by primary pigment cells, **G** Nuclear level of the corona of distal retinula cells surrounded by primary pigment cells, **H** Nuclear level of ring of proximal retinula cells. Drawings modified after Müller (2008).

ax axonic process of a retinula cell; **bm** basal matrix; **cc**; crystalline cone (compartment); **ccp** distal (or proximal) process of a cone cell; **co** corneal lens; **coc** eucone cell; **drc** distal retinula cell; **drh** distal rhabdom; **idrc** irregular (small) distal retinula cell; **ipc** interommatidial pigment cell; **ppc** primary pigment cell; **prc** proximal retinula cell; **prh** proximal rhabdom



The proximal retinula cells are always four in number and create a closed rhabdome with wider, less ordered microvilli (diameter 100-150 nm). The first to third cells are of equal size, the fourth is smaller and of irregular shape (Figs. 12.2D, 12.3G). The rhabdomeric microvilli of both distal and proximal retinula cells are closely attached to each other and do not interdigitate. Because of the absence of interdigitating rhabdomeres in the proximal retinula in *Scutigera* ommatidia, a homology of the dual type retinulae of notostigmophoran ommatidia and pleurostigmophoran lateral ocelli is not convincingly supported (Müller and Rosenberg, 2006). A homology of dual type retinulae

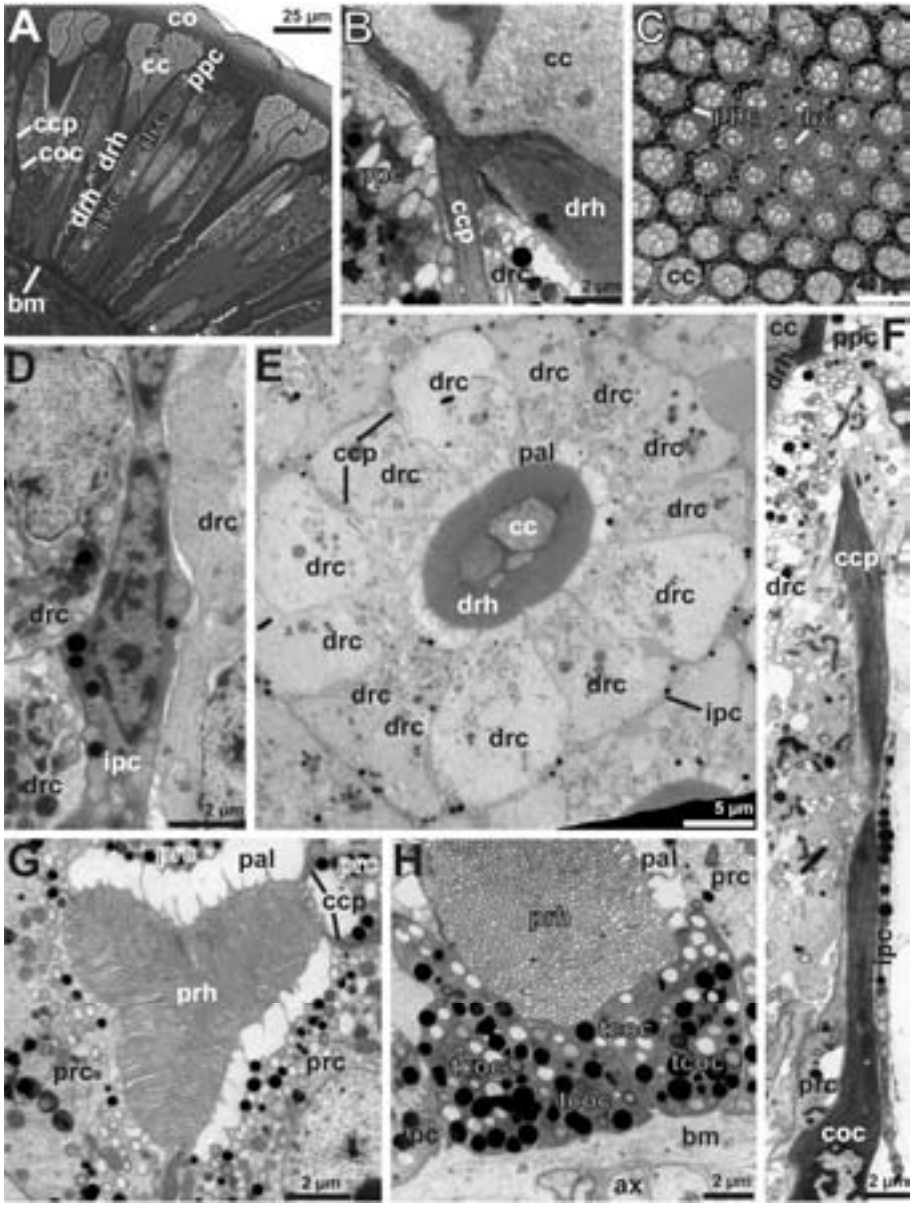


Fig. 12.3 Organization of compound eye and ommatidia of *Scutigera coleoptrata* as observed by light microscopy (A,C) and transmission electron microscopy (B,D-H). **A** Longitudinal section through the middle of the compound eye showing several ommatidia in longitudinally and tangentially cut perspective. **B** Distal process of a cone cell connecting to associated cone compartment. **C** Trans-

of Notostigmophora and some Hexapoda could be questioned due to different absolute numbers of retinula cells. Additionally, there is a slight intraspecific variation of distal retinula cell numbers among the Notostigmophora, a condition unknown in crustacean and hexapod ommatidia.

Accessory pigment cells

The pigment shield is composed of several elongated cells surrounding the entire ommatidium. Interommatidial pigment cells, 14-16 in number, extend from the cornea down to the basal matrix (Figs. 12.2A, 12.3D-E). In its most distal and proximal regions, an interommatidial pigment cell is densely filled with pigment granules (diameter 0.4-0.8 µm), mostly rather osmiophilic. Other typical organelles are large vesicles of lower electron density (diameter 0.5-1.2 µm). A distinct nuclear zone begins on the proximal level of the primary pigment cells (Fig. 12.3D). With their specific equipment of cytoplasmic granules, the interommatidial pigment cells may provide optical isolation between adjacent ommatidia. Moreover, the elaborated system of microtubules may offer a stabilisation to the extended ommatidia.

Basal matrix and optic neuropils

The entire compound eye is lined by a complex basal matrix separating the retinal region of the eye from the subjacent neuropil and the hemolymphatic space (Fig. 12.2A,

verse section through a compound eye showing ommatidia cut at in their distal part at different levels of the crystalline cone, the deepest cuts being found in the cetre of the image. **D** Longitudinal section through an interommatidial space occupied by interommatidial pigment cells that here surround the distal retinulae of neighbouring ommatidia. **E** Overview of a corona of 13 distal retinula cells at the proximal level of the crystalline cone. Distal processes of the four cone cells are found in the infraretinular spaces. **F** Oblique-longitudinal section through the periphery of a distal retinula cell providing an almost complete view of a distal cone cell process extending from the proximal soma to related cone compartment. **G** Cross section through the corona of proximal retinula cells shortly below the transition zone to distal retinula. Note the four proximal cone cell projections running down to the basal matrix through infraretinular spaces. **H** Oblique-longitudinal section through proximal tip of the proximal rhabdom produced by proximal retinula cells. Swollen and pigmented terminations of proximal cone cell processes are assembled below the rhabdom. A,C,D-E,G originals Müller, B,F,H micrographs modified after Müller et al. (2003b).

ax axonic process of a retinula cell; **bm** basal matrix; **cc**; crystalline cone (compartment); **ccp**; distal (or proximal) process of a cone cell; **co** corneal lens; **coc** eucone cell; **drc** distal retinula cell; **drh** distal rhabdom; **ipc** interommatidial pigment cell; **pal** swollen cisternae of the perirhabdomeric ('palisade') endoplasmic reticulum (ER); **ppc** primary pigment cell; **prc** proximal retinula cell; **prh** proximal rhabdom; **tcoc** swollen termination of a cone cell

3A). The basal matrix consists of an extracellular (basal lamina of the cone and interommatidial pigment cells) and cellular (terminally swollen processes of the cone and interommatidial pigment cells, retinular axons, underlying muscle bundles, hemocytes, glial cells) portion (Fig. 12.3H). The basal lamina, 0.9-1.5 µm in thickness, is apparently a dense mat of collagen fibrils and microfilaments. The basal matrix is pierced by bundles of retinular axons enveloped by thin sheaths of glial cells (e.g. Fig. 12.3H). The structure and pattern of the basal matrix fit well with the description of the quite simply arranged 'Blattodean-Orthopteran type' (Odselius and Elofsson, 1981).

The optic lobe of *S. coleoptrata* and *T. clunifera* comprises only two retinotopic neuropils, the lamina and medulla. The two neuropils are connected by uncrossed axons. The medulla is equipped with large tangential neurons that send their axons from the inner edge into the protocerebrum, where they terminate at the dendrites of descending neurons (Fahlander, 1938; Strausfeld, 2005).

Interommatidial exocrine glands

There are up to four interommatidial glands of the 'recto-canal' type (cf. Chapter 4) per hexagonal ommatidium (Müller et al., 2003a), located in the interommatidial spaces. Each exocrine gland is capped by a canal cell producing an axial channel lined by cuticle. This conducting canal opens between the corneal facets and terminates into a wall-like opening where the secretion is released to the outside (Fig. 12.1B). An intermediary cell is also found between the distal canal and the proximal secretory cell.

Electrophysiology

The compound eyes enable *Scutigera* to respond to flashes of light of different intensity and quality, including high sensitivity to the UV-A region. Peak sensitivity is at a wavelength of 448 nm (blue). Two, perhaps three kinds of visual pigments are possibly distributed among the distal and proximal retinula cells. It is assumed that the sensitivity to UV radiation may help the animal to avoid open illuminated spaces or to detect exits from concealed hiding places in soil crevices and from the underside of boulders (Meyer-Rochow et al., 2006).

Development

Structures of the distal and proximal retinula cells (including the distal rhabdome)

are observed in histological sections of the first anamorphic instar, which already reacts to light stimuli. Crystalline cone cells are not observable. During the following anamorphic stages the size and the number of ommatidia increase continuously. In 7 mm juveniles, eye anatomy corresponds to that of the adult. The growing eyes of adults are entirely surrounded by a proliferation zone of high mitotic activity. Differentiated functional ommatidia do no exhibit further trace of cell proliferation (Harzsch et al., 2007).

Vision and behaviour

Bright lateral light at different times of the day has no influence on the running direction of the nocturnal *S. coleoptrata*, which thus does not exhibit negative phototaxis, but under bright light conditions the animals move preferentially towards dark-coloured plates (positive skototaxis). A reaction to polarized light has never been observed (Görner, 1959), even though at least the rhabdomeres of some distal and proximal retinula cells have strictly parallel, unidirectional microvilli that in principle should enable the photoreceptors to detect light polarization (Müller et al., 2003b).

Based on experiment, Klingel (1960) stated that *S. coleoptrata*, although a fast-running predator, does not seem to use its eyes for detecting prey, only recognizing its prey when stimulated by direct physical contact (antenna or legs) or chemical attraction.

The lateral ocelli of the Pleurostigmophora

When present, the lateral ocelli in the Pleurostigmophora are located on the frontolateral margins of the head capsule, directly behind the antennae. They can occur as a single pair, like in many Lithobiomorpha (Henicopidae, see Fig. 12.1I, some smaller Lithobiidae), the Craterostigmomorpha (Fig. 12.1J) and the scolopendromorph family Mimopidae. A second pattern is given by four lateral ocelli in a decussate arrangement, quite distant from each other (Scolopendridae, Fig. 12.1K). Finally, there are the so-called ocellar fields containing several or numerous lateral ocelli of varying diameter (most members of the Lithobiidae, Fig. 12.1E,G). Within an ocellar field of a lithobiid, there is a size gradient from the single so-called giant ocellus, confining the field posterioventrally, towards the anteroventral field margin where the smallest ocelli are visible (Fig. 12.1E,G). Some henicopid and lithobiid Lithobiomorpha as well as all Cryptopidae, Plutoniumidae and Scolopocryptopidae (Scolopendromorpha) and Geophilomorpha are blind. The corneal lens' surface often shows the same polygonal sculpturation as seen on a centipede's cuticle in general (Fig. 12.1F,H,J, L).

The cup-like lateral ocelli of the Pleurostigmophora are of fairly uniform structure. The corneal lens, mostly biconvex and deeply vaulted, is produced by an unpigmented epithelium (Fig. 12.4, 6C-D). In *Craterostigmus*, the ellipsoid lateral ocellus is subdivided into two distinct anterior and posterior regions (Fig. 12.4D), whereas the elevated corneal lens covers the entire subjacent retinula in lateral ocelli of the Lithobiomorpha and Scolopendromorpha (Fig. 12.4A-C). A crystalline cone is never present in the pleurostigmophoran lateral ocelli. The often multilayered dual-type retinula is divided into rings of horizontally oriented distal retinula cells with a circumapical, compact rhabdomeres, and a more or less homogeneous epithelium of proximal retinula cells parallel to the optical axis (Fig.4).

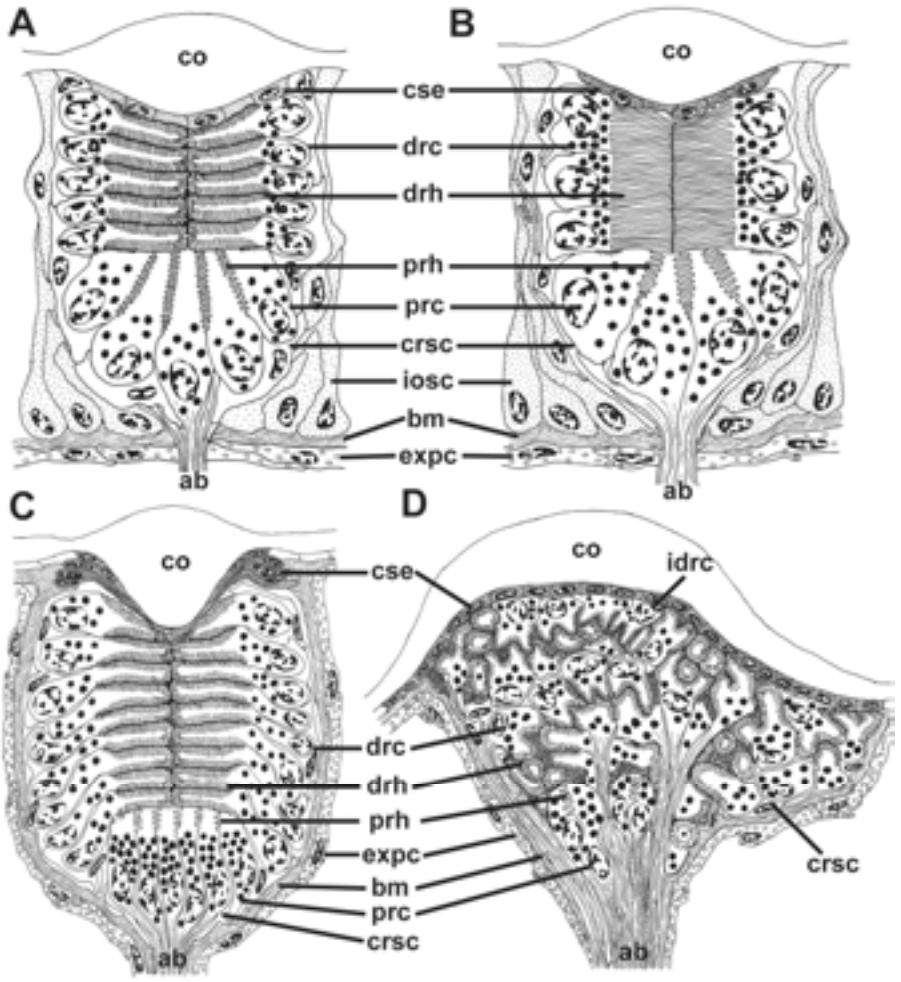
Contiguous proximal rhabdomeres interdigitate and generate diffuse but fused rhabdomes. Nearly all retinula cells belong to the everse type, and only the strongly modified distal retinula of *Craterostigmus* includes some inverse cells (Fig. 12.4D). 1-2 types of sheath cells envelop the dual-type retinula (Fig.4). External pigment cells surround either the whole eye cup (Lithobiomorpha, Craterostigmomorpha) or all the lateral ocelli within a single ocellar field (Scolopendromorpha) (Müller and Meyer-Rochow, 2006a,b, Müller and Rosenberg, 2006, Müller, 2008).

Cornea and corneagenous epithelium

Each corneal lens has a plane-convex (*Craterostigmus*, Fig, 4D) or biconvex longitudinal profile (Fig. 12.4A-C). Seen from above, the lenses look circular or ellipsoid, except for

Fig. 12.4 Schematic reconstructions of different forms of lateral ocelli of various Pleurostigmophora based on light microscopic and transmission electron microscopic examinations. **A** Longitudinal section through a lateral ocellus of *Eupolybothrus fasciatus* (Lithobiomorpha, Lithobiidae) with a multilayered distal retinula, the apex of the distal retinula cells is elongated and carries circumapical fringe of microvilli. **B** Longitudinal section through a lateral ocellus of *Lithobius* sp. (Lithobiomorpha, Lithobiidae) containing a dual type retinula with 2-6 layers of distal retinula cells and two types of sheath cells. **C** Longitudinal section through a lateral ocellus of *Scolopendra* sp. (Scolopendromorpha, Scolopendridae) containing a dual type retinula with up to 20 layers of distal retinula cells. **D** Longitudinal section through the lateral ocellus of *Craterostigmus tasmanianus* (Craterostigmomorpha) consisting of a highly complex network of distalretinula cells interfused by bicellular units of proximal retinula cells. The lateral ocellus is divided into an anterior portion and a larger posterior portion covered by a prominent corneal lens. Drawings modified after Müller (2008).

ab (primary) axon bundle; **bm** basal matrix; **cse** cornea-secreting epithelium (= corneagenous cell); **co** corneal lens; **crsc** circumretinular sheath cell; **drc** distal retinula cell; **drh** distal rhabdom; **expc** external pigment cell; **idrc** inverse distal retinula cell; **iosc** interocellar sheath cell; **prc** proximal retinula cell; **prh** proximal rhabdom



the postero-lateral ocellus in *Scolopendra* which may adopt an 8-shape (Fig. 12.1E-L). In lithobiomorph and scolopendromorph ocelli, the internal lens surface is either sunk into the eye cup, moderately sunk (slighty asymmetrical) as in *Lithobius* and *Eupolybothrus* or deeply invaginated (very asymmetrical) as in *Scolopendra*. The corneal lens is produced by a corneagenous epithelium of 30-2240 mostly flattened cells without pigment granules but showing a very electron dense cytoplasm (Figs. 12.4, 12.6C-D). The homogeneity of the corneagenous epithelium strongly depends on the amount of inner curvature of the corneal lens. In *Craterostigmus*, a layer of cubical cells is homogeneously distributed below the corneal lens. A more complex corneal lens, formed by a partly heterogenous

corneagenous epithelium, is found in *Lithobius*, *Eupolybothrus* and *Scolopendra*. The corneagenous epithelium includes numerous stretched somata that can still be found directly under the corneal vault. There is a tendency to dislocate the thinned somata to the periphery of the cornea. This process of displacement reaches its final stage in *Scolopendra*. Here one can distinguish two types of cornea-secreting epithelial cells. One type is characterized by short, plate-like cells that only surround the lateral margins of the corneal lens and are firmly attached to it by cone-like protusions of the cornea linked to microtubular bundles (Fig. 12.6C). The other type is limited to the most proximal part of the corneal lens. Four to six extremely flattened cells with their nuclei are clustered around the proximolateral region of the corneal lens (Figs. 12.4C, 12.6C). Minute cytoplasmic processes emanate from the corneagenous cell bodies heading towards the central subcorneal zone (Fig. 12.6D). Each cell process becomes thereby attached to a small central sector of the cornea (Müller and Meyer-Rochow, 2006a,b, Müller and Rosenberg, 2006, Müller, 2008).

Dual-type retinula and rhabdome

Depending on the animal's age and the relative position of the lateral ocellus within the ocellar field, the retinula of *Lithobius*, *Eupolybothrus* and *Scolopendra* contains 36-1160 retinula cells. Cell number is the highest in the lateral ocellus of *Craterostigmus* with approximately 1300 photoreceptive cells. All pleurostigmophoran taxa examined have a dual-type retinula comprising distal and proximal retinula cells.

The distal retinula cells are mostly rectangular or cylindrical and are oriented perpendicular to the optical axis. They are aligned in a circle and thus build distinctive horizontal layers. Many of these layers are stacked onto each other (*Lithobius*: 1-6 layers (Fig. 12.4B), *Eupolybothrus*: 4-12 (Fig. 12.4A), *Scolopendra*: 10-20 (Fig. 12.4C)). The distal retinula of *Craterostigmus* deviates considerably from this pattern by having disintegrated the circular arrangement and the stacks of horizontal layers. Here, the distal retinula cells have a laminar extension, and are arranged in clusters (Figs. 12.4D, 12.5G,H).

The pattern of the distal retinula cells and the structure of their rhabdomeres influence the complexity of the likewise multilayered rhabdome. Distal retinula cells with straight apex (*Lithobius*) produce rectangular or orthogonal rhabdomeres which get in contact within the axial region of the eye to form a simple fused rhabdome (Figs. 12.4B, 12.5A-D). A second type of distal rhabdome is observed in *Eupolybothrus* and *Scolopendra*, where the cell apices are extended to form circumapical rhabdomeres and a fused but more complex rhabdome, respectively (Figs. 12.4A,C, 12.6A-B,E-F). Knob-like

or bilobed apices forming cap- or W-shaped circumapical rhabdomeres are found in the distal retinula of *Craterostigmus*. The rhabdome of *Craterostigmus* is heavily branched (Figs. 12.4D, 12.5H).

In a pleurostigmophoran lateral ocellus, approximately 10% of all retinula cells belong to the proximal type, differing from the distal ones in shape, orientation, arrangement, pigmentation (*Scolopendra*) and interaction of their rhabdomeres (Figs. 12.4, 12.5F,I, 12.6G). A homogenous layer of proximal retinula cells occupies the bottom of each eye cup in *Lithobius* (Figs. 12.4B, 12.5A), *Eupolybothrus* (Fig. 12.4A) and *Scolopendra* (Figs. 12.4C, 12.6A). In the same taxa, the proximal retinula cells look conical or club-shaped and are aligned parallel to the optical axis (perpendicular to the distal retinula cells, see Figs. 12.4, 12.6G)). Around the apex a uni- or bidirectional rhabdomere is formed which can be very short and unconspicuous (*Scolopendra*, Fig. 12.6G) or elongated (*Lithobius*, *Eupolybothrus*). The microvilli of a proximal rhabdomere are considerably distanced from each other, which allow contiguous rhabdomeres to interdigitate and form an extensively fused, zip-like structure (Figs. 12.4, 12.5F,I, 12.6G). Thus, the entire proximal rhabdome looks stellate (*Lithobius*) or like a complex network in cross sections (*Eupolybothrus*, *Scolopendra*, e.g. Fig. 12.6B). In the latter case, the complexity increases with the total number of proximal retinula cells (*Scolopendra*). Most distally, the tips of the proximal rhabdomeres may abut to the rhabdomeres of the last layer of the distal retinula. Again, the proximal retinula of *Craterostigmus* is very different from those described above because of the disintegration of the multilayered coronal system. There is instead a pattern of loosely dispersed units, each of two drop-like proximal retinula cells clumped together along the entire length (Fig. 12.4D). At the apex of a proximal dual-cell unit, each straight inner contact membrane produces a pectinate rhabdomere which, like in Lithobiomorpha and Scolopendromorpha, intertwine with the one of the partner cell (Fig. 12.5I).

In general, the cytoplasm of both retinula cell types is moderately osmiophilic and is endowed with organelles typical for photoreceptive cells in Arthropoda, such as polymorphous screening pigment granules, vacuoles of various content and osmiophily, cristate mitochondria, Golgi stacks, cisternae of rough and smooth endoplasmic reticulum, and succession stages of lysosomal bodies.

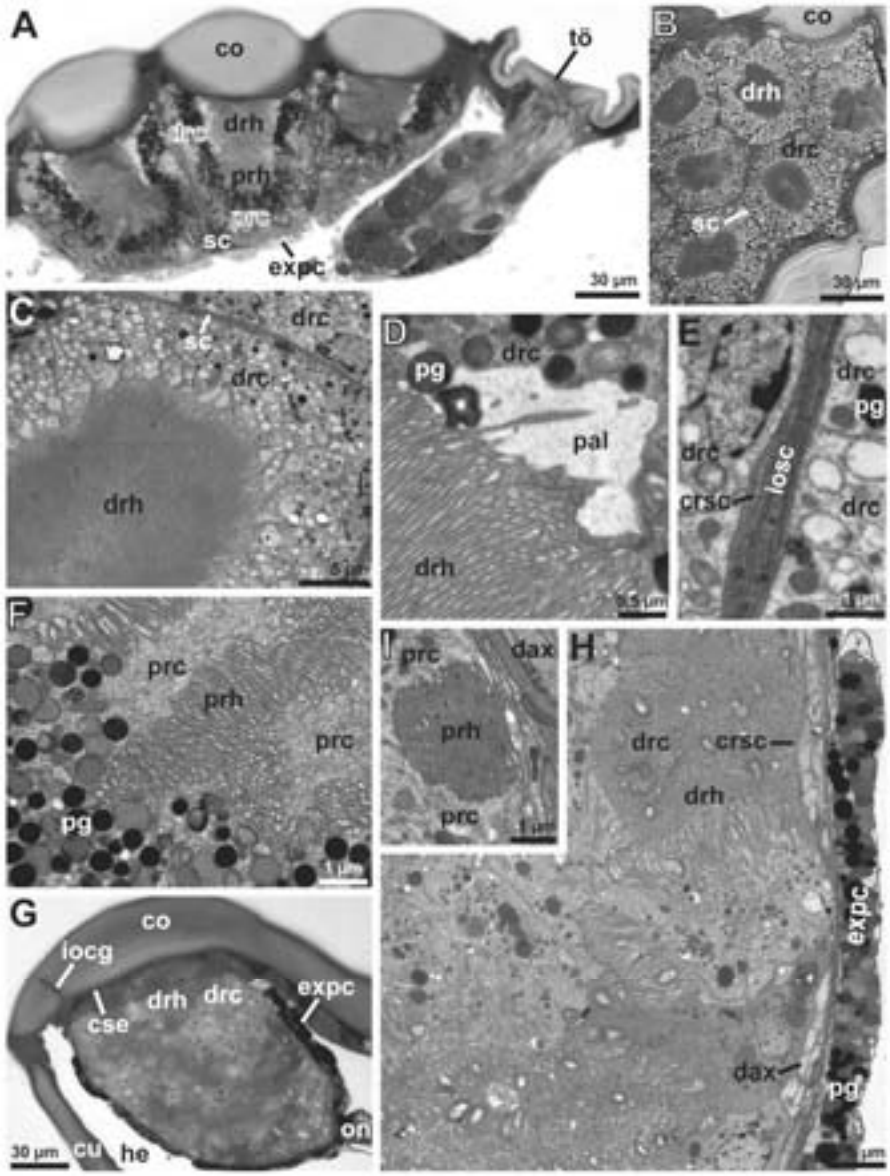
The retinula cells, in particular the distal ones, may also develop a more or less conspicuous system of perirhabdomeric ('palisade') endoplasmic reticulum. In *Lithobius* and *Eupolybothrus*, the cisternae of the latter appear swollen and surround the axial rhabdome as a distinct ring (Bedini, 1968; Bähr, 1971, 1974) (Fig. 12.Fig. 12.5D). The volume of the cisternae varies according to the state of dark-light adaptation (Bähr, 1972). The perirhabdomeric endoplasmic reticulum is also found in the distal retinula cells of *Scolopendra*, where it displays a dense palisade of lengthened cisternae (Fig. 12.6F). In contrast, there is no evidence of swollen perirhabdomeric cisternae in *Craterostigmus*, where a massive and highly ordered smooth endoplasmic reticulum is traversed by thin cytoplasmic bridges in the distal retinula cells (Fig. 12.5H).

Sheath cells

Two types of sheath cells (circumretinular and interocellar) are distinguishable by shape, location in relation to the eye cup and cytoplasmic composition. In all taxa examined, distal and proximal retinulae are tightly enveloped by loosely dispersed circumretinular sheath cells, 20-300 in number (Fig. 12.4). The thin circumretinular sheath cell bodies are usually utricular in shape and have an electron lucent cytoplasm without pigment granules. Small projections are sent in a vertical direction towards the bottom and the tip of the eye cup. Additionally, axial processes may emerge from the soma and penetrate the retinula, running through the infraretinular spaces. Both axial and vertical cell processes often branch and aggregate; as a result, the sheet of circumretinular sheath cells appears multilayered. However, some circumretinular sheath cells of *Lithobius* and *Eupolybothrus* lack axial processes (Figs. 12.4A-B, 12.5E). The

Fig. 12.5 Organization of lateral ocelli of various Lithobiomorpha and Craterostigmomorpha as observed by light microscopy (A-B,G) and transmission electron microscopy (C-F,H-I). **A** Transverse section through the lateral margin of the head capsule of *Lithobius forficatus* providing a longitudinal profile of the lateral ocellar field and anteroventrally adjoined organ of Tömösváry. **B** Cross section through one lateral ocellar field showing severeal ocelli at the level of the distal retinula. *L. forficatus*. **C** Overview of one half of a lateral ocellus placed in the middle of the eye cluster. Note the dense packing of prismatic distal retinula cells contributing to an almost rectangular central rhabdom. *L. forficatus*. **D** Higher magnified view of the apex of a distal retinula cell from which highly ordered rhabdomeric microvilli emanate. *Lithobius dentatus*. **E** Detailed view of an interocellar space between two lateral ocelli filled with a multilayer of circumretinular and interocellar sheath cells. *L. forficatus*. **F** Two proximal retinula cells, each of which forms an apical cytoplasmic process surrounded by interdigitating microvilli. *Lithobius mutabilis*. **G-I** *Craterostigmus tasmanianus*: **G** Posterior part of the lateral ocellus in longitudinal section. **H.** Distal periphery of the lateral ocellus showing the complex network of distal retinula cells and rhabdomeres. **I** Close-up of a bicellular module of proximal retinula cells presenting an interdigitating rhabdom unit. A. LM images modified from Müller (2008), B,F,H originals Müller, C-E modified after Müller and Rosenberg (2006), G.I modified after Müller and Meyer-Rochow (2006b).

co corneal lens; **crsc** circumretinular sheath cell; **cse** cornea-secreting epithelium (= corneagenous cells); **cu** cuticle; **dax** axon of a distal retinula cell; **drc** distal retinula cell; **drh** distal rhabdom; **expc** external pigment cell; **he** hemolymphatic space; **iocg** conducting canal of an intraocellar exocrine gland; **iosc** interocellar sheath cell; **on** optic nerve; **pal** swollen cisternae of the perirhabdomeric ('palisade') endoplasmic reticulum; **pg** highly osmiophilic pigment granule; **prc** proximal retinula cell; **prh** proximal rhabdom; **sc** sheath cell; **tö** organ of Tömösváry



wrapping of retinula cells by axial processes is most elaborated in *Craterostigmus*, where numerous cuneiform clusters of somata of circumretinular sheath cells, located near the margin of the eye cup, emanate bundles of axial processes between the clusters of distal retinula cells. On their way to the centre of the eye cup, these axial processes envelop the

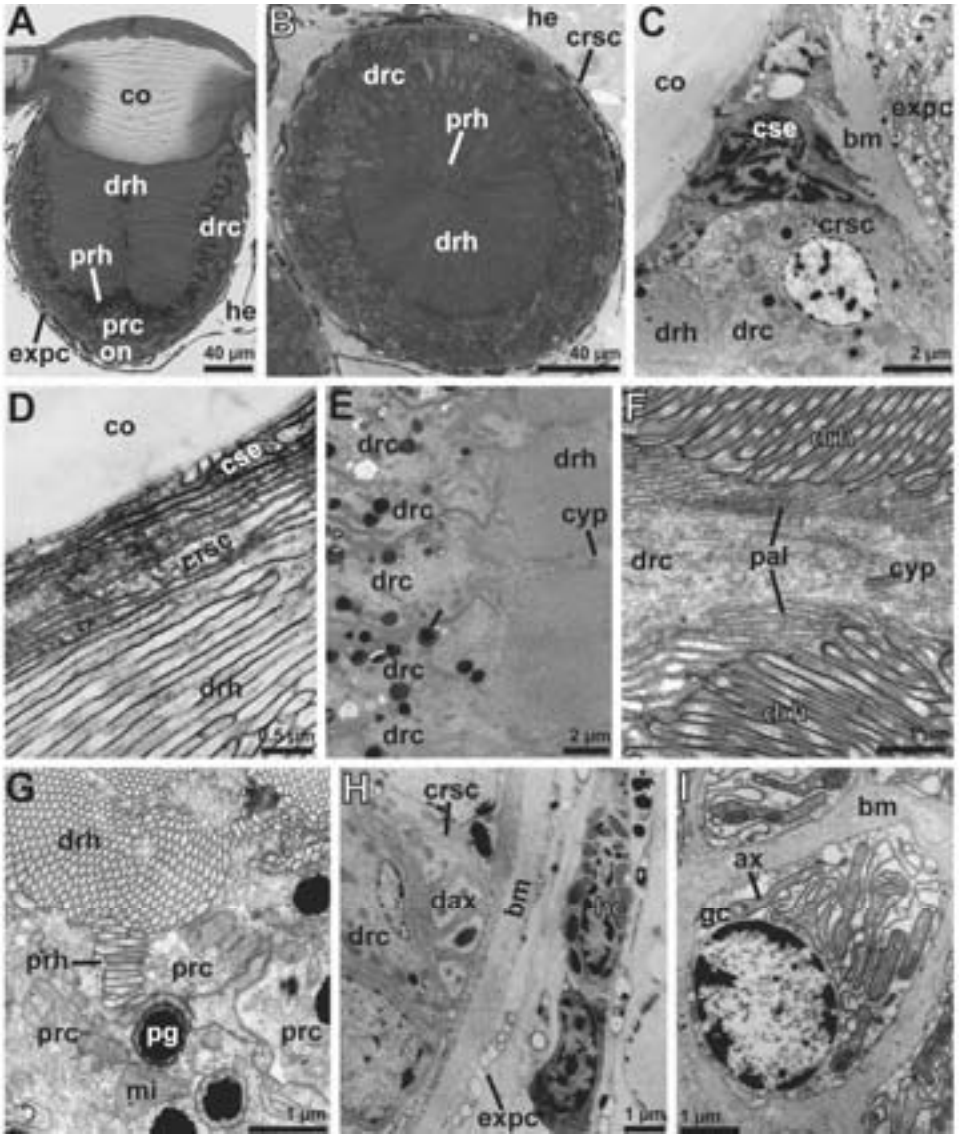
peripheral bundles of retinular axons (Fig. 12.H-I). In *Scolopendra*, the wrapping of the retinula cells by axial processes of the circumretinular sheath cells reaches the highest degree (Figs. 12.4C, 12.6C,H). Processes of distally located sheath cells run between cornea-secreting cells and the distal layer of distal retinula cells (Fig. 12.6D).

Only in *Lithobius* and *Eupolybothrus*, the interocellar space is filled with 20-200 interocellar sheath cells, extending from the cornea down to the basal matrix (Fig. 12.4A-B). Comparable to the circumretinular sheath cells, small vertical and axial cell processes, emanating from the somatic part of the cell, branch and intertwine with processes of neighbouring cells. Thereby, the interocellar sheath cells usually form 6-10 cell layers (Fig. 12.5E). The cytoplasm is generally more electron-dense than in the circumretinular sheath cells. In dark-adapted animals, the entire cytoplasm is heavily endowed with polymorphic, hyaline vacuoles, 0.5-1.2 µm in diameter.

Sheath cells may improve bioelectric isolation between retinula cells and between retinular axon bundles, possibly allowing to transmit rapid, unambiguous signals and to maintain spatial resolution. The sheath cells provide a multilayered system of cytoplasmic compartments with high and low refractive indices. According to Land (1972), only ten of those layers are needed to reflect nearly 100% of the incident light. Since centipedes are strongly nocturnal, having an internal reflecting layer would lead to a significant increase in their eyes' visual sensitivity.

Fig 12.6 Organization of lateral ocelli of Scolopendromorpha reconstructed by the aid of light microscopy (A-B) and transmission electron microscopy (C-I). **A** Longitudinal section the dorsal ocellus of *Scolopendra oraniensis* (Scolopendridae) **B** Cross section through the transition zone of the distal and proximal retinula, the slight axial protrusion of the proximal rhabdomeres is clearly visible by the change from radial to net-like pattern of rhabdomeres. **C** Lateral cluster of cornea-secreting epithelial cells and subjacent soma of a circumretinular sheath cell. Longitudinal view. **D** Detailed view of the complex membrane staples present below the corneal lens representing axial processes of the cornea-secreting cells, circumretinular sheath cells as well as distal rhabdomeric microvilli. **E** Longitudinal overview of a couple of horizontally stapled distal retinula cells at the base of the axial cytoplasmic processes and distal rhabdomeres. **F** Higher magnification of proximal end of finger-like cytoplasmic process of a distal retinula cell. Note the vestigial perirhabdomeric ER apparatus. **G** Transition zone of distal and proximal retinula, two interdigitating proximal rhabdomeres are illustrated. **H** Lateral margin of a distomedian region of a lateral ocellus bordered by a complex, ramified basal matrix and a diffuse multilayer of external pigment cells. **I.** Cross section through a subretinal axon bundle entering the optic nerve. Note the extensive glial sheathing of the retinular axons. A modified from Müller (2008), B-C,E-F,H originals Müller, D,G,I. modified after Müller and Meyer-Rochow (2006a).

ax axonic process of a retinula cell; **bm** basal matrix, **co** corneal lens; **crsc** circumretinular sheath cell; **cse** cornea-secreting epithelium (= corneagenous cells); **cyp** thin cytoplasmic process of a distal retinula cell; **dax** axon of a distal retinula cell; **drc** distal retinula cell; **drh** distal rhabdom; **expc** external pigment cell; **gc** glial cell sheath; **hc** hemocyte; **he** hemolymphatic space; **mi** mitochondrion; **on** optic nerve; **pal** swollen cisternae of the perirhabdomeric ('palisade') endoplasmic reticulum (ER); **pg** highly osmiophilic pigment granule; **prc** proximal retinula cell; **prh** proximal rhabdom



External pigment cells/ basal matrix

The lateral ocelli of the Scolopendromorpha and Craterostigmomorpha are lined along the entire periphery of the eye cup by a basal matrix (Fig. 12.4C-D) which consists of an extracellular portion (basal laminae of sheath cells, cornea-secreting epithelial cells,

external pigment cells, occasionally also epidermal cells) and a cellular portion (peripheral sheath cells and the projections of the external pigment cells).

In *Craterostigmus*, the extracellular portion is only about 0.5-0.9 µm thick. In contrast, the basal matrix is massively developed in *Scolopendra*. Particularly, the extracellular portion exhibits a broad network of collagen fibres; still a compact layer in the middle and proximal (about 1.4 µm thick) but branched and even more enlarged close to the distalmost part (up to 15 µm thick) of the eye cup (Figs. 12.4C, 12.6C,H-I).

In *Eupolybothrus* (Fig. 12.4A) and *Lithobius* (Fig. 12.4B), a thin basal matrix separates the whole ocellar field from the subjacent plexus of external pigment cells (see below). Therefore, only the bottoms of the mostly contiguous lateral ocelli are bordered by the very thin extracellular portion (< 0.5 µm thick).

In all pleurostigmophoran ocelli, the basal matrix is only perforated near the base of the eye cup. There, the entirety of retinular axon bundles, ensheathed by glial cells, runs into the adjacent optic nerve (e.g. Fig. 12.6I).

A plexus of one or two contiguous rows of external pigment cells delimits the single eye cup (*Craterostigmus*, *Scolopendra*) or the entire ocellar field (*Lithobius*, *Eupolybothrus*) against the hemolymphatic space (Figs. 12.4, 12.5A,H, 12.6A,C,H). The innermost layer contributes to and usually adjoins the extracellular portion of the basal matrix. The cytoplasm of the external pigment cells is filled with small electron lucent vacuoles (0.9-1.4 µm in diameter), polymorphic granules of moderate osmiophily (about 0.5 µm in diameter), and numerous electron dense pigment granules (0.4-0.8 µm in diameter). Large polymorphic granules with an inhomogeneous matrix dominate the cytoplasm in *Lithobius* and *Eupolybothrus*.

Within the external pigment cells of *Scolopendra*, rounded granules of higher electron density are visible (e.g. Fig. 12.6C). In *Craterostigmus*, regions rich in electron lucent granules (distal part of the eye cup) alternate with zones in which more osmiophilic granules are accumulated (Fig. 12.5H). In *Scolopendra* and *Craterostigmus*, the external pigment cells are not restricted to the region around the lateral ocelli(us) as they underlie the adjacent epidermis over a certain distance. External pigment cells cover the distal part of the optic nerve (e.g. *Craterostigmus*, Fig. 12.5G).

Interommatidial and intraocellar organs

In lithobiid centipedes (*Lithobius*, *Eupolybothrus*), the interocellar spaces are sometimes pierced by the openings of interommatidial glands (Müller et al., 2003a). As in *Scutigera*, these glands consist of a canal and a secretory cell. The existence of a third cell type, the

so-called intermediary cell, was however assumed by Müller et al. (2009), based on critical evaluation of broader set of TEM micrographs.

Intraocellar organs are curious structures within the lateral ocellus of the *Craterostigmus*. Pores of flexo-canal epidermal glands are observed near the margins of its eye (Fig. 12.5G). The glandular apparatus breaks through the corneagenous epithelium and extends down to the uppermost layer of the distal retinula. In addition, the anterior, uncurved part of the cornea of *Craterostigmus* is also pierced by sockets and hair shafts of *Sensilla microtrichodea* (see below) (Müller and Meyer-Rochow, 2006b).

Photoreceptor axons and optic neuropils

As shown by Holmgren (1916) and Hanström (1926, 1928) for *Lithobius*, the optic lobes are composed of two optic neuropils (lamina, medulla), situated one after another. Two types of central projections originating from the lateral ocelli have been identified in *L. forficatus* (Melzer et al., 1996/97). Short retinular axons terminate within the first optic neuropil of the lamina. They possess varicose swellings throughout the depth of the neuropil. Long axons pass through the first neuropil and terminate within the medulla, where up to five long collaterals are observed that diverge to various synaptic sites.

Light- and dark adaptive changes and circadian rhythm

Following earlier investigations by Bähr (1972), Müller and Rosenberg (2006) observed fine structural changes within the lateral ocelli of *Lithobius* species in full darkness vs. light adapted state (12:12 h light:dark cycle). When light adapted, the distal retinula cells show a conspicuous ring of swollen cisternae of perirhabdomeric endoplasmic reticulum around the axial rhabdome. In dark adapted lateral ocelli, these cisternae are lesser voluminous. Osmiophilic pigment granules aggregate and build up a screening shield around the retinula. Most important, rhabdomeric microvilli are extended in dark adapted eyes. As typical for arthropod eyes, increase in rhabdome diameter raises the absolute sensitivity of the photoreceptors during the night (Meyer-Rochow, 1999). Bähr (1965, 1967) carried out extracellular recordings from the ocellar field of *Lithobius forficatus*. The electroretinograms reveal that dark adaptation occurs at high stimulus intensities in a biphasic manner and seems to be complete after 20 minutes. Based on the experimental results, Bähr (1967) thought that *L. forficatus* orientates itself from light to dark environments. It could be excluded that *L. forficatus* is able to recognize single images or movements.

Development and regeneration of the ocelli

In embryonic development, the first sign of an anlage of a lateral ocellus is detectable in *Scolopendra* at a time when the embryo has completely folded inward (Heymons, 1901). The spherically shaped eye anlage is covered by the first embryonic cuticle. Before hatching, retinula cells and the nervus opticus are developed. After hatching, the four lateral ocelli are recognizable.

Continuous mitotic activity affects all cellular components of all four lateral ocelli of *Scolopendra oraniensis* in the course of moulting events in adult specimens. Therefore, a persistent proliferation (intercalary growth) goes on within the lateral ocelli ('ocellar ommatidia') of the Scolopendromorpha (Harzsch and Hafner, 2006; Harzsch et al., 2007). The occurrence of intercalary growth can be also suggested for the lateral ocelli of lithobiomorphs (cf. Andersson, 1981).

Ablation of the lateral ocelli in matures junior specimens of *Lithobius forficatus* is followed by regeneration; the new ocelli are innervated by the optic nerve (Joly and Herbaut, 1968). In one case, a heterotypic, antenna-like regenerate was obtained.

Vision and behaviour

Positive skototaxis, i.e. orientation towards dark-coloured objects under bright conditions has been reported for *Lithobius forficatus* (Klein, 1934; Görner, 1959) and *Scolopendra subspinipes* (Plateau, 1887), but also for representatives of the blind Cryptopidae and Geophilomorpha (Plateau, 1886, 1887). Negative phototaxis has been described in *L. forficatus* (Verhoeff, 1902-1925; Klein, 1934; Scharmer, 1935; Bauer, 1955; Görner, 1959; Meske, 1961), but negated in *L. piceus gracilitarsis* (Demange, 1956) and *S. cingulata* (Plateau, 1887). No reaction to polatrized light has been found in either *Lithobius* or *Scolopendra* (Görner, 1959).

Phylogenetic implications of myriapod eye characters

Anatomy and ultrastructure suggest that ommatidia of Myriapoda, Crustacea and Hexapoda share a common origin. This is supported by features like primary pigment cells, a sheath of interommatidial pigment cells, a double-layered retinula built by two distinct retinula cells as well as the successive formation of the compound eye from an anterior proliferation zone, but probably the most important homologous character is the crystalline cone formed by four eucone cells (Müller et al., 2003b, 2007; Harzsch et al., 2007; Müller, 2008). Tripartite crystalline cones, connected to proximally placed cone cell somata by thin infraretinular processes have also been found recently in the penicillate diplopod *Phryssonotus platycephalus* (Müller et al., 2007) (Figs. 12.1C-D, 12.2E-

H). This specific fine structural correspondence suggests that scutigeromorph and penicillate ommatidia are homologous (Müller et al., 2007).

Moreover, the partly constant cell patterns in the ommatidia of scutigeromorph centipedes and in the miniaturized, disintegrated ommatidia of penicillate millipedes are thought to be closer to the ground pattern of Mandibulata than are the ommatidia of the Tetraconata. If so, eucone cells with their nuclei placed outside the light-focusing cone compartments are most likely ancestral (Müller et al., 2003b, 2007; Harzsch et al., 2007; Müller, 2008). In this context, scutigeromorph ommatidia are however also unique, because each distal cone cell process bifurcates and gives rise to the formation of two distinct compartments within the multipartite crystalline cone. One cone cell forming two cone compartments therefore is a potential apomorphy of the Scutigeromorpha.

A phylogenetic analysis carried out by Müller (2008) integrated 28 new eye characters into an updated and corrected matrix of Edgecombe and Giribet (2004) containing 222 morphological characters of various centipedes. The results of this analysis revealed that eye characters, such as 1) unpigmented, flattened epithelial cells producing a more or less vaulted corneal lens, 2) unpigmented sheath cells encompassing the retinula, 3) external pigment cells lining the ocellar field against the hemolymphatic space, 4) a multilayered dual type retinula, and 5) interdigitating rhabdomeres of the proximal retinula cells provide further support for the Pleurostigmophora concept. The assumed derived state of lateral ocelli of Pleurostigmophora implies that cone-less, camera-type eyes surrounded by a mantle of external pigment cells have evolved independently in Chilopoda and Diplopoda. Due to transformation processes, it is only the dual type retinula that remains in favour of homology of scutigeromorph/penicillate ommatidia and pleurostigmomorph/chilognath lateral ocelli.

Epidermal sensilla

Sensory hairs (setae)

Epidermal sensilla are sensory setae, consisting of two structural components: the sensory cells with their dendritic processes and several sheath cells, more or less interspersed into the hair shaft.

Scattered epidermal sensilla

Scattered sensilla are defined here as small epidermal sensilla dispersed on the centipede's cuticle. Sensilla of differing structure and function may be tightly adjoined but do not occur in specialized, encapsulated cuticular compartments to perform specific functions. Scattered sensilla, especially those located on the antennae, cover a wide range of functions, comprising mechano-, chemo-, hygro- and thermoreception. Numerous types of scattered epidermal sensilla have been identified. Eleven sensilla types are presented here in detail. Some of them are restricted to the

antennae. Only five sensillar types are comprehensively understood with regard to their histological anatomy and ultrastructure, examined with light microscopy as well as scanning and transmission electron microscopy. Based on TEM examinations, epidermal sensilla were assumed to be bimodal with regard to their function. The functions of certain types of antennal sensilla have been assumed on the basis of TEM studies. However, experimental evidences (e.g. from behavioural studies or electrophysiology) are still lacking.

1) ***Sensilla trichodea***. – Trichoid sensilla are by far the most common and widespread sensilla found on a centipede's body (Fig. 12.9A). Contrary to many other types of sensilla they occur on any body part lined by a hardened cuticle. However, to date only the antennal trichoid sensilla have been thoroughly investigated. In this review, some TEM observations on the organization of cephalic sensilla trichodea are presented for *Craterostigmus tasmanianus*, *Lithobius dentatus* and *Scolopendra cingulata* (Fig. 12.8B-H). Counting several dozens to hundreds in Scutigeromorpha (e.g. *Scutigera coleoptrata*) (Ernst et al. unpubl.) and Geophilomorpha (e.g. *Geophilus flavus*) (Ernst, 1976, 1994, 2000b), up to 4,000 sensilla of this type can be found on each lithobiomorph (*Lithobius forficatus*), craterostigmomorph (*Craterostigmus tasmanianus*) and scolopendromorph (e.g. *Cryptops hortensis*, *Newportia monticola*) antenna (Keil, 1975, 1976; Ernst, 2000b; Ernst et al., 2006, 2009; Koch et al., 2010). The main information on the fine structural architecture of sensilla trichoidea is owed to the thorough EM studies of Ernst (1976, 1994, 1996, 1999, 2000b) on *G. flavus* and Keil (1975, 1976) on *L. forficatus*.

The elongated hair shaft (length: *L. forficatus*: 100-150 µm, *G. flavus*: 20-120 µm, *C. tasmanianus*: 17-50 µm on median antennomeres, 50-390 µm in other areas, *S. coleoptrata*: 48-73 µm) is often curved to the top and bears numerous spiral ribs on its surface. Ernst et al. (2009) define two subtypes of trichoid sensilla in *Cryptops hortensis*: straight type-1 sensilla, frequently distributed on the lateral side of the antennomeres (length: 17-88 µm long, keenly ribbed) and slightly curved type-2 sensilla, less frequent in the central area of the antennomeres (length: 6-16 µm long, roughly ribbed). Apically, these sensilla have a terminal pore. The highly flexible shaft is fixed to the cuticle surrounding the socket by a membranous cuticular joint. A constant number of 18 uniciliate sensory cells is noticeable in antennal sensilla trichodea of *L. forficatus*. Among these 18 dendritic processes, 17 smaller ones run through the entire sensillum, penetrate the lumen of the shaft and head to the terminal pore opening (cf. Fig. 12.8B), whereas one single and thicker dendrite transforms apically into a tubular body attached to a fibrillous network in the cuticular socket membrane (Figs. 12.7A, 12.8C-D). In contrast, sensory cells vary in composition and number in *G. flavus*. Antennal trichoid sensilla have 15 to 17 uniciliate sensory cells (cf. Fig. 12.8G), those situated on the maxillae are however smaller and

include only 6 to 9 sensory cells, one of which is biciliate and ends up in two tubular bodies at their hair base (Fig. 12.7B).

Each sensillum includes a thecogen cell, a trichogen cell and a tormogen cell as in hexapods. Inner and outer receptor lymph cavities extend deeply into the lumen of the hair shaft (Figs. 12.7A-B, 12.8D-E,G-H). The dendritic sheath is distinct and channels most of the thin outer dendritic segments to the terminal pore opening (Fig. 12.8B-E).

Bimodal sensilla trichodea most likely integrate contact chemoreception and mechanoreception. The extraordinarily high abundances of antennal trichoid sensilla in Geophilomorpha are perhaps correlated with the loss of eyes.

2) ***Sensilla microtrichodea***. – Because of many ultrastructural similarities, the sensilla microtrichodea are easily characterized as miniaturized sensilla trichodea (Fig. 12.9B). To date, only antennal sensilla microtrichodea have been studied in detail. They have never been found on the antennae of Scutigeromorpha, whereas either a few (*Craterostigmus tasmanianus*; Ernst et al., 2006), one hundred (*Cryptops hortensis*; Ernst et al., 2009) or several hundred (*Lithobius forficatus*; Keil, 1975; *Geophilus flavus*; Ernst 1983, 2000b) have been counted. Hair shafts of antennal sensilla microtrichodea are hardly visible externally, even by scanning electron microscopy. This is due to their strict alignment along the basis of each antennomere, which is overlapped by an anterior projection of the subjacent antennomere. In *L. forficatus*, sensilla microtrichodea occur in three distinct groups of 2-5 sensilla, measuring between 5 and 10 µm in length. The sensory apparatus consists of two uniciliate sensory cells surrounded by three sheath cells. Both outer dendritic segments are tightly encased by a thick dendritic sheath produced by the thecogen cell. Both dendrites run up to the slender and smooth hair shaft 5-10 µm in length. Neither tubular bodies nor terminal pores have been found.

More details are known for the microtrichoid sensilla on the antennae of *G. flavus*. The widely smooth hair shafts are longer (7-17 µm), have a terminal pore opening and they are aligned in three circles of 2-5 sensilla at the base of most antennomeres. A slightly variable number of 6-8 sensory cells is surrounded by three sheath cells. Two sensory cells are biciliate, the remaining ones only form a single dendritic process. The slender four outer dendritic segments of the two biciliate sensory cells are connected to the socket membrane via four tubular bodies. The thecogen cell, which is rich in cytoplasmic granula, secretes a thick and far-upreaching dendritic sheath wrapping the dendrites and the inner receptor lymph cavity. An outer receptor lymph cavity, formed by the tormogen sheath cell, is also present. The location at the base of the antennomeres as well as the specific orientation of the tubular bodies relative to the hair shaft and socket membrane may indicate a proprioreceptive function (Ernst, 1983, 2000b).

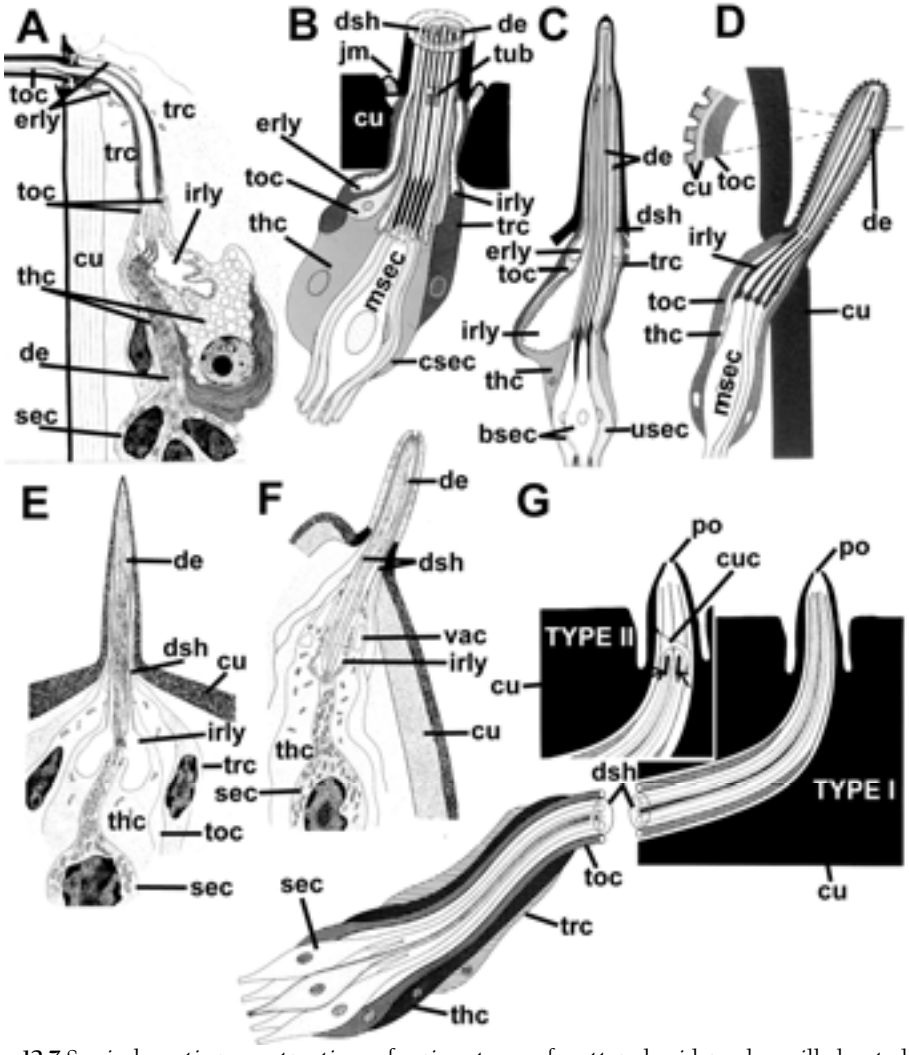


Fig. 12.7 Semischematic reconstructions of various types of scattered epidermal sensilla located on the antennae (A-F) or tip of the forcipules of different chilopod subgroups. **A** Sensillum trichodeum in *Lithobius forficatus*. **B** Sensillum trichodeum in *Geophilus flavus*. The sensillar shaft is cut at its base. **C** Sensillum brachyconicum in *G. flavus*. **D** Sensillum basiconicum in *G. flavus*. **E** Sensillum basiconicum (subtype: long cone shaped sensillum basiconicum) in *Lithobius forficatus*. Perforations of the sensillar shaft are not drawn. **F** Sensillum basiconicum (subtype: short peg sensillum basiconicum) in *L. forficatus*. **G** Two subtypes of sensilla coeloconica found on the forcipules of *L. forficatus* (Types I and II). Tubular bodies of Type II are indicated by small black arrowheads. Drawings modified after following authors: A after Ernst (1976), B,D after Ernst (2000b), C original Ernst, E-F after Keil (1975), G after Ernst and Rosenberg (2003).

3) ***Sensilla basiconica***. – Sensilla basiconica are so far only known from the antennae of lithobiomorph, craterostigmomorph, scolopendromorph and geophilomorph centipedes (Fig. 12.9C). They may appear scattered or, as for instance in Geophilomorpha, ordered in two groups on the terminal antennal article. Keil (1975) distinguished two morphs of sensilla basiconica on the antennae of *Lithobius forficatus*: 1) short pegs, fingerlike, slightly curved sensory cones and 2) long cones, elongated (prismatic) sensory cones. In *L. forficatus*, approximately 40 peg- and cone-shaped sensilla can be counted on each antenna. The number of sensilla basiconica is much less in the Epimorpha (1 in *Craterostigmus tasmanianus*: Ernst et al. 2006; 61-114 in *C. hortensis*: Ernst et al., 2009; 36-53 in *G. flavus*: Ernst 1979, 2000a,b; at least 2 in *Newportia monticola*: Koch et al., 2010). A common feature is a system of perforations (appr. 0.1 µm in diameter) or deep, longitudinal grooves (*G. flavus*) in the cuticle of the hair shaft. The hair shafts of all sensilla basiconica studied so far are inflexible. The shaft wall continues into the surrounding cuticle without a membranous articulation. Terminal pore openings do occur, but are often inconspicuous.

Studies with transmission electron microscopy, although limited to *L. forficatus* (Keil, 1975) and *G. flavus* (Ernst, 1979, 1996, 2000a,b), show that sensilla basiconica in Chilopoda may vary enormously with respect to the external appearance of the hair shaft and the internal cellular organization. The hair shafts are principally short in peg-shaped sensory cones and vary slightly between the groups: e.g. 10-20 µm in *L. forficatus* (2-4 µm in diameter: Keil, 1975) or 10-14 µm in *G. flavu* (Ernst, 1979). In contrast, the long cones become much longer, for instance 10-50 µm in *L. forficatus* (Keil, 1975). Further intra- and interspecific variations concern sensory and sheath cells.

In the peg-shaped sensilla basiconica of *L. forficatus*, three biciliate sensory cells are encased by three sheath cells (thecogen, trichogen and tormogen cell) that accompany the dendritic outer segments up the entire shaft to the terminal pore (Fig. 12.7F). The inner receptor lymph cavity (formed by the thecogen cell), delimited along the whole length by the dendritic sheath, likewise extends to the sensillum's tip and houses all dendrites. Outer dendritic segments run peripherally in the shaft, close to the cuticular pores, which are continuous with the shaft lumen.

Peg-shaped sensilla basiconica of *G. flavus* are also innervated by three sensory cells, but the size of their somata is different and each of them produces only one uniciliate dendritic process. Two of the three outer dendritic segments are larger and, while being

bsec biciliate sensillar sense cell; **cu** cuticle; **cuc** cuticular cap; **csec** (contact) chemoreceptor cell; **de** dendrtitic process (inner and outer segment); **dsh** sheath enclosing outer dendritic segments ('Dendritenscheide'); **erly** external receptor lymph cavity; **irly** internal receptor lymph cavity; **jm** joint (socket) cuticle; **msec** mechanoreceptor cell; **po** terminal shaft pore; **sec** sensillar sense cell; **thc** thecogen cell; **toc** tormogen cell; **trc** trichogen cell; **tub** tubular body; **usec** uniciliate sensillar sense cell; **vac** vacuole

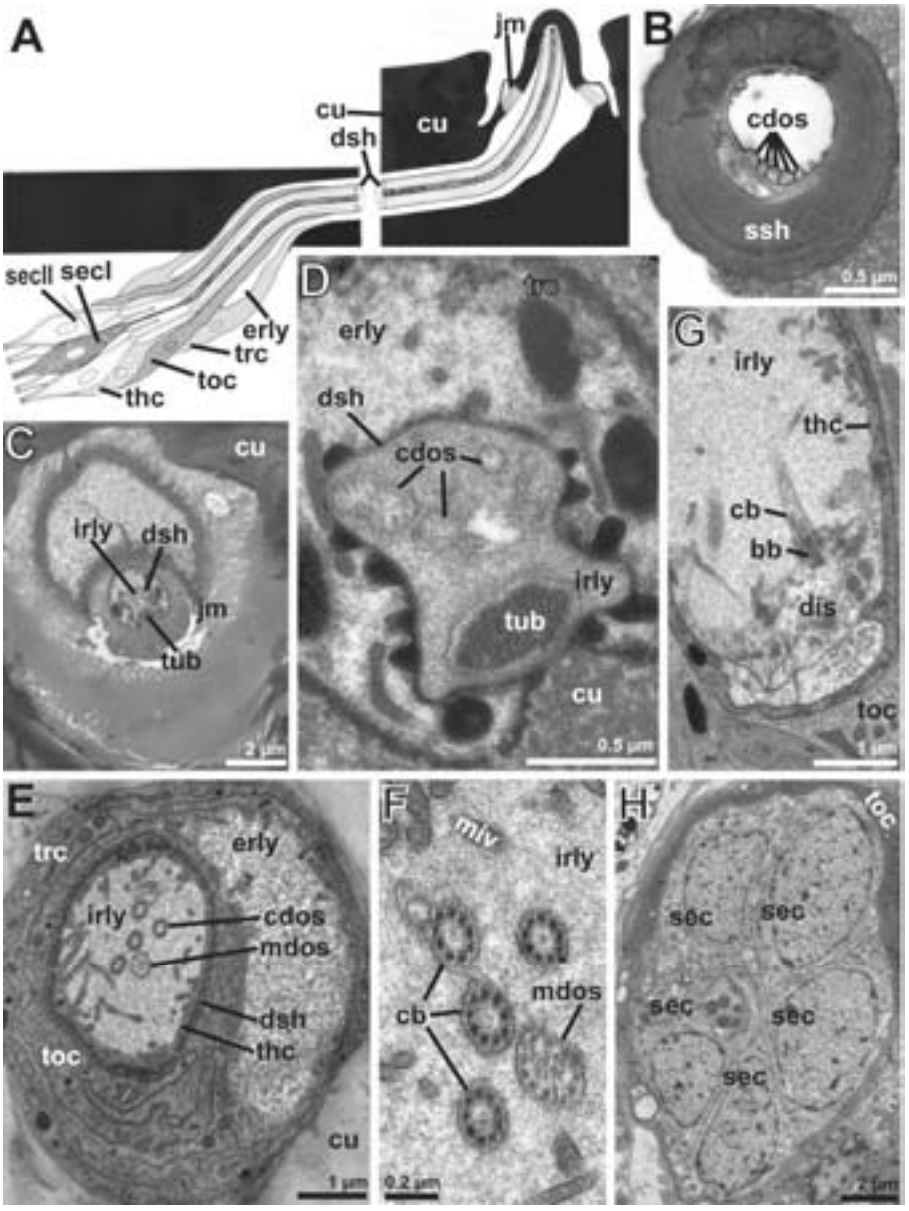
embedded into the inner receptor lymph space, become enveloped by a tube-like, cytoplasmic sheath of the thinner third outer dendritic segment. This constellation likely enlarges the perceptive dendritic surface. The thecogen cell is replaced by a second tormogen cell (1 trichogen cell + 2 tormogen cells). Both tormogen cells send off extensions into the lumen of the hair shaft (Fig. 12.7D).

Cone-shaped sensilla basiconica of *L. forficatus* possess 4-6 uniciliate sensory cells. The 4-6 outer dendritic segments include up to 50 microtubules. The outer dendritic segments enter the shaft lumen. The sheath cell apparatus consists of a thecogen cell surrounding the inner receptor lymph and the proximal part of the dendritic sheath, a trichogen cell surrounding the median and distal parts of the dendritic sheath and running up to the hair shaft, and a tormogen cell enclosing and joining the two latter sheath cells without forming an obvious outer receptor lymph cavity (Fig. 12.7E).

The porous shaft of the sensillum basiconicum seems to enable volatile odors to reach the tips of the thinned and extended outer dendritic segments and thereby supports an interpretation in favour of olfaction. Passing of volatile odor molecules through the perforated wall of peg-shaped sensilla basiconica of *G. flavus* is also considered possible, presuming at least a selective permeability of the thin cuticle at the bottom of the hair shaft grooves (Tichy and Barth, 1992). Hygro- and/or thermoreception are assumed for the sensilla basiconica in *L. forficatus* (Keil, 1975).

Fig. 12.8 **A** Semischematic reconstruction of type-I sensilla coeloconica inserted on the tip of forcipules of *Geophilus flavus*. **B-H** Collection of original transmission electron micrographs showing sections of sensilla trichodea located near-by the lateral ocellar field of *Lithobius dentatus* (B), *Craterostigmus tasmanianus* (C-G) and *Scolopendra cingulata* (H): **B** Cross section through the sensillar shaft loaded with several profiles of outer dendritic segments running through the shaft lumen (probably contact chemoreceptors). **C** Oblique cross section on the level of the cuticular socket showing dendrites enclosed by dendritic sheath structure. **D** Cross section through same region, but with higher magnification of dendritic outer segments, one tubular body is clearly visible in the inner receptor lymph cavity. **E** Cross section through sensillum trichodeum at the level of ciliate bodies. Note the presence of inner and outer receptor lymph cavities, three types of sheath cells and several dendritic outer segments. **F** Close-up of the center of the inner receptor lymph cavity housing several mechano- and (contact-) chemoreceptive dendrite populations distinguishable by their size and occurrence of ciliate bodies. **G** Transition zone of inner and outer segment of a sensillar sensory cell recognizable by the basal body structure. **H** Cluster of five sensillar sensory cells (nuclear level) at the bottom of a sensillum trichodeum. A modified after Ernst and Rosenberg (2003).

bb basal body anchoring the cilium; **cb** ciliate body; **cdos** outer dendritic segment of a putative contact chemoreceptor cell; **csec** (contact) chemoreceptor cell; **cu** cuticle; **dis** inner dendritic segment of a sensillar sensory cell; **dsh** sheath anclosing outer dendritic segments ('Dendritenscheide'); **erly** external receptor lymph cavity; **irly** internal receptor lymph cavity; **jm** joint (socket) cuticle; **mdos**; outer dendritic segment of a mechanoreceptor cell; **miv** microvillus; **sec** sensillar sense cell; **secI** type-I sensillar sensory cell; **secII** type-II sensillar sensory cell; **ssh** sensillar hair shaft; **thc** thecogen cell; **toc** tormogen cell; **trc** trichogen cell; **tub** tubular body



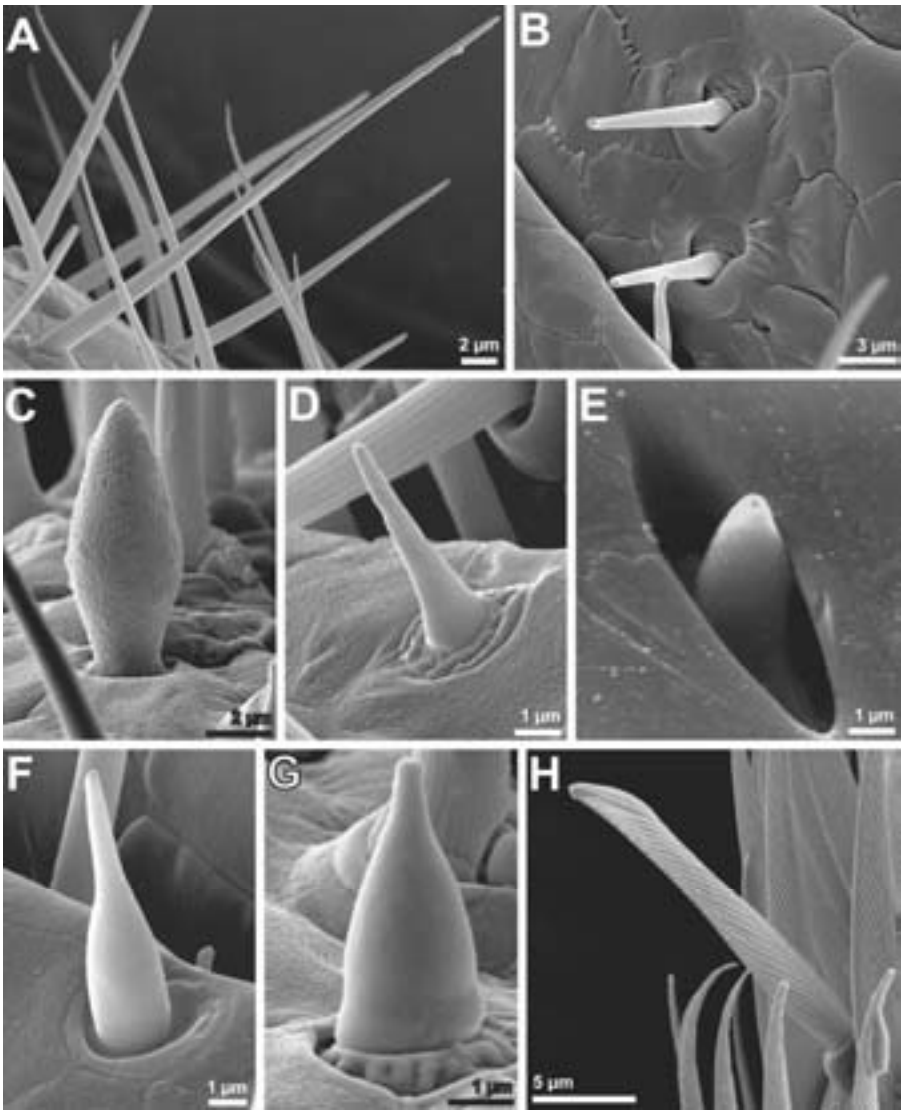
4) ***Sensilla brachyconica***. – In *Cryptops hortensis*, Ernst et al. (2009) distinguish two classes of sensilla brachyconica depending on hair length and insertion place on the antennae: terminal sensilla brachyconica and upper-edge sensilla brachyconica (compare

also Fig. 12.9D). Based on external morphology, the upper-edge sensilla brachyconica are here homologized with the large and small sensory cones on the nodes of the antennae of *Scutigera coleoptrata* (Ernst et al., unpubl.). With numbers of 72 per antenna in *L. forficatus* (Keil, 1975) and up to 15 per antenna in *C. hortensis* (Ernst et al., 2009), sensilla brachyconica with short, spine-like hair tapering continuously to the tip (diameter ranging from 1.6-2.1 µm at the base to 0.3 µm at the tip) are frequent on the anteroventral and anterodorsal edge of antennomeres 2 to 16. Their length is approximately 10 µm in *L. forficatus* and between 4.4 and 6.8 µm in *C. hortensis* (Fig. 12.9D). At the base, upper-edge sensilla brachyconica are inserted in a wide and shallow cavity allowing for movements in all directions. The existence of a terminal pore opening is mostly uncertain, only in *Scutigera coleoptrata* does such a pore opening seem to exist (Ernst et al., unpubl.).

The sensilla brachyconica on the distal tip of the terminal antennomere may become considerably longer, e.g. those of *S. coleoptrata* (5.5 µm), *G. flavus* (14-18 µm: Ernst, 1981, 1999, 2000b) or the so-called 'Sinneskegel' of *L. forficatus* (50 µm: Keil, 1975). Terminal spine-shaped sensilla are fewer in number, e.g. 3 per antenna in *S. coleoptrata*, 7 per antenna in *G. flavus*, 8 in *L. forficatus*, 5-11 in *C. hortensis*. The shape of the hair is slender, sometimes slightly curved towards the tip, which bears a rounded pore opening. In *C. hortensis*, the hair looks smooth and velvety, decreasing in diameter from 1.6 to 2.1 µm at the occasionally striated base to 0.3 µm at the distal tip. Scanning electron microscopy showed that the socket of both terminal and upper-edge sensilla brachyconica is firmly connected to the surrounding cuticle (*G. flavus*, *L. forficatus*, *C. hortensis*).

A transmission electron microscopic description of sensilla brachyconica is only available for *G. flavus* and *L. forficatus* (Ernst, 1981, 2000b; Keil, 1975). In *G. flavus*, Ernst (1981, 2000b) described a bimodal sensory apparatus made up of one uniciliate and two biciliate sensory cells enclosed by three sheath cells (Fig. 12.7C). The total of five outer dendritic segments have different diameters and terminate on different levels in relation to the hair shaft. Tubular bodies are absent, indicating the inability of these sensilla to process mechanosensory stimuli. A higher number of 4-6 uniciliate sensory cells is present in *L. forficatus* (Keil, 1975). The exposed position on the tip of the terminal antennomere suggests a thermoreceptive function in *L. forficatus*, whereas the bimodal arrangement of sensory cells enables the addition of hygroreceptors in *G. flavus* (Ernst et

Fig. 12.9 Compilation of types of epidermal sensilla in Chilopoda, documented on the antennae of *Cryptops hortensis* (A-D,F-G) and *Scutigera coleoptrata* (H) as well as recessed on the maxillipedes of *Lithobius forficatus* (E). The sensilla have been ordered according to their appearance in the text. **A** Sensillum trichodeum. **B** Sensillum microtrichodeum. **C** Sensillum basiconicum. **D** Sensillum brachyconicum. **E** Sensillum coeloconicum. **F** Club-shaped sensillum. **G** Hat-shaped sensillum. **H** Beak-shaped sensillum.



al., 2009). The possible existence of a terminal pore in *C. hortensis* suggests a functional combination of hygro- or thermoreceptors with contact chemoreceptors (Ernst et al., 2009).

5) **Sensilla coeloconica**. – Sensilla coeloconica are structurally highly diverse and seem to be widespread among the five main chilopod subtaxa. Although variations in hair shape do occur, these sensilla are reliably distinguishable from all other types (Fig.

12.9E). The majority of sensilla coeloconica is described on the forcipular claw (Jangi and Dass, 1977, Ménez et al. 1990; Ernst, 1995; Rosenberg and Ernst, 2001; Ernst et al., 2002; Ernst and Rosenberg, 2003). Others are known from the antenna of *Craterostigmus tasmanianus* (Ernst et al., 2006) or the epi- and hypopharyngeal regions of some Scutigeromorpha (Koch and Edgecombe, 2006) and many Scolopendromorpha ('nipple- and/or bullet-shaped' sensilla: Edgecombe and Koch, 2009; Koch et al., 2010). The sensory cone is centered into a deep cavity of ovoid or spherical shape. The cone mostly has a terminal pore and barely overlaps the level of the surrounding cuticle. We distinguish three subtypes according to size differences and local patterns of distribution.

Type-I: Sensilla coeloconica with broad and conical hair shafts (2-5 µm in length) can be seen in large numbers on the ventral and dorsal side of the forcipular tarsungulum (Fig. 12.9E). Sensory cells and sheath cells are connected to the sensory cone via a long, S-shaped channel. In *L. forficatus*, 7-9 uniciliate sensory cells are surrounded by one thecogen cell, one trichogen cell, and one tormogen cell. Thick (0.6-1.2 µm in diameter) and thin (0.2-0.4 µm in diameter) outer dendritic segments pass through the cuticular channel and center of the sensory cone where they abut the apical pore opening. On their way up the channel, the dendrites are wrapped by the dendritic sheath and the thecogen sheath cell. (Rosenberg and Ernst, 2001; Ernst and Rosenberg, 2003) (Fig. 12.7G). In *G. flavus*, approximately 60 type-I sensilla coeloconica occur at the tip of the tarsungulum. Deviations from type-I sensilla in *L. forficatus* are a mostly lesser number of sensory cells (n=3-8), smaller diameters of outer dendritic segments (thick dendrites: 0.4-0.7 µm, thin dendrites: 0.2-0.3 µm) and absence of a terminal pore on the sensory cone. The basis of the sensory cone is hinged to the cuticle of the socket cavity by a membranous joint and stiff radial microfibrils, limiting shaft mobility (Ernst and Rosenberg, 2003) (Fig. 12.8A).

Type-II: Sensilla coeloconica with slender hairs shafts and rounded tips (1.5-2.7 µm in length) can be observed in low numbers on the uppermost tip region of the forcipular tarsungulum. Type-II sensilla are similar to those of type-I in general configuration but there are considerable differences in receptor composition. In hemianamorphic larval stages and adults of *L. forficatus*, the two outer dendritic segments with widest diameters are associated with tubular bodies (mechanoreception), allowing for stable connection of the dendrites to a cap-like differentiation of the socket cuticle. All remaining, putatively chemoreceptive dendrites run further up, enter the hair shaft and terminate close to the apical pore opening (Ernst and Rosenberg, 2003) (Fig. 12.7G).

Type-III: Sensilla coeloconica with long and acute hair shafts (2-4.5 µm in length) can be found in almost elliptical cavities at the inferior edge of the tarsungulum tip. A terminal pore opening exists.

With exception of the antennae of *C. tasmanianus* where the existence of terminal pores on tumbler switch-shaped sensilla is dubious, type-I sensilla coeloconica are thought to function as contact chemoreceptors (Rosenberg and Ernst, 2001; Ernst and Rosenberg, 2003). This assumption is supported by behavioural experiments conducted by Jangi and Dass (1977) in *S. morsitans*. The existence of dendritic outer segments of thick and thin diameters in type-I sensilla coeloconica in Lithobiomorpha and Geophilomorpha indicates a dual function, possibly a combination of thermo- and hygroreceptors (Ernst, 1995, 2000b; Ernst and Rosenberg, 2001). In type-II sensilla coeloconica of *L. forficatus*, mechano- and chemoreceptors are coupled, and the former receptor function appears obvious because of the existence of tubular bodies (Rosenberg and Ernst, 2001).

6) **Collared bottle-shaped sensilla**. – Based on SEM data, collared bottle-shaped sensilla, hitherto exclusively found on the antennae of *Craterostigmus tasmanianus*, are compound sensory hairs distinguished by a smooth, collar-like shaft and a smooth, tapered distal flagellum without apical pore opening (Ernst et al., 2006). Owing to a joint-like structure connecting its base to the shaft, the flagellum is assumed to be movable. Variations of antennal bottle-shaped sensilla do occur in *C. tasmanianus*. Edgecombe and Giribet (2004) illustrated a short as well as a long subtype to be frequent on the dorsal side at the anterior edge of antennomere 16 and a single long bottle-shaped sensillum on the ventral side at the anterior edge of the same antennomere. Sensilla of both subtypes are abundant on the anterior edges of antennomeres 2, 6 and 11.

7) **Collared tube-shaped sensilla**. – Based on SEM data, tube-shaped sensilla are hitherto only known from the apical tip of the terminal antennomere of *Craterostigmus tasmanianus* (Ernst et al., 2006). Like bottle-shaped sensilla, they appear compound, being divided into a shaft and flagellar region. In contrast to the latter type, however, tube-shaped sensilla have a long and slender shaft contrasting with a much shorter flagellum without an apical pore opening. Sensilla of similar collared form have been documented on the antenna of *Scolopocryptops sexspinosus*. Here, the length ratio between shaft and flagellum is about 1:2. The hair looks striated as in typical antennal trichoid sensilla (see above and Edgecombe and Giribet, 2004). Bipartite sensilla also seem to occur in other *Scolopocryptops* species (Attems, 1930) as well as in *Dinocryptops miersi* (Lewis, 2000).

8) **Club-shaped sensilla**. – SEM observations reveal that simple and blunt club-shaped sensilla of *Cryptops hortensis* are superficially similar to bottle-shaped sensilla of *Craterostigmus tasmanianus*. Club-shaped sensilla are not subdivided into distinct segments (Ernst et al., 2009). The base of the sensillum is inserted in a plain cavity (Fig. 12.9F). Only a few of them (2–4) have been mapped on each antenna of *C. hortensis*. At the tip of the sensillum, a terminal pore opening is present. Termed as 'bottle-shaped sensilla',

club-shaped sensilla have also been documented grouped together on the apical antennomeres of the scolopendromorphs *Newportia monticola* and *Ectonocryptoides quadrimeropus* (Koch et al., 2010). The cavity around the socket is spacious.

9) **Hat-shaped sensilla**. – To date, hat-shaped sensilla were exclusively found on the antennae of various Scolopendromorpha. The 2-6 hat-shaped sensilla, observed in *Cryptops hortensis*, are by far the smallest sensilla ever found on chilopod antennae (up to 5.7 µm in diameter, see Fig. 12.9G)) (Ernst et al., 2009). Often, the bulky and smooth sensory hair sits on a furrowed, plate-like pedestal embedded into a flat, bowl-like depression of the cuticle. The form of the sensory hairs rather resembles a mitre. A terminal pore is present. This type of sensillum seems to be also present on the terminal antennomere of *Newportia monticola* ('bottle-shaped sensilla', Koch et al., 2010).

10) **Button-shaped, rimmed sensilla**. – This rather unique type of epidermal sensilla belongs to the group of hypopharyngeal sensilla and was found by Koch and Edgecombe (2006) on the tongue of scutigeromorphs (e.g. *Thereuopoda longicornis*) in paired paramedial rows, innervated by paired neuropils sitting below the frontal epidermis of distal hypopharynx. Cuticular structures formed by these sensilla display resemble a buzzer structure ('button') on which the small, peg-like sensilla are mounted. According to Koch and Edgecombe (2006), inturned cuticular rims of these sensilla bear an irregularly folded, whereas the outer rim displays a more regular, concentric ornament. Subsequently Koch and Edgecombe (2008) discovered distinct rows of button-like sensilla on the hypopharynx of Lithobiomorpha.

11) **Beak-shaped sensilla**. – Based on SEM data alone (Ernst et al., unpubl.), over 4.000 beak-like sensilla were observed on the antennae of *Scutigera coleoptrata* (Fig. 12.9H). The long, broad and flattened sensory hairs resemble an elongated beak. The shaft bears a pattern of spiralled, strongly convex ribs separated from each other by deep furrows, probably the roughest rib pattern among all hitherto described types of chilopod sensilla. The tip of the shaft is thickened, often curved, and carries a terminal pore opening. By being inserted on the anterior margin of every second or third antennomere and by keeping contant distance from each other in this circular arrangement, beak-shaped sensilla are aligned accurately along the entire antenna, probably forming a grid pattern of chemoreceptors.

Large or compound epidermal sensilla

These are complex structures with more or less closely assembled sensilla which may contain one or several types of sensilla. Compound epidermal sensilla often appear in

module-type arrangements and are sunk into a deep depression of the epidermis, only visible from outside by a more or less spacious pore opening. The axons of their sensory units are grouped together in a specialized nerve targeting in one or several parts of the brain.

Tömösváry organ

The Tömösváry organ is a sense organ assumed to function as a carbon dioxide detector. The name Tömösváry organ refers to the first discoverer, the Romanian myriapodologist Ödön Tömösváry (1852-1884), and was introduced by Vogt and Yung (1883). Common English synonyms are 'temporal organ' or 'postantennal organ'. In accordance with Haupt (1979) and Tichy and Barth (1992), the Tömösváry organ is assigned here to the category of compound sensilla organs.

The Tömösváry organ is located behind the basis of each antenna in the Scutigeromorpha (*Scutigera coleoptrata*: Tichy and Barth, 1992; *Thereuonema tuberculata*: Yamana and Toh, 1990) and Lithobiomorpha (Lithobiidae: *Lithobius forficatus*: Tichy, 1972, 1973a; Tichy and Barth, 1992; various Henicopidae: Edgecombe, 2004). In Lithobiomorpha, the broad, crater-like Tömösvary organs are variously placed anteriolaterally to the ocellar field (or posteriolaterally to the antennal basis in blind species) often nested against the edge of the head capsule (most Lithobiidae; e.g. Müller and Rosenberg, 2006) (see also Fig. 12.5A). In some Henicopidae of the genus *Paralamyctes*, the openings to the Tömösváry organ are placed in a depression of the bending edge of the head capsule (Edgecombe et al., 2002, Edgecombe, 2004), but in *Lamyctes caeculus* and *Haasiella* spp. they are displaced posteriorly to the cephalic pleurite and are thereby not visible from above (Edgecombe, 2004). In Craterostigmomorpha, a triangular plate surrounded by a prominent cuticular ring lateral to the clypeus is possibly a Tömösváry organ (Dohle, 1990).

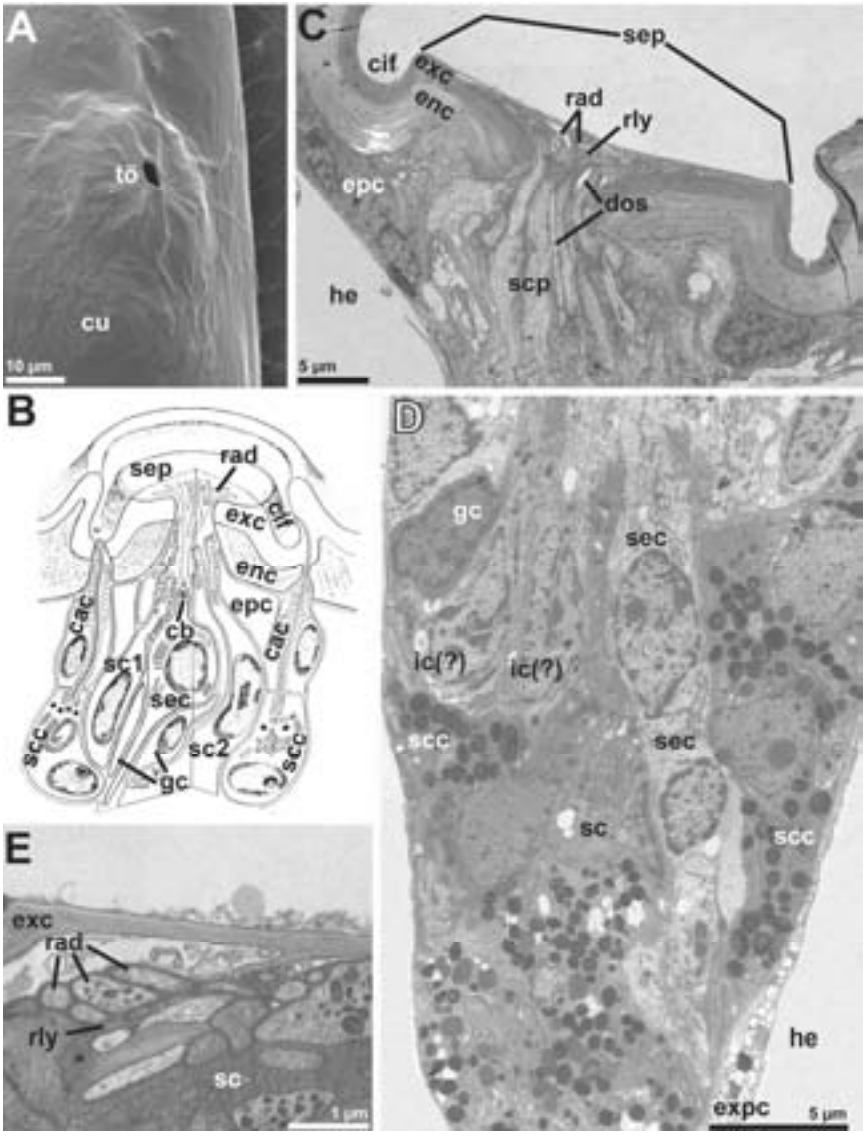
Besides the Chilopoda, Tömösváry organs are found in all remaining subgroups of the Myriapoda as well as in Protura and Collembola (see summary in Haupt, 1979).

The most thoroughly studied Tömösváry organ in Chilopoda is that of *Lithobius forficatus* (Tichy, 1972, 1973a, b; Tichy and Barth, 1992). It consists of a sensory apparatus of twelve closely packed biciliate sensory cells enveloped by an outer mantle of at least twice as many sheath cells (Fig. 12.10B,D). The dendritic processes emanating from each sensory cell are divided into three compartments according to the 1) distance to the soma, 2) the occurrence of ciliary structures and 3) the extension of the inner receptor lymph cavities (Fig. 12.10B). The outer dendritic segment consists of an unpaired ciliary segment and paired dendrites that project distally and finally come into contact with the cuticle. The cuticle forms an elevated sensory plate lining the bottom of the crater-

shaped opening (Figs. 12.5A, 12.10B-C). On the level of the ciliate body, the sheath cells transform into elongated distal extensions that withdraw from the dendrites but form small inner receptor lymph cavities around one pair of cilia, the third dendrite compartment, the so-called outer segment. The liquid content of the receptor lymph cavities is controlled by continuous secretion activity by the sheath cells. When approaching the sensory plate, the small inner receptor lymph cavities fuse to build the outer receptor lymph cavity. More distally, however, the ciliary processes divide into second order branches (Fig. 12.10C,E). All branches and subbranches are still embedded in the outer receptor lymph cavity and finally make contact to the inner surface of the thin exocuticle that lines the bottom of the crater-shaped opening of the organ. In its center, the sensory cuticular plate is raised above the level of the surrounding circular furrow, which is covered by a cuticular bulge. The circular furrow is rich in pore openings of numerous flexo-canal epidermal glands that enclose the entire sensory apparatus (Figs. 12.5A, 12.10B-C). Tichy (1972, 1973a) described these accessory flexo-canal epidermal glands as including only canal cells and secretory cells, but according to new observations the presence of intermediary cells cannot be excluded. Potential candidates for intermediary cells are marked in Fig. 12.10D. From the bottom of the sensory cell's soma, an axonic process is sent off to enter the nervus Tömösváryi, with thick neurilemm and glial cell sheaths. Tichy (1972, 1973a) described the affererent axonic processes that transmit stimuli to the subjacent neuropils without synaptic connections, but the absence of synapses appears problematic. The Tömösváry organ afferents innervate three compartments within the ipsilateral protocerebrum: the second

Fig 12.10 External morphology (SEM: A) and cytomorphology (TEM: C-E) of the Tömösváry organ in *Scutigera coleoptrata* (A) and *Lithobius forficatus* (B-E). **A** Tömösváry organ, note the dome-like elevation of the cuticle leaving a small central pore opening on the summit. **B** 3D cut-away drawing showing the ultrastructural organization of the Tömösváry organ in *L. forficatus* (adapted from Tichy, 1973a). **C** Longitudinal section through the apical region of the Tömösváry organ with the sensory plate crossed by numerous ramified projections of the outer dendritic segments, processes of the sheath cells and receptorlymph interspaces. **D** Organization of the median and proximal regions of the Tömösváry organ housing a high diversity of cell types, same individual as in C. **E** Highly magnified sector within the sensory plate region (longitudinal view). Ramifications of outer dendritic segments abut the thinned cuticle. A original Ernst, Sombke; C-E originals Müller.

cac canal cell (of an accessory flexo-canal epidermal gland); **cb** ciliate body; **cif** circular furrow around the sensory plate; **cu** cuticle; **dos** outer dendritic segment; **enc** endocuticle; **epc** epidermal cell; **exc** exocuticle; **expc** external pigment cell; **gc** glial cell; **he** hemolymphatic space; **ic(?)** putative intermediary cell(s); **rad** ramified tip of an outer dendritic segment; **rly** receptor lymph space; **sc**; sheath cell; **sc1** type-1 sheath cell; **sc2** type-2 sheath cell; **scc** secretory cell (of an accessory flexo-canal epidermal gland); **scp** sheath cell process (with microvillar fringe); **sec** sensory cell constituting Tömösváry organ; **sep** sensory plate; **tö** opening of the Tömösváry organ



optic neuropile (medulla), the dorsolateral protocerebrum, and the pedunculi of the so-called mushroom bodies (Petykó et al., 1996).

The fine structure of Tömösváry organs in *T. tuberculata* (Yamana and Toh, 1990) and *S. coleoptrata* (Tichy and Barth, 1992) widely corresponds to conditions in *L. forficatus*.

Similar are number, proportions, compartmentalization and dendritic ramification of the sensory cells. So too is the arrangement of sheath cells and the merging of inner receptor lymph cavities into one distal outer receptor lymph cavity. However, scutigeromorph Tömösváry organs have both mono- and biciliate sensory cells and strongly differ from those in Lithobiomorpha by their outer appearance and distal expansion of the sensory apparatus. In *T. tuberculata* and *S. coleoptrata*, the sensory apparatus, which is lined by thin, fibrous cuticle, forms a mushroom-like bulbus protruding into a spacious cuticular cavity covered by a cuticle with a small central opening only (Fig. 12.10A).

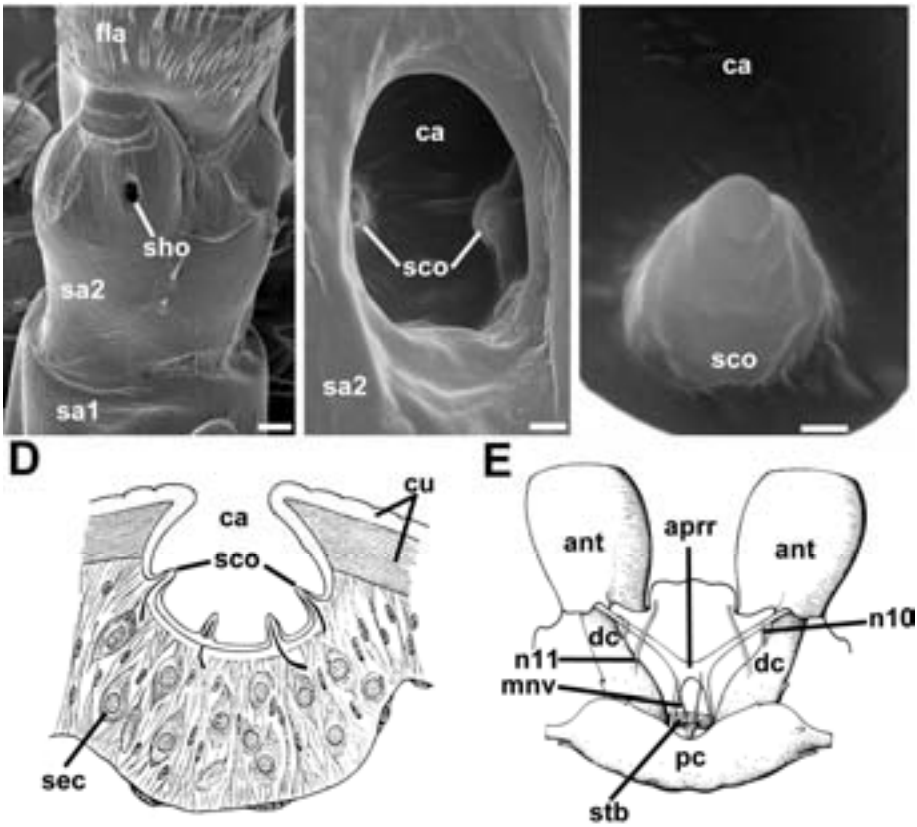
There have been speculations on the function of Tömösváry organs, ranging from hygroreception (Bauer, 1955; Tichy, 1973a,b) to vibration detection (Meske, 1960, 1961). However, electrophysiological recordings on *T. tuberculata* have shown the sensitivity of Tömösváry receptor cells against H_2O and, in particular, CO_2 (Yamana et al., 1986, 1998; Yamana and Toh, 1987). The receptor cells are assumed to detect changes in ion concentrations within the receptor lymph cavities. Sensitivity to CO_2 molecules is considered to be especially advantageous for predators living in or close to the soil (e.g. hidden underneath stones or in rocky/wooden crevices). Functional constraint to tune and enhance the sensitivity towards H_2O and/or CO_2 perception may have led to enormously enlarged Tömösvary organs in cave-inhabiting centipedes, just like described by Hennings (1904, 1906). Some blind Henicopidae (e.g. *Paralamyctes (Haasiella) trailli*, *Lamyctes caeculus*) carry larger Tömösváry organs than their relatives equipped with eyes (Serra, 1981, Edgecombe et al., 2002).

Shaft organ ('Schaftorgan')

Verhoeff (1904) described a dorsomedian pit on the distal (second) antennomere of the antennal shaft of scutigeromorph centipedes and termed it the 'Schaftorgan' (see also Fig. 12.11A-C). The English term 'shaft organ' was introduced by Lewis (1981). Within this

Fig 12.11 External morphology (SEM data: A-C) and anatomy (LM data: D) of the shaft organ on the antennae of *Scutigera coleoptrata*. **A** Overview of the second shaft antennomere of one antenna photographed from dorsal perspective. The shaft organ is clearly visible as a round breakthrough in the cuticle. **B** Higher magnification of the cavity of the shaft organ from dorsal perspective. Bumps at the bottom of the cavity represent sensory cones. **C** Detail of the cavity and close-up of one sensory cone. **D** Longitidinal section of the shaft organ and schematic reconstruction of the sensory cell pattern situated below the bottom of the cavity. **E** Cut-away drawing of a dissected antennal muscle sense organ in *Lithobius forficatus*. Example for a proprioreceptor in Chilopoda. A-C, originals Ernst, Sombke; D modified after Fuhrmann (1922); E modified after Rilling (1968).

ant antenna; **aprr** antennal proprioreceptor (muscle sense organ); **ca** cuticular cavity of the shaft organ; **cu** cuticle; **dc** deutocerebrum; **fla** proximal antennomere of the flagellum; **mnv** motoric nerve; **n10-11** 10th and 11th nerve; **pc** protocerebrum; **sa1** first antennal (shaft) article; **sa2** second antennal (shaft) article; **sec** sensory cell constituting the shaft organ; **sco** sensory cone; **sho** opening of the shaft organ; **stb** stomodeal bridge (=frontal connective)



pit, approximately 20 cone-shaped sensilla with a length of 3 µm are present in *Scutigera coleoptrata* (Fig. 12.11D). The function of the shaft organ is completely unkown. Fuhrmann (1922) suggested that these sensilla may have an olfactory function. However, due to their insertion within the shaft organ it is not likely that the sensilla are gustatory or mechanoreceptors (Ernst et al., unpubl.). However, in SEM studies the sensillar shaft seems to be unstructured and the rounded tip does not possess a terminal pore (Ernst et al., unpubl.). Typologically, the sensilla assembled in the shaft organ resemble the sensilla brachyconica that have also been observed on the antennae of other chilopods.

Proprioreceptors

Proprioreceptors have been described by Rilling (1960, 1968) in *Lithobius forficatus*, as specialized muscle receptors and sensory cells with free terminations structures. Proprioreceptive sensory cells are often constant in number and show equal distribution

patterns on their target structures. Ramified dendritic processes of the sensory cells cover wide parts of the unsclerotized cuticle typically found in the intersegmental or interpodomeric membranes. Compression or stretching of the articular membranes then results in depolarization of the proprioreceptors. Otherwise, proprioreceptive nerve elements are associated with thin muscular bands. These filigree muscular bands connect moveable cuticular plates (tergites, pleurites, sternites) and tentorial structures with each other. The terminology of these so-called muscle receptors is based on their location in the centipede's body. For instance, there are pedal muscle receptors that consist of a simple muscle band innervating a pair of primary sensory cells and receive input from the coxa-trochanter-joint in the walking legs. Pedal muscle receptors of principally identical structure are likewise present at the bases and within the maxillae II, the maxillipedes as well as the antennae, there forming the so-called antennal muscle sensory organ (Fig. 12.11E). Furthermore, there are tergal muscle receptors that are placed at the attachment points of the dorsal longitudinal musculature which extends between two succeeding large tergites. Afferents of tergal muscle receptors extend to the ventral nerve chord.

Besides Scutigeromorpha, tergal muscle receptors have also been reported in the dorsal longitudinal musculature of *Scolopendra morsitans*. Varma (1972) found receptors consisting of 13 bi- and multipolar sensory cells. Each receptor is innervated by the N-IV nerve projecting from the ventral ganglia 1-20. Muscle receptors arranged at different parts of the trunk have been described in a further *Scolopendra* species as well as in *Lithobius* sp. and the geophilomorph *Haplophilus* sp. (Osborne, 1961; Finlayson, 1976).

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III

Antennal sensilla of *Scutigera coleoptrata*

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The source of chilopod sensory information: External structure and distribution of antennal sensilla in *Scutigera coleoptrata* (Chilopoda, Scutigeromorpha)

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Abstract

The investigation of the antennae of *Scutigera coleoptrata* (Linnaeus, 1758) by scanning electron microscopy revealed the presence of five types of sensilla: sensilla trichodea, beak-like sensilla, cone shaped sensilla brachyconica on the terminal article, sensory cones at the antennal nodes, and the shaft organ. Alongside the presence and absence of antennal sensillar types, three unique characters are found in the Scutigeromorpha: the presence of long antennae with nodes bearing sensory cones, the presence of a bipartite shaft including the shaft organ, and the presence of beak-like sensilla. Neuroanatomical data show that the animals' brains are equipped with well-developed primary olfactory and mechanosensory centers, suggesting that the antennae must be equipped with specialized sensilla to perceive chemo- and mechanosensory cues. With the evidence provided in this paper for the Scutigeromorpha, SEM data are available at last on the antennal sensilla for all five chilopod taxa, allowing a comparative discussion of antennal morphology in Chilopoda.

Key words: Scanning electron microscopy, antennal sensilla, Chilopoda

Introduction

Early investigation on the antennae and sensilla of Chilopoda were conducted during the 19th and early 20th century using only light microscopy. The antennal sensilla were classified as touch bristles and pale cones (Leydig 1860, Sazepin 1884, Fuhrmann 1922, Verhoeff 1902-1925). Using electron microscopic methods, various chilopods were investigated with regard to their sensillar equipment, e.g. *Lithobius forficatus* (Lithobiomorpha) (Keil 1975, 1976), *Geophilus flavus* (Geophilomorpha) (Ernst 1976, 1979, 1981, 1983, 1996, 1997, 1999, 2000), *Craterostigmus tasmanianus* (Craterostigmomorpha) (Ernst et al. 2006), and *Cryptops hortensis* (Scolopendromorpha) (Ernst et al. 2009). However, the antennal sensilla of a representative of the Scutigeromorpha were never investigated in detail with these methods.

Scutigera coleoptrata (Linnaeus, 1758) is a crepuscular to nocturnal, ground-living centipede with long walking legs and body-long antennae. The representatives of the Scutigeromorpha are predators and feed on various living arthropods using their poisonous forcipules to kill their prey. The Scutigeromorpha display many unique characters, such as the dorsal respiratory openings (Hilken 1998) and compound eyes (Müller et al. 2003, 2011). The eyes are characterized by high UV-sensitivity which may serve as an alarm signal to avoid bright light environments or to detect exits from concealed hiding places in soil crevices (Meyer-Rochow et al. 2006). However, behavioral experiments suggest that for hunting, visual cues are much less important than an olfactory guided search (Klingel, 1960, Sombke et al. 2011). Considering that many Chilopoda including *S. coleoptrata* are active mostly during night time (Rosenberg 2009), it seems reasonable to argue that mechanoreception and chemoreception are the major sensory modalities involved in hunting for prey. Recent neuroanatomical studies show that the animals' brains are equipped with well-developed primary olfactory and mechanosensory centers (Sombke et al. 2009, 2011), suggesting that the antennae must be equipped with specialized sensilla to perceive chemo- and mechanosensory cues. According to a majority of phylogenetic analyses, the Scutigeromorpha are the sister group to all remaining chilopod subgroups, the latter collectively forming the taxon Pleurostigmophora (Dohle 1985, Borucki 1996, Edgecombe and Giribet 2007, Shear and Edgecombe 2010). Therefore, sensory systems in Scutigeromorpha may have retained characters that are plesiomorphic with respect to the ground pattern of the Chilopoda and even the Myriapoda. For instance, as mentioned above, in the visual system, it is assumed that the ommatidia of Scutigeromorpha comprising eucone cells with their nuclei placed outside the cone compartments may reflect a state already present in the last common ancestor of the Mandibulata (Müller et al. 2003, Harzsch et al. 2007). This scanning electron microscopic study continues the comparative investigation on the antennal sensilla of Chilopoda (e.g. Ernst 2000) and presents the classification, distribution, and in part new definitions of antennal sensilla of *Scutigera coleoptrata*.

Material and methods

Specimens of *Scutigera coleoptrata* were collected on the Balearic Island Ibiza, mainly in the litter of pine forests. If not fixed directly after capture, individuals were kept in plastic tubes (Falcon tubes, 50 ml) at room temperature. All specimens had developed all 15 leg pairs and a body length of 14 to 16 mm, which classifies them as Pseudomaturus (Rosenberg 2009). The length of six investigated antennae ranged between 15 and 16 mm. After anesthetization and decapitation, the heads were fixed and preserved in 70 % ethanol. Following dehydration in a graded series of ethanol, several antennae were critical point dried and mounted on Leitz-Taps (Plano), sputter coated with a 25 nm gold film (BAL-TEC SCD 005), and examined by scanning electron microscopy (LEO 1450 VOP). The dorsal and ventral antennal side was observed on different antennae.

Results

Structure of the antennae

In *Scutigera coleoptrata*, the length of the antennae exceeds the length of the specimens' body. Each antenna consists of a shaft and a long flagellum (Fig. 1 A, B). The shaft is composed of two large articles, which are approximately 260 µm (the proximal one) and 150 µm (the distal one) in length. The distal article bears the oval to round opening of the shaft organ with a diameter of 10 – 15 µm (Fig. 1 A-C). The long flagellum consists of round 500 (450 – 519) small antennal articles (or antennomeres, annuli, flagellomeres) (Fig. 1 A).

The antennal articles diminish in size along the length of the flagellum from the base (64.8 µm in length) to the end (18 µm in length). The diameter of the articles ranges from 152 µm at the antennal base to 38.4 µm at the apex. Along the flagellum, smaller and larger articles can be observed (Fig. 1 A, D, E). At the end of the flagellum, two to four small articles are followed by a large terminal article with an invagination at its top (Fig. 2 F). Along the flagellum, three nodes (Verhoeff 1902-1925: nodus; Lewis 1981: node; Bonato et al. 2010: node) are visible that are considerably longer than the antennal articles (about 318 µm in length and up to 219 µm in diameter) and pin-joint the rigidly coupled articles (Fig. 1 D). Each nodus consists of a longer and a shorter part. In the investigated specimens they were observed in different positions (see table 1). Even in the same specimen, the position of the node can vary between the right and the left antenna.

The antennal articles are covered by rows of flattened trichomes (approx. 20 µm in length): small articles possess a single transversal row; large articles bear two to three transversal rows of trichomes (Fig. 1 A, D-F, 2 D). Near the end of the flagellum, only a single row of trichomes is present (Fig. 2 F). The solid trichomes are slightly bent and firmly inserted to the antennal cuticle. Its surface shows a characteristic texture and the apex often appears slightly hooked (Fig. 1 F, Fig. 2 A-F). The shaft is striated to ribs, which end in a longitudinal edge (Fig. 2 A-C).

	Specimen 1 dorsal		**Specimen 2 ventral**		**Specimen 3 dorsal**	
	Left	Right	Left	Right	Left	Right
1st node	50	49	52	51	42	42
2nd node	165	157	178	149	126	136
3rd node	?	337	?	?	299	?
Total number	?	519	450	?	465	?

Table 1. Total number of antennal articles and position of antennal nodes from three different specimens of *Scutigera coleoptrata*. Left and right antennae were investigated. Specimen 1 and 3 were examined on the dorsal side, specimen 2 on the ventral side.

Five types of antennal cuticular sensilla can be differentiated by scanning electron microscopy (Fig. 4), and are described below.

Sensilla trichodea

Two types of trichoid sensilla can be differentiated: (1) long sensilla trichodea (Fig. 2 B, C) with a length between 48 and 73 µm (basal diameter: 4.2 – 7.8 µm), and (2) shorter sensilla mesotrichodea (Fig. 2 A) with a length between 27 and 40 µm (basal diameter: 2.9 – 5.6 µm). The diameter at the tip of the shaft ranges between 0.6 and 1.4 µm. Rarely, shorter trichoid sensilla (10 to 20 µm in length) are noticeable. The sensilla trichodea are concentrated on parts of the shaft and on the antennal articles one to 16 in a repetitive arrangement (Fig. 1 A asterisks). The total number of s. trichodea ranges between 95 and 105 per antenna (dorsal side: 50 – 60, ventral side: 45 – 47). On the left and right side of the distal shaft article, the trichoid sensilla are arranged in groups mainly on its dorsal part. The major portion of the surface is free of sensilla. For instance, the proximal shaft article bears ten s. trichodea and four s. mesotrichodea, the distal shaft article bears six s. trichodea and nine s. mesotrichodea. The number of s. trichodea ranges from one to four per flagellar article. Sometimes, few s. trichodea are found at more distant articles up to the first node. The long and straight shaft of the s. trichodea is distinctly striated (Fig. 2 B, C, 4) and bears a single terminal pore (Fig. 2 A). The base of the shaft is set in a small depression of the antennal cuticle. The cuticle forms a bidentate scale at the base of the shaft which restricts the movement of the sensillum in the basal direction (Fig. 2 B).

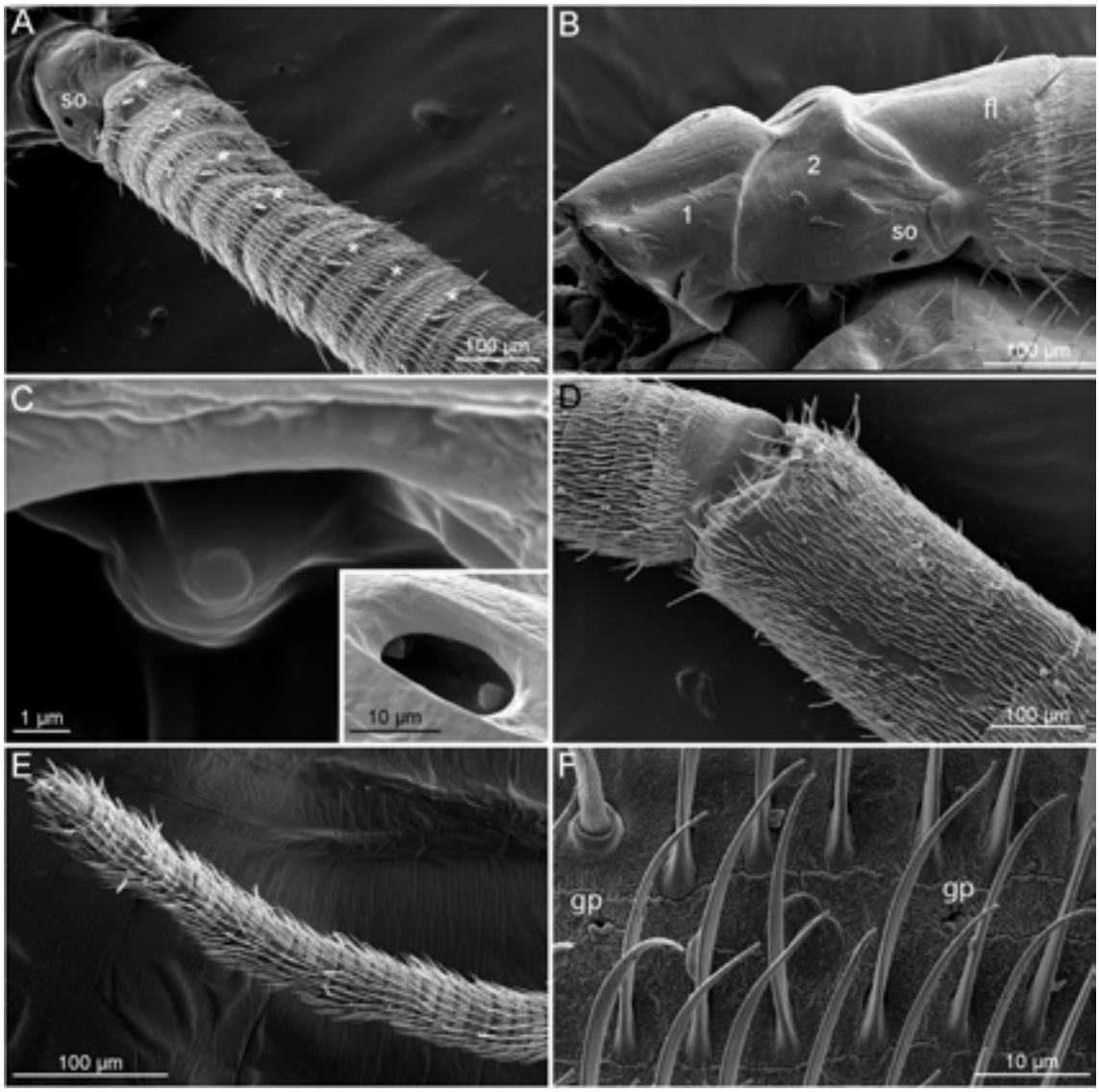

Figure 1. A Dorsal view of the right antenna with shaft articles and shaft organ (so), and the proximal articles of the flagellum. Asterisks mark a row of trichoid sensilla. **B** Dorsolateral view of the two shaft articles (left antenna, 1 and 2) with the shaft organ (so) and the proximal part of the flagellum (fl). **C** View inside the shaft organ with one of the cones covered with a fine liquid film. Inset: The shaft organ with two observable cones. **D** The first node of the right antenna from ventrolateral. Lower node part = 59th antennal article. **E** Distal articles of the right antenna. **F** Rows of trichomes on the 22nd antennal article. In the upper left, the base of a single beak-like sensillum is visible. Glandular pores (gp) are visible on the antennal articles.

Beak-like sensilla

The most frequent sensilla in *S. coleoptrata* are longish sensilla, whose apex reminds of the beak of a bird. Therefore, we term these characteristic sensilla “beak-like sensilla”. On single antennae of two specimens, 1,192 sensilla of this type were counted at the dorsal side and 1,033 sensilla at the ventral side, which results to a total number of more than 2,200 of beak-like sensilla per antenna. The length of the beak-like sensilla varies between 16.5 and 51 µm and decreases from the basal to distal articles of the flagellum. However, the shape is constistent along the antenna. The basal diameter of the sensilla ranges between 1.78 and 4.85 µm, the diameter at the apex ranges between 0.7 and 1.3 µm. One to five beak-like sensilla are present per article of the flagellum (Fig. 2 D). They are absent on the shaft (Fig. 1

D). In the distal part of the flagellum beak-like sensilla are rare (Fig 1 E). The longish terminal article bears a circle of seven beak-like sensilla around at its tip (Fig. 2 F). At the nodes, the number of beak-like sensilla ranges between 4 and 20 (see table 2); the first node bears more sensilla than the two remaining. They are arranged in a transversal row near the upper edge of each node (two to five sensilla). At the lateral edges of the articles, the sensilla project outwardly (Fig. 3 B). The beak-like sensillum consists of a long and slightly curved shaft that appears triangular in SEM. Its end has a beak-like form with a single terminal pore (Fig. 2 E arrow, 4). The base of the shaft is inserted into a rounded basal pedestal of the antennal cuticle (Fig. 2 E, 4). The surface of the shaft shows an irregular cuticular pattern (Fig. 3 A) and is covered by longitudinal ribs, which end in an acute edge of the triangle (Fig. 2 E).

	Specimen 1 dorsal		**Specimen 2 ventral**		**Specimen 3 dorsal**	
	Left	Right	Left	Right	Left	Right
1st node	20	17	16	10	11	17
2nd node	11	6	6	8	7	6
3rd node	-	6	-	-	4	-

Table 2. Number of beak-like sensilla per antenna at the antennal nodes from three different specimens. Left and right antennae were investigated. Specimen 1 and 3 were examined on the dorsal side, specimen 2 on the ventral side.

Sensilla brachyconica

Sensilla brachyconica are only present at the end of the terminal article. Here, three sensilla are situated in a terminal depression, possibly a shrinking artefact, and are surrounded by a circle of beak-like sensilla (Fig. 2 F, 3 C). Each sensillum is composed of a sensory cone (approximately 5.3 to 5.8 µm in length, base diameter: 2.32 – 2.67 µm) that is tapered to its end (diameter: 0.47 – 0.6 µm). A terminal pore is always present (Fig. 3 C, D, 4). The surface of the cone appears velvety. At its base, the sensillum is inserted into the antennal cuticle without any specialization. It is likely that the cone is not movable.

Sensory cones

Several sensory cones are observable, which are located in a small cuticular fold at the upper edge of a proximal nodal article and near the following one (Fig. 3 E, F). Their number ranges from five to nine on the dorsal and ten to eleven on the ventral side. Sometimes, the third node is free of sensory cones. However, occasionally, up to four sensilla are present on the dorsal side.

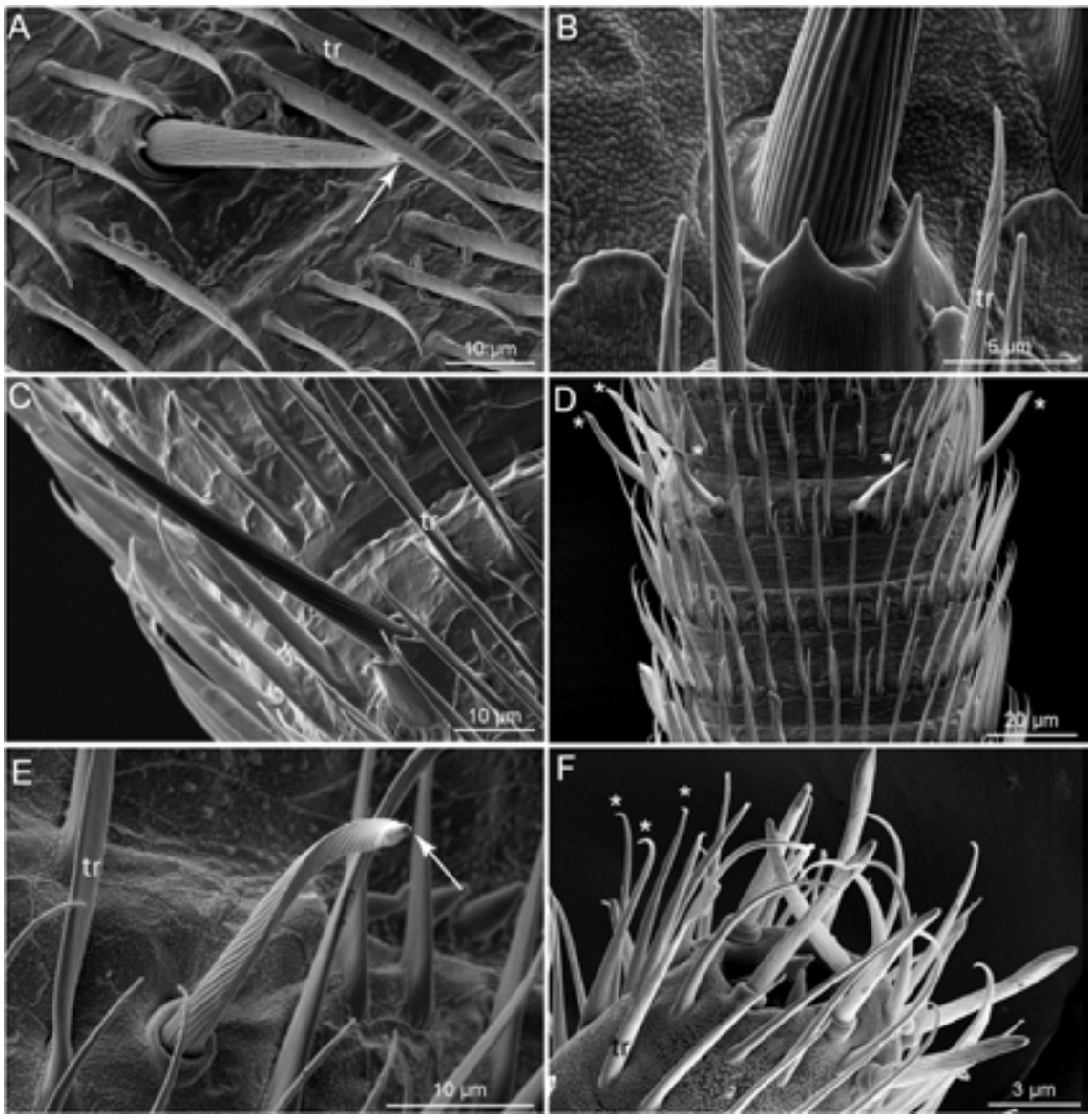

Figure 2. A Sensillum mesotrichodeum on the 11th antennal article surrounded by trichomes (tr). The arrow marks a terminal pore. **B** The base of a sensillum trichodeum with bidentate scale. **C** Sensillum trichodeum on the dorsal side of the 18th antennal article. **D** Transversal row of five beak-like sensilla (asterisks) on the 302nd antennal article. **E** Trichomes (tr) and a beak-like sensillum on the 49th antennal article. The arrow marks a terminal pore. **F** Trichomes (tr) and a circle of beak-like sensilla with sensilla brachyconica on the terminal antennal article. Asterisks mark several hooked trichomes.

According to their length, two types of sensory cones are distinguishable: a larger (4.99 to 6.11 µm, basal diameter 2.93 to 3.4 µm) and a smaller type (1.41 to 2.4 µm, basal diameter 1.02 to 1.19 µm), situated in close proximity to one another (Fig. 3 F, 4). The diameter of the tip ranges between 0.68 and 1.00 µm in both types. Each sensillum possesses a shaft that tapers to a rounded apex. It is unclear if a terminal pore exists; sometimes, a flat depression is observable which might display a terminal pore (Fig. 3 F-H, 4). The distal part of the shaft of larger cones appears smooth whereas the basal part is structured by faint longitudinal stripes or bands (Fig. 3 G) resembling the nano-sculpture of the surrounding cuticle. The shaft of the smaller cones is completely structured by protuberances, similar to the surrounding cuticle. The base of the shaft is inserted into the antennal cuticle without articulation (Fig. 3 F, H).

Shaft organ

The shaft organ on the distal shaft article consists of a small circular opening of about 10 to 15 µm in diameter (Fig. 1 A-C). The opening leads to a more or less deep cuticular invagination. From the bottom, several spine-like sensillar cones arise which are covered by a fine liquid film (Fig. 1 C). Due to the small opening, it is difficult to explore the entire cavity of the shaft organ by SEM. One can see sensillar cones inserted tightly to the cuticular bottom of the organ. The short shaft seems to be unstructured; the rounded tip does not seem to possess a terminal pore (Fig. 1 C inset).

Discussion

Antenna

The most obvious feature of the antennae of *Scutigera coleoptrata* is their enormous length (Fig. 5). The flagellum may consist of more than 500 antennal articles, which is much more than in Lithobiomorpha (17 to more than 100 articles), Craterostigmomorpha (18 articles), Scolopendromorpha (17 to 27 articles), and Geophilomorpha (14 articles) (reviewed in Lewis 1981, Rosenberg 2009). In *S. coleoptrata*, the flagellum is subdivided into four parts by three different nodes (Verhoeff 1902-1925: flagellum primum, f. secundum, f. tertium, f. quartum). Each nodus is divided into two different articles: a larger (Verhoeff 1902-1925: Nodale) and a smaller one (Verhoeff 1902-1925: Postnodale). An articulated joint allows movements of the different antennal sections in a horizontal plane (Fuhrmann 1922). The movement is accomplished by muscle fibres inserted at the smaller nodal articles (Verhoeff 1902-1925, Fuhrmann 1922). The position of the nodes is not fixed; even in the same specimen, the arrangement and position is not always the same between right and left antenna (see table 1). Similar observations are known from *Pilbarascutigera incola* (Edgecombe and Barrow 2007). The presence of extremely long antennae with articulated nodes and the bipartite shaft (Verhoeff 1902-1925, Fuhrmann 1922: Schaftglied; Lewis 1981: shaft, Bonato et al. 2010: scape) in Scutigeromorpha are unique characters within the Chilopoda. Fuhrmann (1922) assumed that the bipartite shaft results from a fusion of two articles. It allows the articulation of the antenna with the head capsule and, by muscle insertion, movement of the flagellum. Parts of the shaft and the flagellum are covered by rows of trichomes (Verhoeff 1902-1925: Häutungshaare). Verhoeff (1902-1925) supposed these trichomes may prevent the antennae from getting stuck during moulting.

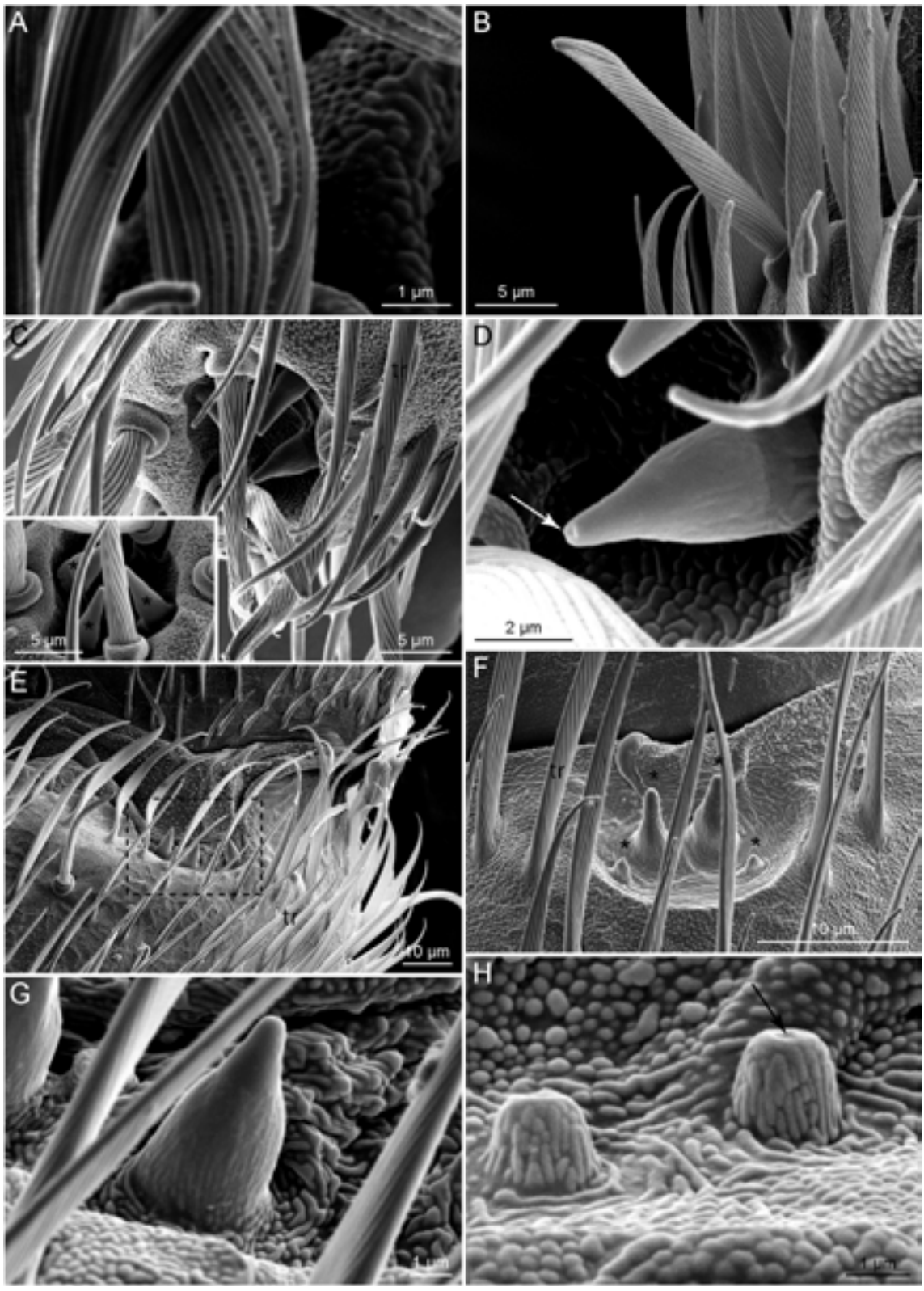

Figure 3. A The ribbed structured surface of a beak-like sensillum with small grains between the ribs. **B** Beak-like sensillum on the lateral edge of the 259th antennal article. **C** View onto the cuticular depression of the terminal antennal article with three sensilla brachyconica and several trichomes (tr). Inset: different view into the cuticular depression. Asterisk mark the three sensilla brachyconica. **D** A sensillum brachyconicum with arrow pointing to a terminal pore. **E** The second node (173th antennal article) with a group of sensory cones (in dashed rectangle). **F** A group of two large and two small sensory cones (asterisks) at the third node (301st article). **G** Large sensory cone with ribbed base and smooth tip at the second node. **H** Two ribbed small sensory cones with a flat depression at the tip which may be a terminal pore (arrow).

Antennal sensilla and sense organs

Verhoeff (1902-1925) described in *S. coleoptrata* the occurrence of two different hairy sensilla (“Tastborsten”), situated between the trichomes. In a parallel investigation Fuhrmann (1922) stated that the antennae are poor in sensory organs except for the shaft organ. Contrary, our SEM-investigation reveals the presence of five different types of antennal sensilla: s. trichodea, beak-like sensilla, s. brachyconica, sensory cones, and those assembled in the shaft organ. In addition, Minelli (1993) figured a sensory plate of unknown function from a distal antennomere of *Scutigera coleoptrata* (Minelli 1993: Fig. 22 D). We interpret this plate as two pore openings of epidermal glands covered by a mass of secretion.

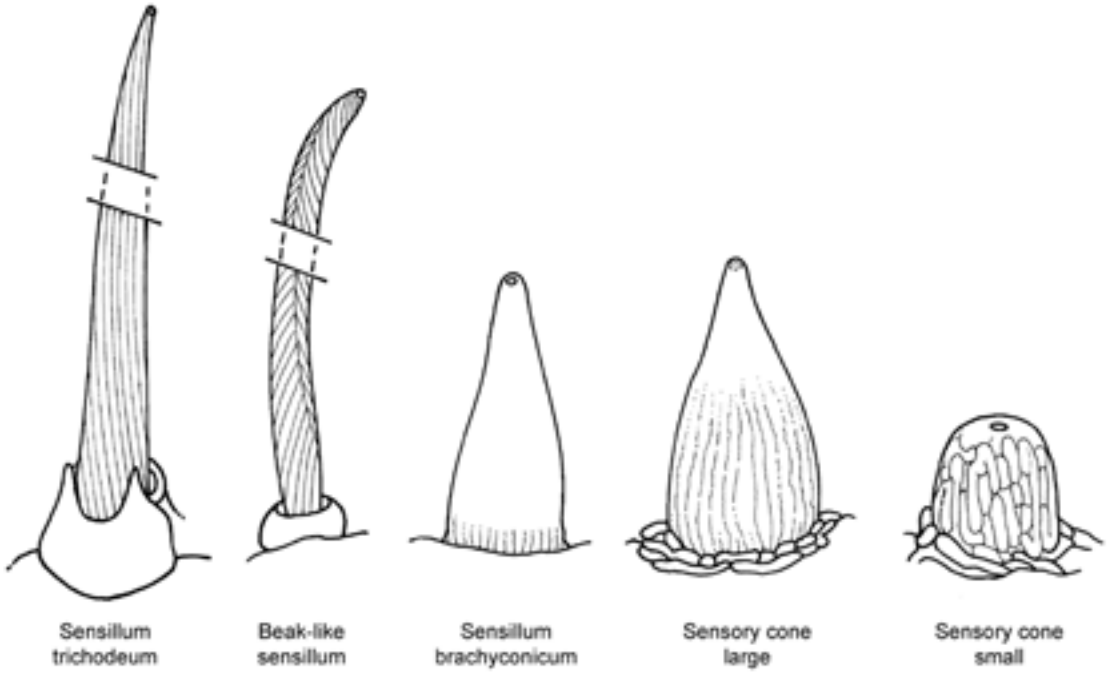

Figure 4. Schematic representation of the different sensillar types in *Scutigera coleoptrata*. Originals: Jochen Jacobi.

Sensilla trichodea

Sazepin (1884) first described “hairs” (“dornähnliche antennale Haare”) on the antennae of *S. coleoptrata*. Verhoeff (1902-1925) reported strong tapering touch-sensitive trichomes (“Tastborsten mit gerader Spitze”) and Fuhrmann (1922) figured these as hollow trichomes (“Borsten”), but denied a sensory character. All these structures correspond to the sensilla trichodea and are similar to trichoid sensilla of all hitherto investigated representatives of centipedes (see table 3) (Ernst 1976; Keil 1975, 1976; Ernst et al. 2006, 2009). The surface of the hair shaft is always striated and a terminal pore is present. The mobility of the shaft is restricted in a basal direction by a bidentate scale. There are great differences in total number of antennal trichoid sensilla in centipedes. In *S. coleoptrata*, a number of 95-105 s. trichodea was counted per antenna. The number differs significantly from those of *Geophilus flavus* (620 to 660 per antenna) (Ernst 1976), *Craterostigmus tasmanianus* (about 1.100) (Ernst et al. 2006), *Lithobius forficatus* (about 2.000) (Keil 1975, 1976) and *Cryptops*

hortensis (up to 4,000) (Ernst et al. 2009). The length varies from 27 to 73 µm in *S. coleoptrata* which is in the range to those of *C. hortensis* (17 to 87 µm), whereas the length in *G. flavus* (up to 120 µm), *L. forficatus* (up to 150 µm), and *C. tasmanianus* (up to 390 µm) is considerably greater. In all cases, the antennal s. trichodea show a terminal pore opening. Similar trichoid sensilla with a bidentate scale were described from the tergal plates of *Scutigerina weberi* and *Scutigera coleoptrata* (Edgecombe and Giribet 2006: Fig. 2C-E).

TEM investigations of the internal fine structure of antennal s. trichodea were hitherto done only in *Geophilus flavus* (Ernst 1976, 1996, 2000) and in *L. forficatus* (Keil 1975, 1976). Here, they bear two types of sensory cells: uniciliate cells with long dendritic outer segments and uniciliate (*L. forficatus*) or biciliate (*G. flavus*) sensory cells with short dendritic outer segments combined with tubular bodies. The first functions as chemoreceptive, the second as mechanoreceptive element. As in all other centipedes, the presence of a terminal pore and the mobility of the hair base suggests that these sensilla should be interpreted as contact chemoreceptors. A similar integration of mechanoreceptive and chemoreceptive function is known for the four apical cones at the top of the last antennal article in some species of Diplopoda (Nguyen Duy-Jacquemin 1981, 1985, 1996, 2004; Schönrock 1981) and from the gustatory sensilla of hexapods (Adams et al. 1965, Hansen & Heumann 1971).

Beak-like sensilla

The most frequent type of sensilla in *S. coleoptrata* is the beak-like sensillum with about 2,200 sensilla per antenna. On the base of light microscopy, these sensilla were first recognized by Verhoeff (1902-1925) as touch sensitive trichomes (“Tastborsten mit gebogener Spitze”). They are concentrated on the articles along the flagellum, whereas the shaft is free of them. A remarkable feature is a circle of about seven beak-like sensilla at the tip of the terminal antennal article. Similar sensilla have been found in other scutigeromorph species: in *Pilbarascutigera incola* on the antennae (Edgecombe and Barrow 2007: Figs. 19, 20); in *Thereuopoda clunifera* on the antennae (Edgecombe and Barrow 2007: Fig. 64) and presumably on tarsal segments in *Scutigerina malagassa* (Edgecombe and Giribet 2006: Fig. 5B). In addition, Edgecombe and Giribet (2006) showed possibly similar sensilla on tarsal segments in *Scutigera coleoptrata* (Edgecombe and Giribet 2006: Fig. 5E). The presence of this sensillar type exclusively in scutigeromorph species leads to the assumption, that beak-like sensilla could be a unique feature of this group. One can argue that the beak-like sensillum is either a peculiar type of sensilla in centipedes or a variation of a sensillum trichodeum. In *Scutigera coleoptrata*, antennal beak-like sensilla are slightly smaller than the s. trichodea. There are some similarities in the structure: the surface of the shaft is striated, a terminal pore is always present, and the basal insertion possibly allows a movement of the sensilla although the morphology of the basal pedestal is different. An obvious difference is the slightly curved tip of the shaft in contrast to the straight shaft in the s. trichodeum. The function of both types of sensilla seems to be similar: the terminal pore points to chemoreception and the mobility of the hair base suggests a mechanoreceptive function.

Sensilla brachyconica

S. brachyconica are cone-shaped antennal sensilla of variable length. In Chilopoda, they are concentrated mainly on the tip of the terminal article as well as on distal parts of antennal

articles. In *Scutigera coleoptrata*, the three cone-like s. brachyconica are found at the tip of the terminal antennal article. Corresponding sensilla have also been found in representatives of other chilopod subtaxa, but there are remarkable differences in the external shape. There are seven cone-shaped s. brachyconica in *Geophilus flavus* (Ernst 1981, 1996, 2000), eight smooth sensory cones in *Lithobius forficatus* (Keil 1975, 1976: “Sinneskegel”), and eight s. brachyconica in *Cryptops hortensis* (Ernst et al. 2009). In *Craterostigmus tasmanianus*, s. brachyconica seem to be absent. Here, only one single terminal antennal s. basiconicum is developed, which is centrally located and encircled by 6-13 tube-like sensilla (Ernst et al. 2006). The smooth s. brachyconicum in *S. coleoptrata* is the smallest type of terminal antennal sensilla. A terminal pore exists, therefore a chemoreceptive function is assumed. The exposed position of these sensilla could indicate a hygroreceptive function. Contrary to this, s. brachyconica in *G. flavus* and *L. forficatus* do not bear a terminal pore. Nevertheless, they are thought to function as hygroreceptors like in hexapods (compare Altner et al. 1983, Altner & Loftus 1985). Fine structural investigation for s. brachyconica only exists for *G. flavus* with one uniciliate and two biciliate sensory cells (Ernst 1981) and *L. forficatus* with four to six uniciliate sensory cells (Keil 1975).

Sensory cones

The sensory cones at the upper edge of the nodes are similar to the s. brachyconica on the terminal antennal articles of *S. coleoptrata*. Both have approximately the same length, but the diameter is larger in the sensory cones. Differences also exist in the shaft surface: it appears velvety with a ribbed base in s. brachyconica compared to a smooth shaft at the tip in the sensory cones (Fig. 4). A terminal pore is present in s. brachyconica, but uncertain in sensory cones. It is difficult to suggest a function of the sensory cones. Their localization in a cuticular fold on all antennal nodes indicates a proprioreceptive function, similar to the s. microtrichodea at the base of antennal articles in *L. forficatus* and *G. flavus*. Fine structural investigations reveal two (*L. forficatus*) or four (*G. flavus*) mechanoreceptive sensory cells (Keil 1975, 1976; Ernst 1983, 1996, 1997) supporting the presumed proprioreceptive function for these sensilla. In some hexapods, the small hair sensilla in the bristle fields with one uniciliate mechanoreceptive sensory cell are able to detect changes in the position of neighboring parts of the body (Thurm 1964, Markl 1965).

Shaft organ

Verhoeff (1904) was the first to describe a simple sensory organ of unknown significance ("ein einfaches Sinnesorgan von unbekannter Bedeutung") on the distal article of the shaft ("Schaftorgan"; Lewis 1981: shaft organ; Bonato et al. 2010: shaft organ). Subsequently, histological investigations were carried out by Fuhrmann (1922). He found several cone-like sensilla on the bottom of the cavity and suggested an olfactory function. The small opening of the shaft organ makes it difficult to dissolve the structure of the sensilla at the bottom by SEM technique. The sensilla possibly resemble the s. brachyconica or the sensory cones. The cuticle of their shafts seems to be unstructured and a terminal pore is not recognizable. A shaft organ is also present in *Pilbarascutigera incola* (Edgecombe and Barrow 2007: Fig. 4) and it is likely that all representatives of the Scutigeromorpha possess one. At present, it is not possible to provide any functional information of the sensilla of the shaft organ, as we have no information about their cellular architecture. Due to their position, the sensilla inside the shaft organ are likely neither contact chemoreceptors nor mechanoreceptors. It is possible that the thin liquid film overlaying the sensory cones allows volatile molecules to bind to the surface of these sensilla.

Chilopod antennal sensilla

With the evidence provided in this paper for the Scutigeromorpha, SEM data are available at last on the antennal sensilla for all five chilopod higher taxa (see table 3). Overall, ten types of scattered and collared sensilla as well as sensory cones and the shaft organ have been described (reviewed in Müller et al. 2011). With only three types of sensilla as well as sensory cones and the shaft organ, the Scutigeromorpha bear the lowest diversity of antennal sensillar types in the Chilopoda. All investigated chilopod species possess sensilla trichodea. Sensilla microtrichodea (proprioreception) are present in the Pleurostigmophora (Lithobiomorpha, Craterostigmomorpha, Scolopendromorpha, and Geophilomorpha) (Ernst 1983, Ernst et al. 2006, 2009, Keil 1975) but lacking in the Scutigeromorpha. Antennal s. coeloconica with a proprioreceptive function are only present in the Craterostigmomorpha and Geophilomorpha (Ernst 2000, Ernst et al. 2009). The presence of sensilla basiconica was proven for *Craterostigmus tasmanianus*, *Cryptops hortensis*, and *Geophilus flavus* (Ernst 1979, Ernst et al. 2006, 2009). In *Lithobius forficatus*, Keil (1975) was unsure if the long cones ("Sinneskegel") are basiconic sensilla because pores in the shaft were not detected definitely. However, Tichy and Barth (1992) classified them as olfactory sensilla. For the function of the short pegs ("Sinneszapfen"), Keil (1975) assumed hygroreception. At the moment it is not clear if these sensilla are comparable to basiconic or brachyconic sensilla. Either way, on the antennae of Scutigeromorpha basiconic sensilla seem to be reduced. Sensilla brachyconica (thermo- and/or hygroreception) are present in all investigated species, except for *C. tasmanianus*. Club-shaped and hat-like sensilla are only present in *Cryptops hortensis*. Here, a chemosensory function is assumed (Ernst et al. 2009). Collared sensilla are only present in *Craterostigmus tasmanianus* (tube-like, and bottle-like sensilla) (Ernst et al. 2006: Fig 11, 19) and in the genus *Scolopocryptops* (Edgecombe and Giribet 2004: Fig. 8 h).

Alongside the presence and absence of antennal sensillar types, three unique characters are found in the Scutigeromorpha: (1) the presence of long antennae with nodes bearing sensory

cones, (2) the presence of a bipartite shaft including the shaft organ, and (3) the presence of beak-like sensilla.

	Scutigera coleoptrata	*Lithobius forficatus*	*Craterostigmus tasmanianus*	*Cryptops hortensis*	*Geophilus flavus*
	present study	Keil 1975,1976	Ernst et al. 2006	Ernst et al. 2009	Ernst 1976, 1979, 1981, 1983
S. trichodeum	95-105	2,000	~ 1,100	~ 4,000	620-660
S. microtrichodeum	-	240	few	~ 100	80-114
Beak-like sensillum	~ 2,200	-	-	-	-
S. coeloconicum	-	-	5-8	-	few
S. brachyconicum	3	80	-	20-26	7
S. basiconicum	-	80	1	61-114	36-53
Bottle-like sensillum	-	-	4-16	-	-
Club-shaped sensillum	-	-	-	2-4	-
Hat-like sensillum	-	-	-	2-6	-
Tube-like sensillum	-	-	6-13	-	-
Sensory cones	+	-	-	-	-
Shaft organ	+	-	-	-	-

Table 3. Number of antennal sensilla and presence of sensory cones and shaft organ in representatives of the five chilopod subtaxa. Numbers per antenna. Plus symbol: present; minus symbol: not present.

Mechano- and chemoreception

Klingel (1960) proposed that *S. coleoptrata* detects its prey by contact-chemoreception exclusively. There are only few examples suggesting a sense of olfaction in chilopods, e.g. that of Meske (1961) in *Lithobius forficatus*. However, in contrast to Klingel (1960), recent behavioral experiments with *S. coleoptrata* revealed that this species is also able to perceive airborne stimuli from living prey as well as from chemical extracts (Sombke et al. 2011).

The present investigation of the external structure and distribution of sensilla by SEM-techniques clarifies that most of the antennal sensilla of *S. coleoptrata* may function both as mechanoreceptors and contact chemoreceptors and are therefore involved in detection of

prey. In *Geophilus flavus* (Ernst 1979, 2000), *Lithobius forficatus* (Keil 1975), *Craterostigmus tasmanianus* (Ernst et al. 2006), and *Cryptops hortensis* (Ernst et al. 2009), sensilla basiconica exist as multiporous sensilla. In *G. flavus*, these sensilla are suggested to function as olfactory sensilla (Ernst 1979). Nevertheless, in *Scutigera coleoptrata* one or several of the detected types of antennal sensilla with a terminal pore must be responsible for an olfactory perception as shown by behavioral essays (Sombke et al. 2011), but this issue needs to be clarified by electrophysiological and ultrastructural studies. The sensilla on the antennal tip are most likely contact chemoreceptors and used for probing the surrounding environment. This interpretation is also substantiated by the relatively high diversity of sensilla types. Interestingly, the beak-like sensilla do not make complete contact to the soil because (1) they are present in transversal and longitudinal rows on all sides of the antenna (Fig. 2 D) and (2) the antenna is rarely in complete contact with the soil. In fact, the antennae are mainly kept in a parallel position to the soil while the first node bends the distal part of the flagellum into that direction (Fig. 5) and only the distal-most part of the antenna gets actually in contact to the soil (Fig. 5 inset). Therefore, an olfactory function might be possible for the beak-like sensillum. Most likely, both the sensilla trichodea and the beak-like sensilla are capable of mechanoreception. In addition, sensilla on the mouth parts and on the walking appendages play an additional important role in the detection of mechanosensory stimuli (Rosenberg 2009).

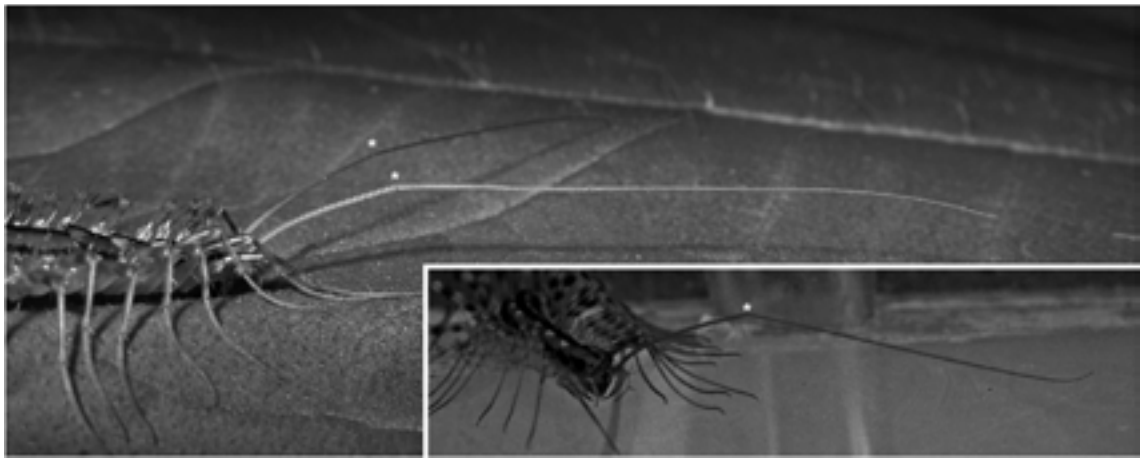

Figure 5. Photographs of the anterior body and antennae of *Scutigera coleoptrata.* Typical position of the antennae with the proximal part directed slightly upward. Next to the first node (asterisks), the antennae are in more or less parallel position to the ground. Only the distal part is often bent to the bottom (inset).

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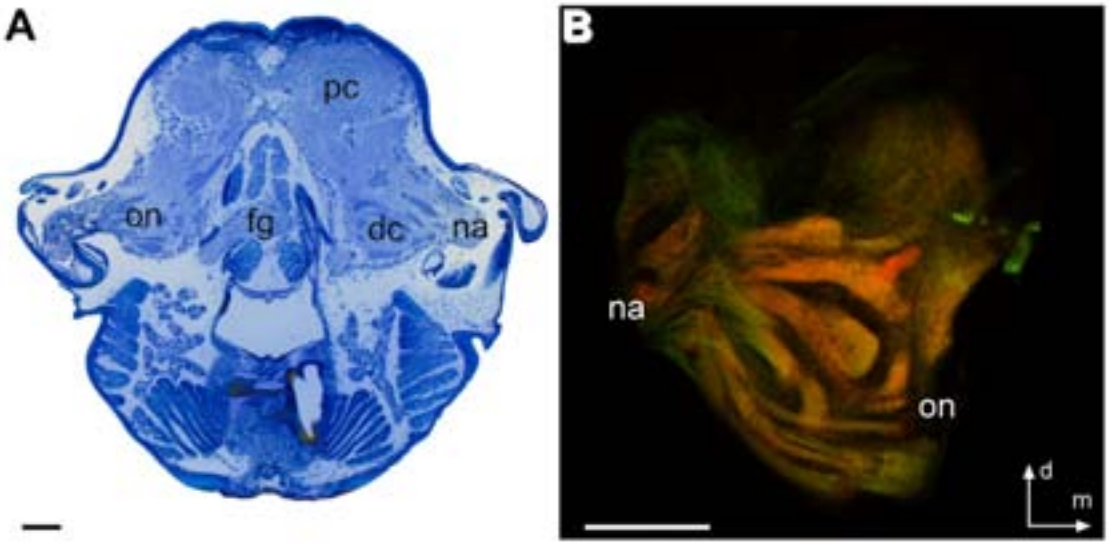
IV

Anatomy of the deutocerebum of *Scutigera coleoptrata*

Sombke A, Harzsch S, Hansson BS. 2009. Brain structure of *Scutigera coleoptrata*: New insights into the evolution of mandibulate olfactory centres – short communication. Soil Organisms 81 (3): 319–325.



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Brain structure of *Scutigera coleoptrata*: New insights into the evolution of mandibulate olfactory centres – short communication –

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Abstract

Myriapods represent an arthropod lineage, originating from a marine arthropod ancestor that most likely conquered land independently from hexapods. The successful transition from marine to terrestrial life requires a number of physiological adaptations important for survival out of water. The sensory organs of terrestrial species must be able to function in air rather than in water. In chemoreception, establishing aerial olfaction requires molecules to be detected in gas phase instead of in water solution. In general, the neuroethology of myriapods and the architecture of their central nervous systems are poorly understood. In a set of preliminary experiments with the centipede *Scutigera coleoptrata*, we analysed the central olfactory pathway with serial semi-thin sectioning combined with 3D reconstruction, antennal backfilling with neuronal tracers, and immunofluorescence combined with confocal laser-scanning microscopy. These experiments indicate that *S. coleoptrata* possess the neuronal substrate for a good sense of aerial olfaction. However, the architecture of its olfactory system is clearly distinct from hexapods and also from terrestrial crustaceans, indicating independent evolution of its olfactory sense.

Keywords: Chilopoda, nervous system, olfaction

1. Introduction

As descendants from marine ancestors, Hexapoda and Myriapoda represent two clades that, considering the latest molecular analyses as well as neurophylogenetic studies (e.g. Harzsch 2006, 2007), conquered land independently from each other. The successful transition from marine to terrestrial life requires a number of physiological adaptations e.g. gas exchange, salt and water balance, nitrogenous excretion, thermoregulation, moulting, and reproduction (Powers & Bliss 1983, Burggren & McMahon 1988). Concerning the nervous system, the sensory organs of terrestrial species must be able to function in air rather than in water. For olfaction, a transition from sea to land requires molecules to be detected in gas phase instead of in water solution. The odour stimulus also changes from mainly hydrophilic molecules in aqueous solution to mainly hydrophobic in the gaseous phase. In addition, the olfactory system is, like the rest of the organism, very prone to desiccation and

mechanical abrasion in the terrestrial environment. All of these new selection pressures may participate in reshaping the sense of smell (see e. g. Harzsch & Hansson 2008 for an example on terrestrial Crustacea). Furthermore, the structure of the central nervous system may also mirror functional adaptations of the olfactory system to the terrestrial life style. Studying the olfactory system in Myriapoda and comparing it to that of Hexapoda may therefore provide insights into how the arthropod nervous system evolved in response to new environmental and ecological challenges.

A wide variety of structures in the arthropod brain such as the central complex and the optic neuropils as well as eye development have been analysed against a phylogenetic background (Harzsch 2002, Loesel et al. 2002, Sinakevitch et al. 2003, Strausfeld 2005, Harzsch et al. 2007). However, the comparative anatomy of the olfactory pathway has received less attention, and conflicting hypotheses on the evolution of structures involved have been brought forward (Strausfeld & Hildebrand 1999 versus Schachtner et al. 2005). We have chosen the centipede *Scutigera coleoptrata* (Linnaeus 1758) as a model for terrestrial olfactory pathways. *S. coleoptrata* is a raptorial animal that preys on live arthropods and is among the fastest-moving terrestrial arthropods (Rosenberg 2009). According to phylogenetic analyses, the Scutigeromorpha are the most basal myriapod taxon (Edgecombe & Giribet 2007) and therefore, their sensory systems may have retained aspects that are plesiomorphic for the Myriapoda. In the present preliminary report, we provide a first overview over morphological studies on the central olfactory pathway of this animal.

2. Materials & methods

Specimens were collected on the Balearic island Ibiza and kept in plastic boxes. For section series, several individuals were decapitated and prefixed for 24h in a solution of 80 % ethanol, 37 % formaldehyde and 100 % acetic acid. After washing in sodium hydrogen phosphate buffer (PBS, pH 7.4) specimens were postfixed for 1h in 2 % OsO_4 solution (same buffer) at room temperature and, following dehydration in a graded series of acetone, embedded in Araldite. Serial semi-thin sections (1.5 µm) were prepared with a Microm HM 355 S and stained using 1 % toluidine blue and Pyronin G in a solution of 1 % sodium tetraborate. Overall, section series of 5 specimens were investigated. The alignment and 3-dimensional reconstruction was made using AMIRA. For immunohistochemistry, specimens were fixed for 4h at room temperature in 4 % parafomaldehyde in sodium hydrogen phosphate buffer (PBS, pH 7.4). The brains were dissected, washed 4 x 30 min in PBS, embedded in agarose, and subsequently sectioned (80 µm) with a vibratome (Microm HM 650 V). Permeabilisation of the brains in PBS-TX (PBS, 0.3 % Triton X-100) for 1 h was followed by incubation in primary antibodies against synaptic proteins (Synapsins, Klagges et al. 1996), the neuropeptide FMRF-amide (compare Harzsch and Hansson 2008), Allatostatin (generously provided by Prof. Dr H. Agricola, Vitzthum et al. 1996), and Tyrosine-Tubulin (Sigma), as well as in phalloidin (a probe against actin), and the HOECHST stain (nuclear marker). Brains were incubated over night at 4 °C, washed 4 x 30 min in PBS and incubated for 1 h in a cocktail of secondary antibodies conjugated with Alexa 488 or Alexa 564 (compare Harzsch and Hansson 2008 for a detailed description of the methods). After incubation, the sections were washed 4 x 30 min in PBS and mounted. For antenna-backfills, the left antenna was cut leaving app. 3 mm. Then, a capillary filled with Dextranbiotin (Molecular Probes) dissolved in aqua dest. was placed over the cut tip of the antenna. After incubation for 4 hours, the

specimen were decapitated and fixed in 4 % paraformaldehyde. Following dissection, brains were washed 4 x 30 min in PBS and subsequently sectioned (80 µm) with a vibratome. Specimens were incubated in streptavidin conjugated to Alexa 488 (1:2500 in PBS) over night at 4 °C. After washing in PBS sections were subsequently mounted in Mowiol.

3. Results and discussion

Studies on the anatomy and microarchitecture of the nervous system of *Scutigera coleoptrata* have been conducted by Saint-Remy (1889) and Hörberg (1931). Fahlander (1938) described the central nervous system of *Thereuopoda clunifera* (Wood, 1862) (Scutigeromorpha). The deutocerebrum of Scutigeromorpha is filled with two glomerular masses. The structure of the anterior mass was described by Saint-Remy (1889) and Hörberg (1931) as irregular, 'sausage-like', or as convoluted ribbons. In *T. clunifera*, Fahlander (1938) specified the deutocerebral lobe neuropils as numerous, dense glomerular masses that are circular in diameter. Our neuroanatomical data show that the deutocerebral lobes of *S. coleoptrata* are filled with dense neuropils that take on the shape of elongate cylinders (Figs 1A, B, C). In histological sections, the neuropils are highly contrasted. Because of their elongate shape we suggest they be referred as olfactory neuropils rather than gomeruli or glomerular masses. The olfactory neuropils are bilaterally symmetrically arranged, so that it is possible to match the corresponding neuropils in the left and right deutocerebral lobe (Fig. 2C). In three histological section series, we found 34 individually identifiable neuropils in each lobe (Fig. 2D). All of them had a more or less cylindrical form, while the distal end was thickened or bent posteriorly. At least one neuropil extended a contralateral connection (Figs 1D clc, 2C) as described by Fahlander (1938) as anterior antennal commissure. In contrast to crustacean olfactory neuropils (Harzsch & Hansson 2008), in all preparations, both core and periphery of all observed neuropils were uniformly stained. No evidence for subcompartments was found.

Immunolocalisation of synaptic proteins and phalloidin histochemistry confirmed the cylindrical form of the neuropils (Figs 1B, C, E). The immunolocalisation of FMRF-amide in the deutocerebral lobes showed a diffuse distribution pattern and an absence of labelling in the olfactory neuropils (Fig. 1E). Instead, only fibres surrounding the olfactory neuropils were stained. Projecting tracts were not visible. Allatostatin-immunoreactivity was present throughout the deutocerebral lobes. The olfactory neuropils were filled with numerous fine immunolabeled profiles (Fig. 1F).

By backfilling the antennal nerve with the anterograde axonal marker Dextranbiotin, we obtained reliable information concerning the innervation pattern of the neuropils (Fig. 1D). Based on these preparations, we were able to verify that antennal input does in fact target all deutocerebral lobe neuropils. The olfactory neuropils and the contralaterral connection were clearly stained. Filled tracts also project to the dorsal protocerebrum and deep into the suboesophageal ganglion. These experiments suggest that *S. coleoptrata* possesses the neuronal substrate for a good sense of aerial olfaction.

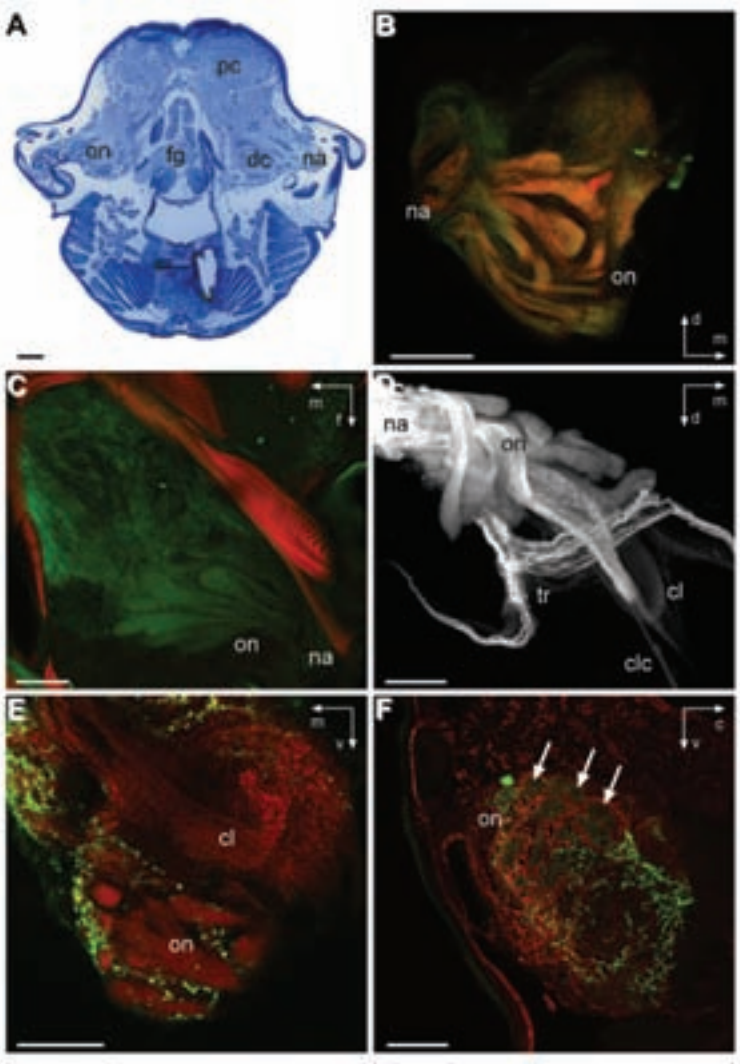

Fig. 1 **A**: Histological cross-section of the head of *Scutigerea coleoptrata*. dc: deutocerebrum; fg: frontal ganglion; na: nervus antennalis; on: olfactory neuropils; pc: protocerebrum; **B**: Immunolocalisation of synaptic proteins (red) and phalloidin labelling (green) in the right antennal lobe; **C**: Immunolocalisation of synaptic proteins (green) and phalloidin labelling (red) in the left antennal lobe (horizontal section); **D**: Dextranbiotin backfill of the left antennal lobe. cl: corpus lamellosum; clc: contralateral connection; tr: tracts; **E**: Immunolocalisation of synaptic proteins (red) and FMRF-amide (green) in the left antennal lobe; **F**: Immunolocalisation of Tubulin (red) and Allatostatin (green) (sagittal section). Arrows mark colocalisation in single olfactory neuropils.

All scale bars 100 µm. Arrow abbreviations: d: dorsal; m: median; f: frontal; c: caudal; v: ventral.

The posterior deutocerebral mass is innervated by the ventrocaudal part of the antennal nerve and is called corpus lamellosum or masse lamelleuse (after Saint-Remy 1889). Hörberg (1931) described it as glomerular structure composed of parallel tracts. Immunolocalisation of synaptic proteins and phalloidin histochemistry as well as antenna backfill experiments revealed a composition of approx. ten lamellae (Figs 1D, E, 2A, B). The lamellae can be divided into two different types: (1) outer lamellae that form a loop (not shown) and (2) inner lamellae that project dorsomedially (Fig. 2B). Fahlander (1938) observed that the corpora are connected over a tract within the posterior antennal comissure. In contrast to observations in Scolopendromorpha (Sombke, unpublished), in *S. coleoptrata* this contralateral connection was not present in any of our preparations.The function of the corpus lamellosum is unknown. We speculate that it may process mechanosensory input from the antennae comparable to the lateral neuropil in Crustacea and the mechanosensory neuropil in Hexapoda.

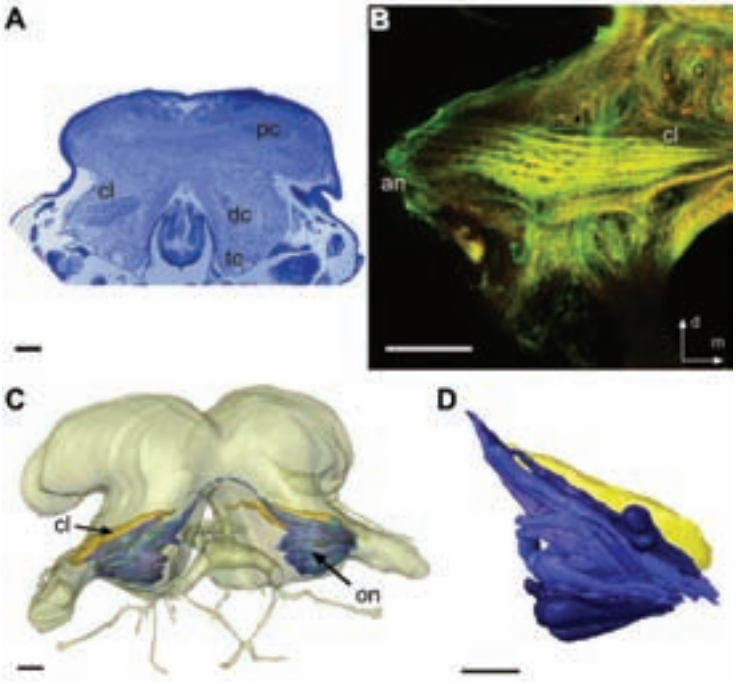

Fig. 2 **A**: Histological cross-section. cl corpus lamellosum, dc deutocerebrum, pc protocerebrum, tc tritocerebrum; **B**: Immunolocalisation of synaptic proteins (red) and phalloidin labelling (green) in the right antennal lobe; **C**: 3D-reconstruction of the brain. cl corpus lamellosum (yellow), on olfactory neuropils (blue); **D**: 3D-reconstruction of the neuropils in the left deutocerebral lobe.

All scale bars 100 µm. Arrow abbreviations: d: dorsal; m: median.

In hexapods and malacostracan crustaceans, the axons of the olfactory receptor neurons target the primary chemosensory neuropil where the receptor axons synapse with local olfactory interneurons and projection neurons (reviewed in Schachtner et al. 2005). The neuropils in the deutocerebral lobes are organised in numerous spherical glomeruli in most insects and in elongate columnar glomeruli in crustaceans. In-depth comparison of species within and across tetraconate taxa, however, demonstrates that many characters of the organisation of tetraconate olfactory centres are shared among distantly related clades, but have been modified in various taxon-specific ways (reviewed in Schachtner et al. 2005). In contrast, Strausfeld & Hildebrand (1999) are doubtful concerning a homology of the primary olfactory neuropils in insects and crustaceans. Our results show that, clearly, the shape of the olfactory neuropils in *S. coleoptrata* is different from both that in hexapods and malacostracan crustaceans. This suggests that upon conquering land, the Myriapoda followed their own distinct pathway in evolving an olfactory system that is suited for aerial olfaction. Nevertheless, in our view the possession of distinct neuropils for chemosensory and mechanosensory procession in *Scutigera coleoptrata*, malacostracan Crustacea and Insecta could indicate a common architectural principle within the Mandibulata.

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Deutocerebral organization and olfactory behavior in *Scutigera coleoptrata*

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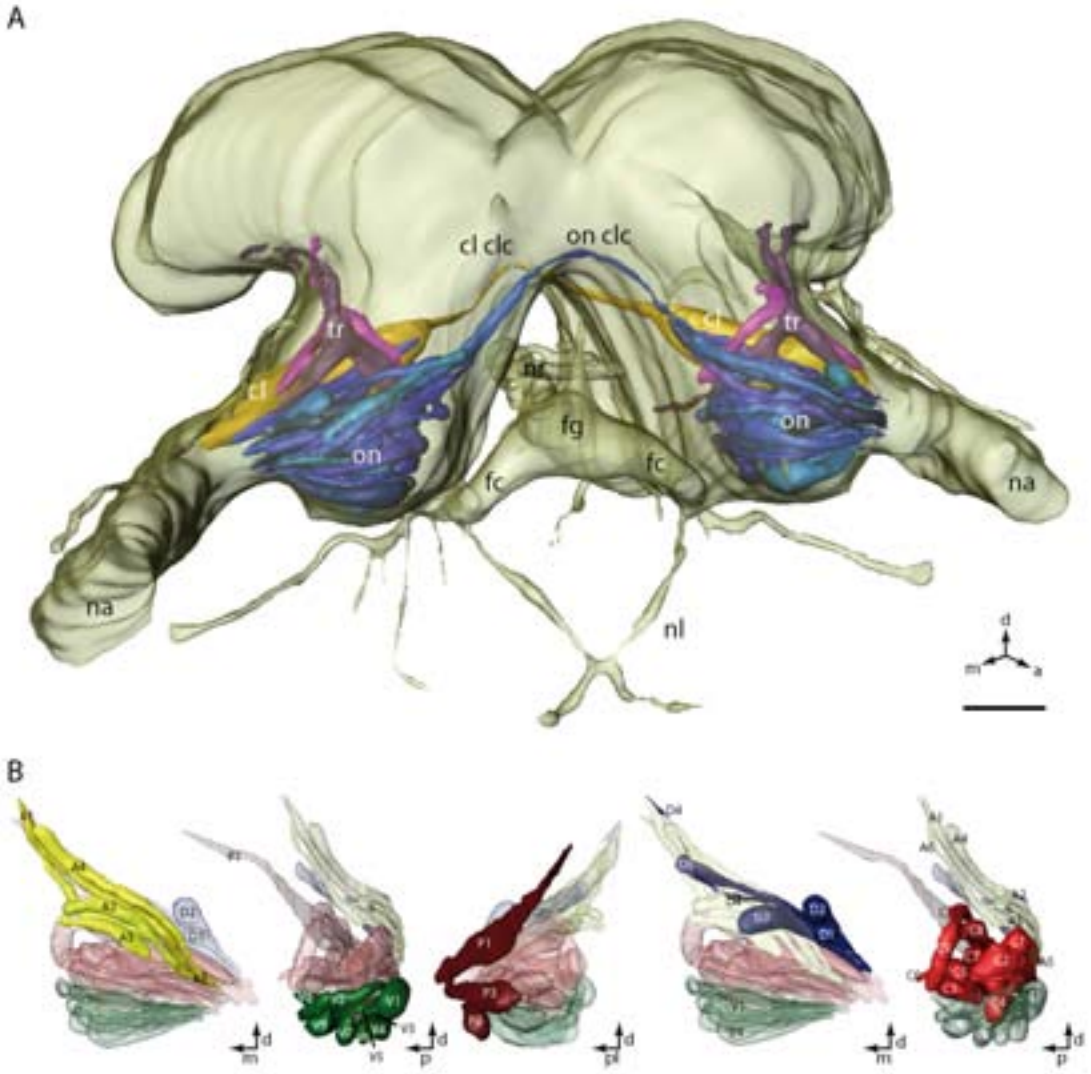

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Organization of Deutocerebral Neuropils and Olfactory Behavior in the Centipede *Scutigera coleoptrata* (Linnaeus, 1758) (Myriapoda: Chilopoda)

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Abstract

Myriapods represent an arthropod lineage, that originating from a marine arthropod ancestor most likely conquered land independently from hexapods and crustaceans. Establishing aerial olfaction during a transition from the ocean to land requires molecules to be detected in gas phase instead of in water solution. Considering that the olfactory sense of myriapods has evolved independently from that in hexapods and crustaceans, the question arises if and how myriapods have solved the tasks of odor detection and odor information processing in air. Comparative studies between arthropod taxa that independently have established a terrestrial life style provide a powerful means of investigating the evolution of chemosensory adaptations in this environment and to understand how the arthropod nervous system evolved in response to new environmental and ecological challenges. In general, the neuroethology of myriapods and the architecture of their central nervous systems are insufficiently understood. In a set of experiments with the centipede *Scutigera coleoptrata*, we analyzed the central olfactory pathway with serial semi-thin sectioning combined with 3-dimensional reconstruction, antennal backfilling with neuronal tracers, and immunofluorescence combined with confocal laser-scanning microscopy. Furthermore, we conducted behavioral experiments to find out if these animals react to airborne stimuli. Our results show that the primary olfactory and mechanosensory centers are well developed in these organisms but that the shape of the olfactory neuropils in *S. coleoptrata* is strikingly different when compared with those of hexapods and malacostracan crustaceans. Nevertheless, the presence of distinct neuropils for chemosensory and mechanosensory qualities in *S. coleoptrata*, malacostracan Crustacea, and Hexapoda could indicate a common architectural principle within the Mandibulata. Furthermore, behavioral experiments indicate that *S. coleoptrata* is able to perceive airborne stimuli, both from live prey and from a chemical extract of the prey. These results are in line with the morphological findings concerning the well-developed olfactory centers in the deutocerebrum of this species.

Key words: behavior, Chilopoda, deutocerebrum, nervous system, neurophylogeny, olfaction

Introduction

In many arthropods, primary olfactory centers are characterized by their subdivision into structural and functional subunits (Schachtner et al. 2005). In Hexapoda, the subunits of the neuropil that is targeted by the axons of olfactory sensory neurons (OSNs), the antennal lobe, are called glomeruli (reviewed in Homberg 1994; Anton and Homberg 1999; Vosshall and Stocker 2007). In malacostracan crustaceans, these subunits in the olfactory lobe are also called glomeruli or sometimes ''olfactory columns'' (Sandeman et al. 1992, 1993; Schmidt and Ache 1996b; Harzsch and Hansson 2008). Within the glomeruli of both Hexapoda and malacostracan Crustacea, first-order integration of olfactory input takes place, which is then relayed to secondary brain centers. Within the primary centers, the afferents of OSNs target dendritic arborizations of local interneurons and projection neurons (Schachtner et al. 2005) representing the first step in the complex mechanisms of odor recognition and discrimination (Hansson and Christensen 1999; Ignell and Hansson 2005). The glomerular array in Hexapoda is thought to represent a chemotopic map, which forms the basis of the olfactory code (Galizia and Menzel 2000, 2001; Ignell and Hansson 2005). Due to the common architecture of primary olfactory

brain centers (antennal lobe in Hexapoda, olfactory lobe in Crustacea) in corresponding sets of olfactory interneurons, the glomeruli may represent homologous structures of Hexapoda and malacostracan Crustacea (Schachtner et al. 2005; Strausfeld 2009). However, differences in their neuronal organization may also suggest independent origins and thus convergent evolution (Strausfeld 2009). Similarities can also be found when comparing vertebrate and arthropod olfactory neuropils (ONs). These conformities in architecture across widely separated phyla could be the result of common selective pressures to perform the same tasks (Strausfeld and Hildebrand 1999; Eisthen 2002). The deutocerebrum also processes mechanosensory input. In Hexapoda, the antennal mechanosensory and motor center (AMMC) integrates information from antennal mechanoreceptors and other areas of the head capsule (Homberg 2005). In Crustacea, the lateral and median antennular neuropils (LAN and MAN) receive mechanosensory and nonolfactory chemosensory input from the antennules (antenna 1; e.g., Schmidt et al. 1992; Schmidt and Ache 1996a).

Many recent phylogenetic studies have suggested a sister group relationship between Crustacea and Hexapoda, the 2 taxa together being called Tetraconata (reviews Dohle 2001; Richter 2002). The position of Myriapoda in the system of arthropods is still in debate (reviewed in Edgecombe and Giribet 2007). Traditionally, Myriapoda, Crustacea, and Hexapoda have been united in a taxon called Mandibulata. However, some molecular studies suggest a sister-group relationship of myriapods and chelicerates (together called ''Paradoxopoda'' after Mallatt et al. 2004 or ''Myriochelata'' after Pisani et al. 2004) including analyses of nuclear ribosomal genes (Mallatt et al. 2004), mitochondrial genes (Popadic et al. 1998; Hwang et al. 2001; Negrisolo et al. 2004), Hox genes (Cook et al. 2001), and 18S and 28S rRNA (von Reumont et al. 2009). The latest studies in this field analyzed the DNA sequence from 62 nuclear protein-coding genes from 75 arthropod species to reliably retrieve the Mandibulata again (Regier et al. 2010). All recent phylogenetic studies have in common that they strongly suggest a convergent conquest of land of Myriapoda and Hexapoda from a marine ancestor of Mandibulata.

The successful transition from marine to terrestrial life requires a number of physiological adaptations, for example, related to gas exchange, salt and water balance, nitrogenous excretion, thermoregulation, moulting, and reproduction (Powers and Bliss 1983; Burggren and McMahon 1988). Living on land also raises new questions regarding the evolution of chemical communication, as a transition from sea to land means that molecules need to be detected in gas phase instead of in water solution. Furthermore, the odor stimulus changes from mainly hydrophilic molecules in aqueous solution to mainly hydrophobic in the gaseous phase. How do arthropods that conquer land solve the tasks of odor detection and odor information processing, and how have the new selection pressures reshaped the sense of smell? Comparative studies between arthropod taxa that independently have established a terrestrial life style provide a powerful means of investigating the evolution of chemosensory adaptations in this environment. Studying the architecture of the olfactory system in Myriapoda and comparing it with that of Hexapoda may therefore provide insights into how the arthropod nervous system evolved in response to new environmental and ecological challenges.

Compared with hexapods and crustaceans, the architecture of the myriapod nervous system is not well understood. For *Scutigera coleoptrata* as a representative of the Chilopoda (''centipedes''), early investigations on the anatomy and microarchitecture of the nervous system include those of Saint-Remy (1887, 1889) and Hörberg (1931). Fahlander (1938) described the central nervous system of *Thereuopoda clunifer* (Scutigeromorpha). These studies showed that the deutocerebrum of Scutigeromorpha contains 2 glomerular masses. The structure of the anterior mass was described by Saint-Remy (1887, 1889) and Hörberg (1931) as irregular, ''sausage-like'', or as convoluted ribbons. In *T. clunifera*, Fahlander (1938) specified the deutocerebral lobe neuropils as numerous, dense glomerular masses that are circular in diameter. A more recent brief description of the deutocerebral neuropils of *Lithobius forficatus* (Lithobiomorpha) was given by Strausfeld et al. (1995). Some other aspect of the myriapod nervous system such as the organization of the central complex (Loesel et al. 2002) and the optic neuropils (Strausfeld 2005) as well as eye development (Harzsch et al. 2007) have received attention more recently and were analyzed against a phylogenetic background. However, little detailed information is available about the primary olfactory centers in Myriapoda. We have chosen the centipede *S. coleoptrata* (Linnaeus, 1758) for our analysis of the myriapod central olfactory pathway. This centipede is a raptorial animal that preys on living arthropods and is among the fastest moving terrestrial arthropods (Rosenberg 2009). According to the recent most phylogenetic analyses, the Scutigeromorpha are the sister group to all remaining chilopod subgroups that are summarized in the taxon Pleurostigmophora (Edgecombe & Giribet 2007; Sheer and Edgecombe 2010) and therefore sensory systems may have retained aspects that are plesiomorphic for the Myriapoda. For instance, in the optic system, it is assumed that the ommatidia of Scutigeromorpha with eucone crystalline cone cells with their nuclei placed outside the core compartments may reflect a state already present in the last common ancestor of the Mandibulata (Harzsch 2006; Müller et al. 2007). Olfaction in myriapods is almost *terra incognita,* and many questions can thus be asked: Is the nervous system suited to process olfactory signals from the antenna like in hexapods or crustaceans? Does *S. coleoptrata* respond to olfactory stimuli in behavioral experiments? In the present study, we have analyzed the architecture of the central olfactory pathway of *S. coleoptrata* and have conducted behavioral tests with prey and chemical stimuli to answer these questions.

Materials and methods

Experimental animals

Specimen of *S. coleoptrata* (Linnaeus, 1758) (Figure 1) were collected on the Balearic island Ibiza mainly in pine forests. If not fixed directly after capture, individuals were kept in plastic tubes (Falcon tubes 50 ml) at room temperature. For keeping, animals were transferred into standard *Drosophila* rearing tubes supplied with bark and water. They were fed with *Drosophila melanogaster* or juveniles of *Acheata domesticus*. Only fully adult specimens were used for experiments. Fully adult specimens have developed all 15 leg pairs and a body length of at least 20 mm (reviewed in Rosenberg 2009).

Histology

For section series, several individuals were decapitated and prefixed for 24 h in a solution of 10 parts 80% ethanol, 4 parts 37% formaldehyde and 1 part 100% acetic acid. After washing in sodium hydrogen phosphate buffer (phosphate buffered saline [PBS], pH 7.4), specimens were postfixed for 1 h in 2% OsO_4 solution (same buffer) at room temperature and, following dehydration in a graded series of acetone, embedded in Araldite (Araldite epoxy resin kit, Agar Scientific). Serial semi-thin sections (1–1.5 μm) were prepared with a Microm HM 355 S and stained using 1% toluidine blue and Pyronin G in a solution of 1% sodium tetraborate. Overall, section series of 5 specimens were investigated.

Immunohistochemistry

For immunohistochemistry, specimens were fixed for 4 h at room temperature in 4% paraformaldehyde in PBS. The isolated brains were dissected, washed 4× 30 min in PBS, embedded in agarose (Agarose Broad Range, Carl Roth), and subsequently sectioned (80 μm) with a HM 650 V vibratome (Microm). Permeabilization of the brains in PBS–TX (PBS, 0.3% Triton X-100) for 1 h at 4 °C was followed by incubation in primary antibodies overnight at 4 °C. The antisera used were polyclonal rabbit anti-FMRFamid (1:1000, DiaSorin), monoclonal mouse anti-synapsin "Synorf1" antibody (1:30 in PBS–TX, antibody provided by E. Buchner, Universität Würzburg, Germany), rabbit anti-Dip-allatostatin 1 (AST-A, 1:2000, provided by H. Agricola), and mouse anti-tyrosinated tubulin (1:1000, Sigma). After incubation in the primary antisera, tissues were washed in several changes in PBS for 2 h at room temperature and incubated in a secondary Alexa Fluor488 or secondary Alexa Fluor546 antiserum (1:50, Invitrogen) overnight at 4 °C. All sections were counterstained with the nuclear marker bisbenzimide (0.1%, Hoechst H33258) for 1 h at room temperature. Some sections were processed with a histochemical counterstain, a high-affinity probe for actin, by adding Phallotoxins conjugated to Alexa Flour546 (1:50, Molecular Probes) during the incubation in the secondary antibody. Finally, all tissues were washed for 2 h in several changes of PBS and mounted in Mowiol (Calbiochem). Overall, immunohistochemical and histochemical experiments were conducted with 15 specimens (synapsin + phalloidin $n = 5$, synapsin + RFamid $n = 5$, tyrosinated-tubulin + A-type allatostatin $n = 5$).

Figure 1 *Scutigera coleoptrata* (Linnaeus, 1758). Scale bar = 10 mm. Original kindly provided by S.Fischer. This figure appears in color in the online version of *Chemical Senses*.

Specificity of the antisera

The tetrapeptide FMRFamide and FMRFamide-related peptides are widely distributed among invertebrates and vertebrates and form a large neuropeptide family with more than 50 members all of which share the RFamide motif (reviews: Price and Greenberg 1989; Greenberg and Price 1992; Nässel 1993; Homberg 1994; Dockray 2004; Nässel and Homberg 2006; Zajac and Mollereau 2006). The antiserum we used was generated in rabbit against synthetic FMRFamide (Phe-Met-Arg-Phe-amide) conjugated to bovine thyroglobulin (DiaSorin, Cat. No. 20091, Lot No. 923602). According to the manufacturer, staining with this antiserum is completely eliminated by pretreatment of the diluted antibody with 100 μg/ml of FMRFamide. We repeated this experiment and preincubated the antiserum with 100 μg/ml FMRFamide (Sigma; 16 h, 4 °C) and this preincubation abolished all staining. We conclude that the DiaSorin antiserum that we used most likely labels any peptide terminating with the sequence RFamide. Therefore, we will refer to the labeled structures in our specimens as "FMRFamide-like immunoreactive (RFir) neurons" throughout the paper.

The monoclonal anti-tyrosine tubulin (mouse IgG3; Sigma Product Number T 9028, Clone TUB-1A2) was raised against a peptide containing the carboxy terminal amino acids of α-tubulin. According to the manufacturer, this antibody reacts with tyrosine tubulin from bovine brain, African monkey green kidney cells, dog kidney, marsupial kidney, mouse pituitary tumor (AtT-20), yeast, and *Xenopus*, indicating that the antigen that this antibody recognizes is evolutionarily conserved across a broad range of species. Tubulin is the major building block of microtubules and represents a heterodimer of α-tubulin and β-tubulin. Microtubules grow and turn over rapidly, but some populations

of interphase microtubules are more stable, such as acetylated α-tubulin (Kreis 1987). Tubulin tyrosinylation is involved in the assembly status of tubulin. Tyrosinated tubulin (tyr-tubulin) represents a relatively dynamic subclass of interphase microtubules (Kreis 1987).

The monoclonal mouse anti-*Drosophila* synapsin "Synorf 1" antibody (provided by E. Buchner, Universität Würzburg, Germany) was raised against a *Drosophila* Glutathione S-Transferase(GST)-synapsin fusion protein and recognizes at least 4 synapsin isoforms (ca. 70, 74, 80, and 143 kDa) in western blots of *Drosophila* head homogenates (Klagges et al. 1996). In western blot analysis of crayfish homogenates, this antibody stains a single band at approximately 75 kDa (Sullivan et al. 2007). In a western blot analysis comparing brain tissue of *D. melanogaster* and the terrestrial hermit crab *Coenobita clypeatus*, the antibody provided identical results for both species, staining 1 strong band around 80–90 kDa and a second weaker band slightly above 148 kDa (Harzsch and Hansson 2008). Furthermore, the antibody consistently labels brain structures in representatives of malacostracan crustaceans (Beltz et al. 2003; Vilpoux et al. 2006; Harzsch and Hansson 2008) in a pattern that is consistent with the assumption that this antibody does in fact label synaptic neuropil in Crustacea. We also conducted a western blot analysis comparing brain tissue of *D. melanogaster* and *S. coleoptrata*. The antibody provided similar results for both species staining 1 strong band around 80–90 kDa. Our analysis strongly suggests that the epitope that Synorf 1 recognizes is strongly conserved between the fruit fly and the chilopod. Beyond the arthropods, this antibody even labels synaptic neuropil in ancestral taxa of protostomes such as the Chaetognatha (Harzsch and Müller 2007; Rieger et al. 2010) and Plathelminthes (Cebia 2008) suggesting that the epitope that this antiserum recognizes is highly conserved over wide evolutionary distances.

Allatostatin peptides share the conserved C-terminal sequence -YXFGL-amide (Bendena et al. 1999) and are present in the nervous system of various insects analyzed so far (reviewed in Kreissl et al. 2010). In hexapods, allatostatins are often colocalized with other transmitters, for example with γ-aminobutyric acid (GABA) and other peptides in antennal lobes of moths (Berg et al. 2007, 2009). Corelease with GABA is likely related to the inhibitory function of AST. The antiserum against *Diploptera punctata* (Pacific beetle cockroach) A-type allatostatin I (Dip-allatostatin I, APSGAQRLYGFGL-amide) was provided by H. Agricola (Friedrich-Schiller Universität, Jena, Germany). Vitzthum et al. (1996) have characterized the Dip-allatostatin I antibody. It recognizes all Dip-allatostatins. No cross-reactivity was found with corazonin, crustacean cardioactive peptide (CCAP), FMRFamide, leucomyosuppression, locustatachykinin 11, perisulfakinin, and proctolin as tested by noncompetitive enzyme-linked immunosorbent assay. Vitzthum et al. (1996) showed that preadsorption of the diluted antisera against Dip-allatostatin I, GMAP, and *Manduca sexta* allatotropin with 10 μM of their respective antigens abolished all immunostaining in brain sections of *Schistocerca gregaria*. We performed a preadsorption test and preincubated the antiserum with 200 μg/ml A-type allatostatin I (Sigma, A9929; 16 h, 4 °C), and this preincubation abolished all staining. We conclude that the antiserum that we used most likely labels any peptide terminating with the sequence YXFGL-amide. Therefore, we will refer to the labeled structures in our specimens as "allatostatin A–like immunoreactive (ASTir) neurons" throughout the paper.

Antennal backfills

For anterograde antennal backfill stainings, specimens were immobilized by slowly cooling down and mounted in plastic Petri dishes. The left antenna was then cut, leaving a stump of approximately 3 mm. A glass capillary filled with 2% Dextran-biotin (dextran, biotin, 3000 MW, lysine fixable; Molecular Probes, Cat. No. D-7135) dissolved in aqua dest. was placed over the antennal stump. Animals were kept alive for 4 h at room temperature, then decapitated and the head subsequently fixed in 4% paraformaldehyde in PBS for 4 h at room temperature. Thereafter, brains were dissected out in PBS and either processed as whole mounts or sectioned (80 μm). Vibratome sections and brains were washed 3× 20 min in PBS and incubated for 8 h in Streptavidin conjugated to Alexa 488 (1:2.500 in PBS, Molecular Probes Cat. No S-11223) over night at 4 °C. The sections and brains were then rinsed 3× 20 min with PBS and finally mounted in Mowiol. We performed 5 successful backfills all of which yielded a homogeneous representation of primary sensory neuropils with only little variation between the structural patterns.

Golgi impregnation

Heads of *S. coleoptrata* were prefixed in a solution containing 5 parts 2.5% potassium dichromate, 1 part 25% electron microscopy-grade glutaraldehyde and 3% glucose for 4 days at 4 °C. After dissection, brains were washed in 2.5% dichromate for 1 h, followed by 4 days incubation in 99 parts 2.5% potassium dichromate and 1 part 1% osmium tetroxide, and then immersed in 0.75% silver nitrate. Finally, brains were dehydrated through a graded series of ethanol and propylene oxide and embedded in Durcupan (Fluka Chemicals), which was polymerized over 2 nights at 60 °C. Serial 30 μm sections were cut with a sliding microtome (Jung HN 40 and Leica SM 2000R). For details on the method, see Strausfeld (1980).

Microscopy

Whole mounts and brain sections were viewed with a Zeiss AxioImager equipped with the Zeiss Apotome structured dillumination device for optical sectioning. Digital images were processed with the Zeiss AxioVision software package. In addition, specimens were analyzed with a laser scanning microscope (Zeiss 510 Meta) using 10× (Zeiss EC

Plan-Neofluar 10×/0.3) and 20× (Zeiss Plan-Apochromat 20×/0.8) objectives. Double-labeled specimens were generally analyzed in the multitrack mode, in which the 2 lasers operate sequentially. Narrow band pass filters were used to assure a clean separation of the labels and to avoid cross-talk between the channels. Images based on *Z*-stacks of several optical sections were obtained using the LSM 510 SP2 Software (Zeiss). All images were obtained at 1024 × 1024 pixel resolution and processes in Adobe Photoshop using global contrast and brightness adjustment features.

Three-dimensional reconstruction

For the alignment and 3-dimensional (3D) reconstruction, AMIRA 4.1 (Mercury Systems) operated on a FS Celsius work station was used. In each section, contours of the neuropils were demarcated and a 3D model, in which individual neuropils could be defined, was generated. Reconstructions from antennal backfills were generated by using the Isosurface function. A nomenclature of the olfactory subunits was developed based on the general position of single neuropils in the deutocerebrum, where capital letters give the position: anterior (A), posterior (P), ventral (V), dorsal (D), and central (C).

Ethological experiments

To test whether *S. coleoptrata* is able to detect airborne stimuli, an ethological test was designed. Two holes (1 cm in diameter) were drilled into the bottom of a plastic box (25 × 20 × 8 cm) in opposite quadrants (Figure 9A). Two round containers with a height of 5 cm were placed underneath the holes, one of them containing a stimulus. As stimuli, living juvenile crickets (*A. domesticus*) or an extract of the same species was presented. To produce the extract, 50 juvenile specimens of *A. domesticus* were immersed in 1 ml methylene chloride for 1 h at room temperature in darkness. The decanted liquid was dried with sodium sulfate decahydrate and concentrated under a light nitrogen stream to 100 µl. Ten microliters (the equivalent to 5 living juvenile specimens) were then pipetted onto a filter paper (10 × 5 mm) that was placed in one of the containers. Specimens were tested individually in the arenas. The position of the stimulus was changed randomly between the experiments. After the experiment, all arenas and handling containers were cleaned, rinsed in aqua dest., and dried.

The behavioral experiments were conducted over night in darkness starting round 10.00 PM. Single individuals were placed in individual arenas, which were then inspected on the following morning. A positive choice was recorded when *S. coleoptrata* had moved into one of the containers. Otherwise, the trial was recorded as "no choice." Five-to-ten specimens of *S. coleoptrata* were tested per night, and new specimens were used for each replicate. The choices were analyzed with a binominal test to investigate whether the distribution of the choice differed from 50:50. Individuals that did not make a choice were excluded from statistical analyses.

In addition, a field experiment was conducted on the island of Ibiza using a glass terrarium (50 × 30 × 25 cm) in the field with the same substrate and physical conditions as the surrounding area (Figure 9B). Three filter papers (10 × 5 mm) were placed in the center of the terrarium at a distance of 5 cm to each other and impregnated with 1) 10 µl cricket extract or 2) 10 µl water. The third paper served without any impregnation as a control. Five specimen of *S. coleoptrata* were placed into the terrarium. The experiments were run during 3 nights from about midnight until about dawn. The terrarium was filmed in the dark with a Sony DCR-SR70 equipped with night shot optics and infrared illumination. The videos obtained were evaluated using VLC media player. In the video presented as Supplementary material online, the stimulus is to the left, the control in the middle, and the water-soaked paper to the right.

Results

Overview of the *Scutigera coleoptrata* brain

A dorsal to ventral series of vibratome sections reveal that the head of *S. coleoptrata* is densely packed with musculature (Figure 2). The brain is sandwiched between an anteriorly located system of muscles and more posteriorly located musculature that operates the mouthparts (Figure 2A,B). The protocerebrum expands as a huge mass dorsally to the deutocerebrum (Figures 2A,B,C and 3A; terminology according to the body axis). The well-developed elongated optic lobes extend laterally from the protocerebrum toward the compound eyes (Figure 2B,C). They comprise 2 neuropils, the distal of which is the lamina and the proximal is called medulla (Melzer et al. 1996) or visual tectum (Strausfeld 2005). The mushroom bodies or corpora pedunculata are located in the dorsal protocerebrum (Figure 7A). The deutocerebrum is the most anterior part of the brain with regard to the body axis but is located ventroposteriorly to the protocerebrum with respect to the neuraxis (Figure 3A). It receives input from the robust antennal nerves at its frontolateral edges (Figures 2E,F and 3A). These nerves, on entering the brain, divide into 2 branches: a dorsoventral part innervates the anterior neuropils (olfactory neuropils; ONs), whereas the ventrocaudal part innervates a posterior neuropil (corpus lamellosum [CL]). As in hexapods, the deutocerebrum is partitioned into many discrete neuropil units that are targeted by antennal OSN axons (Figure 3). Because of their shape, we suggest to call them olfactory neuropils (ON) rather than glomeruli or glomerular masses/neuropils. The sum of the ONs we call antennal lobe as in the Hexapoda. A demarcation between the deutocerebrum and tritocerebrum is not

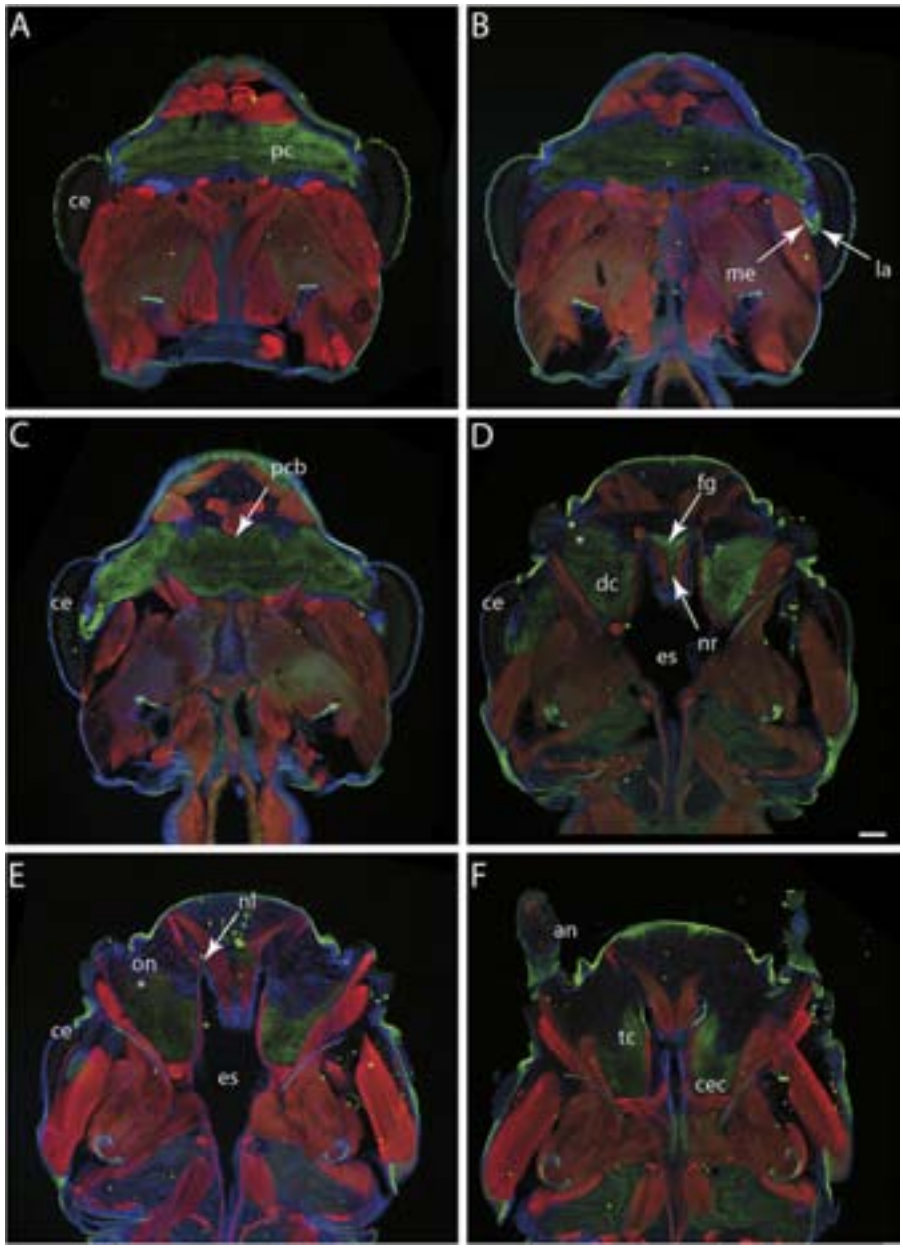

Figure 2 Selected sections from a horizontal vibratom section series through the head of *Scutigera coleoptrata* from dorsal to ventral (epifluorescence microscopy combined with the Zeiss Apotome structured illumination technique for optical sectioning); synapsin immunoreactivity and histochemical labeling of f-actin and nuclei. **(A)** Dorsal section showing compound eyes and the dorsal part of the protocerebrum. **(B)** Region of the midline neuropil. In the lateral protocerebrum, the optic neuropils lamina and medulla are visible. **(C)** Optic neuropils and protocerebral bridge. **(D)** Medial section through the deutocerebrum. The antennal nerve enters the brain and single ONs are detectable (asterisk). The frontal ganglion with the frontal connectives and the *nervus recurrens* are also visible. **(E)** Ventral section of the deutocerebrum and tritocerebrum. Single ONs are detectable (asterisk). The *nervi labrales* are visible in a mediofrontal position. **(F)** Ventral section showing tritocerebrum and circumesophageal connectives. The base of the antenna is visible. an, antenna; ce, compound eye; cec, circumesophageal connective; dc, deutocerebrum; es, esophagus; fg, frontal ganglion; la, lamina; me, medulla; nl, nervus labralis; nr, nervus recurrens; pc, protocerebrum; pcb, protocerebral bridge; tc, tritocerebrum. Scale bar for all images = 100 μm. This figure appears in color in the online version of *Chemical Senses*.

apparent, although the frontal connectives indicate the anterior margin of the tritocerebrum, just posteroventral to the deutocerebral lobes (Seifert 1967). The frontal connectives of the tritocerebrum project slightly caudally and converge medially at the frontal ganglion (Figures 2D and 3A). The *nervus recurrens* extends dorsally of the frontal ganglion on top of pharynx and esophagus (Figures 2D and 3A). A pair of labral nerves emerges in the front of the tritocerebrum (Figures 2E and 3A). These nerves converge and innervate the musculature of the clypeolabral region and the buccal gland. Paired connectives leave the tritocerebrum posteriorly to link it with the subesophageal ganglion.

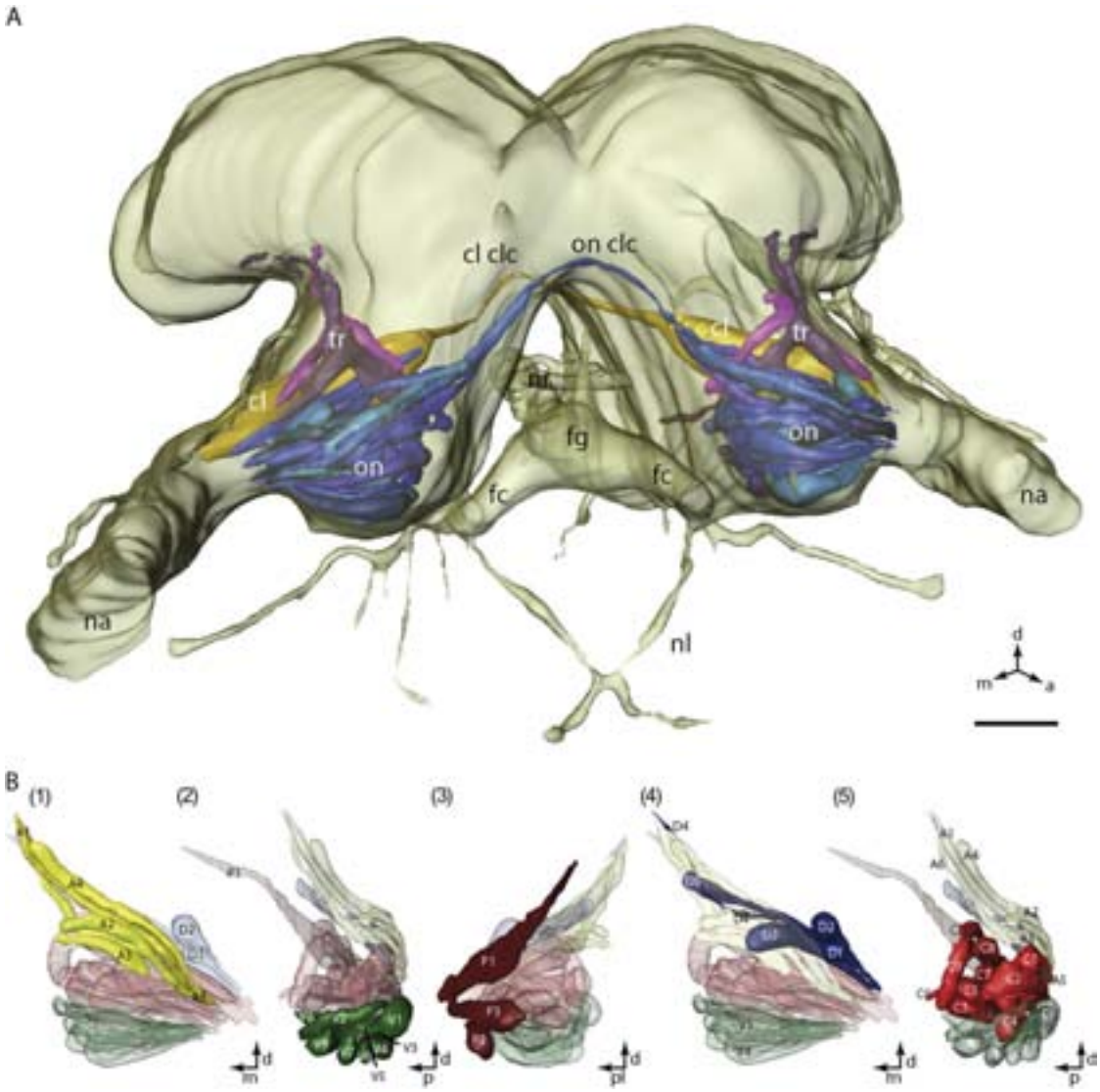

Figure 3 3D reconstructions of the brain and deutocerebral neuropils of *Scutigera coleoptrata* from a histological section series. **(A)** Reconstruction of the brain with the nerves of the deuto- and tritocerebrum. **(B)** Reconstruction of the ONs in various views (frontal, 45° angle frontal, 45° angle cranial). Color coding of neuropil groups: anterior (1), ventral (2), posterior (3), dorsal (4), and central (5). a, anterior; d, dorsal; fc, frontal connective; fg, frontal ganglion; m, median; na, nervus antennalis; nl, nervus labralis; nr, nervus recurrens; on, olfactory neuropils; p, posterior; pl, posteriolateral. Scale bar = 100 µm. This figure appears in color in the online version of *Chemical Senses*.

Backfill experiments

By backfilling the antennal nerve with anterograde axonal markers, we obtained reliable information concerning the innervation pattern of the deutocerebral neuropils. Based on these preparations, we were able to verify that antennal input does in fact target the deutocerebral lobe neuropils, which therefore are first order processing areas in the deutocerebrum. In a fill of the left antennal nerve (Figure 4A,B), the label was transported not only into the deutocerebral lobe but also along tracts projecting into the ventral protocerebrum and into the subesophageal ganglion (arrowheads in Figure 4B,C,D). The afferents separate into 2 tracts, one of which innervates the ONs, whereas the other targets the corpus lamellosum (cl; Figure 4B). These backfills also revealed contralateral connections of both the ONs and the CL (on clc and cl clc; Figure 4B). The isosurface reconstruction (Figure 5A,B) shows the 3D arrangement of this LSM section (108.7 µm from 142 optical sections). The front view displays (Figure 5A) a subset of ONs. The back view (Figure 5B) shows the architecture of the corpus lamellosum with projecting inner lamellae and the anterior closed outer lamellae (ol).

The olfactory neuropils

The dextran-biotin backfills reveal that single ONs have an elongated shape and are arranged in a parallel array

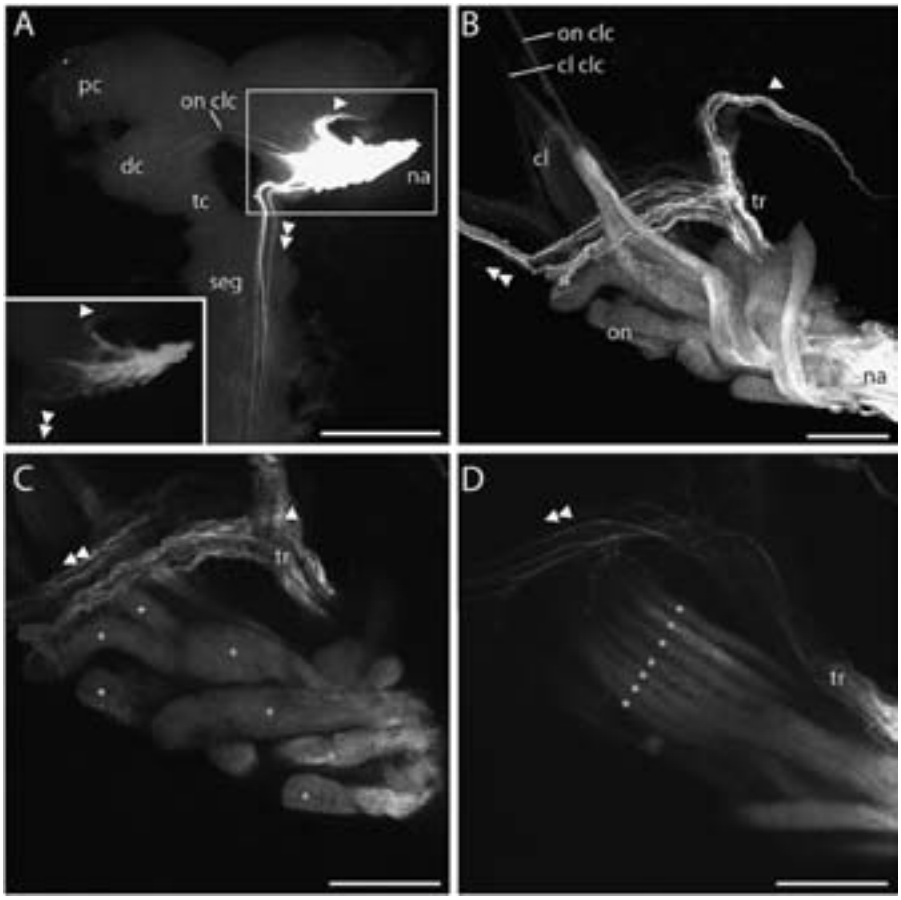

Figure 4 Dextran-biotin backfill of the left antennal nerve, all pictures were taken from the same preparation. **(A)** Epifluorescence microscopy combined with the Zeiss Apotome structured illumination technique for optical sectioning image. Whole-mount preparation of the *Scutigera coleoptrata* brain. Neuropils, contralateral connection of ONs, and projecting tracts (arrowheads) are labeled. The inset shows labeled projections from the antennal nerve (streptavidin). **(B)** Higher magnification of the dextran-biotin backfill of the left deutocerebral lobe. Projection of a confocal stack with 142 single images covering a total thickness of 108.7 μm. Filled neuropils with on clc and cl clc are visible. The base of projecting tr is not visible, nevertheless, the bifurcation into dorsal (arrowhead) and ventral (double arrowhead) tract is detectable. Posterior tracts and corpus lamellosum are weaker stained in this view. **(C)** Single optical section (0.76 μm) of ONs (asterisks) and distal bifurcation of projecting tracts. **(D)** Single optical section (0.76 μm) of the corpus lamellosum. Seven single lamellae are detectable (asterisks). The proximal region of projecting tracts is visible. cl, corpus lamellosum; cl clc, contralateral connection of the corpus lamellosum; dc, deutocerebrum; na, nervus antennalis; on, olfactory neuropils; on clc, contralateral connection of the olfactory neuropils; pc, protocerebrum, tc, tritocerebrum; tr, tracts. Single arrowheads: dorsal projecting tracts and double arrowheads: ventral projecting tracts. All scale bars 100 μm.

(Figure 4C, asterisks). Synapsin immunoreactivity provides a good overview over the general architecture of the ONs and confirms their elongated shape (Figure 6B,C). In horizontal vibratome sections of the whole head, a slight medioposterior direction of the ONs is visible (asterisks in Figure 2D,E). A projection of 43 single confocal images (covering ca. 32 μm) shows that the neuropil of the ONs is densely and uniformly stained (Figure 6B, frontal view). A comparison of the backfill data (Figure 4B,C) with the Synapsin immunoreactivity (Figure 6B) suggests that the antennal afferents extend throughout the entire ONs to establish synaptic connections with interneurons. Not only in a different immunohistochemical preparation (Figure 6C) but also in histological sections (Figure 6F), the neuropil of the ONs appear compact without subcompartments.

The shape and arrangement of the ONs were reconstructed from histological section series stained with toluidine blue, where single neuropils appear highly contrasted (Figure 6F). The roughly parallel array of ONs emerges from the entry point of the antennal nerve and extends in a mediodorsal direction. The 3D reconstruction of the ONs show that they are arranged in a bilaterally symmetrical pattern (Figure 3A), so that it is possible to match the corresponding ONs in the left and right antennal lobe. In 3 histological series from different specimens, we consistently found 34 distinct and uniquely identifiable ONs in a more or less invariant arrangement that seems to be fixed in the 3D space within the deutocerebrum (Figure 6A). This set can be subdivided into 5 groups according to their position: an anterior group (A1–A6, Figure 3B), a ventral group (V1–V9), a posterior group (P1–P3), a dorsal group (D1–D5), and a central

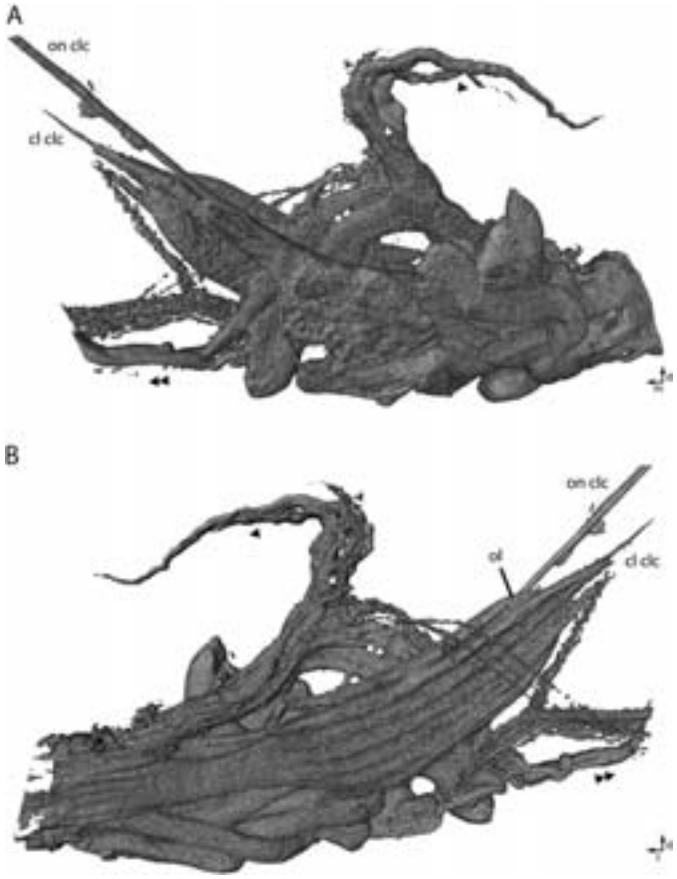

Figure 5 3D isosurface reconstruction of the dextran-biotin backfill shown in Figure 4 from a confocal stack with 142 single images covering a total thickness of 108.7 μm. Filled neuropils with contralateral connections of ONs and corpus lamellosum are visible. **(A)** Front view. **(B)** Back view. The base of projecting tracts is visible dorsal of the base of the corpus lamellosum as well as the bifurcation into dorsal (arrowhead) and ventral (double arrowhead) tr. cl, corpus lamellosum; cl clc, contralateral connection of the corpus lamellosum; d, dorsal; l, lateral; m, median; na, nervus antennalis; ol, outer lammelae of the corpus lamellosum; on olfactory neuropils; on clc, contralateral connection of the olfactory neuropils; tr, tracts.

group (C1–C11). The 2 uppermost ONs (A1, A6) extend into a mediodorsal direction toward the midline whereas the more ventral ones are shorter (Figure 3, groups V, C). The distal end of these ventral ONs is often thickened and/or bent posteriorly (Figure 3B, e.g. V2, C2). The ONs have a length of around 250–400 μm. The cross-sectional profile is more or less 35 μm in diameter. Volume measurements range from approximately 15 500 μm^3 (ON D4) to approximately 1 030 000 μm^3 (ON C3). In average, an ON has a volume of approximately 443 500 μm^3, and the sum of all neuropils is 15 080 000 μm^3.

Localization of RFamide-like immunoreactivity (RFir) reveals mostly fibers that surround the ONs diffusely, the associated somata being located in a mediolateral position to the ONs (arrows in Figure 6E 1 and 2). The ONs exhibited weak RFir (Figure 6E 1 and 2). Allatostatin-like immunoreactivity (ASTir) is expressed more strongly in the deutocerebral lobes (Figure 6D), where a distinct signal is present within the ONs (inset Figure 6D). Twenty to 30 ASTir neurons are located anteriorly to the ONs. Most likely, these neurons give rise to the plexus of immunolabeled fibers in the central area of the neuropils that form distinct synaptic endings. These endings are evenly distributed throughout the neuropil and do not show any regional concentrations.

Golgi impregnations provide information about the morphology of single neurons and, when impregnated in quantity, their contributions to the neuroarchitecture of defined synaptic neuropils. Due to the fact that the Golgi method does not reveal the entire array of neurons, only a subset of axons is visible. Large neurons are often partially impregnated, particularly when several occur together. The neuropils themselves can be resolved as delineated volumes that show up darker than the surrounding tissue even when no neuronal processes have been impregnated. This staining property reveals the 2 kinds of neuropils in the deutocerebrum: the corpus lamellosum (Figure 8A, cl) and the smaller ONs (Figure 8A, on). On entering the brain, axons from the antennal nerve project into a kind of sorting zone from where they follow distinctive trajectories either into the ONs or into the CL (Figure 8A,B). Afferents targeting the ONs are much thinner than those that extend into the CL (Figure 8A,C). Within the ONs, afferent neurons form bundles with more or less parallel neurites (Figure 8C,D,E). Each axon is decorated with numerous bouton-like swellings, here interpreted as presynaptic specializations (arrows in Figure 8D). Larger processes, interweaving among these endings, may belong to local interneurons and, or, relay neurons. Their axons are equipped with distinct synaptic swellings. They branch intensely in the ONs with their synaptic fields overlapping those of the OSN axons (Figure 8D,F). The terminals in ONs are tangled, have many branches, and are generally of very small diameter (Figure 8E).

Corpus lamellosum

The ventrocaudal part of the antennal nerve innervates a presumed mechanosensory neuropil called the corpus lamellosum (CL) that is composed of approximately 8 thin neuropils arranged in parallel, called lamellae (asterisks Figure 4D). Volume measurement of 1 CL revealed a size of approximately 7 900 000 μm^3. Golgi impregnations show that while the OSNs targeting the ONs appear thin, the axons targeting the CL are much thicker (Figure 8A,C) and give off short side branches along their length (Figure 8A,B,C). In histological sections, the neuropil of the CL stands out in high contrast so that the subdivision into the lamellae is clearly recognizable (Figure 7E). Two different types of lamellae are present. The outer lamella medially forms a 180° loop (ol in Figure 7B, arrow in Figure 7 C1, E), whereas the inner lamellae (il in Figure 7B) extend further dorsomedially to project toward the contralateral side. These projections connect to the heterolateral neuropil and form the posterior

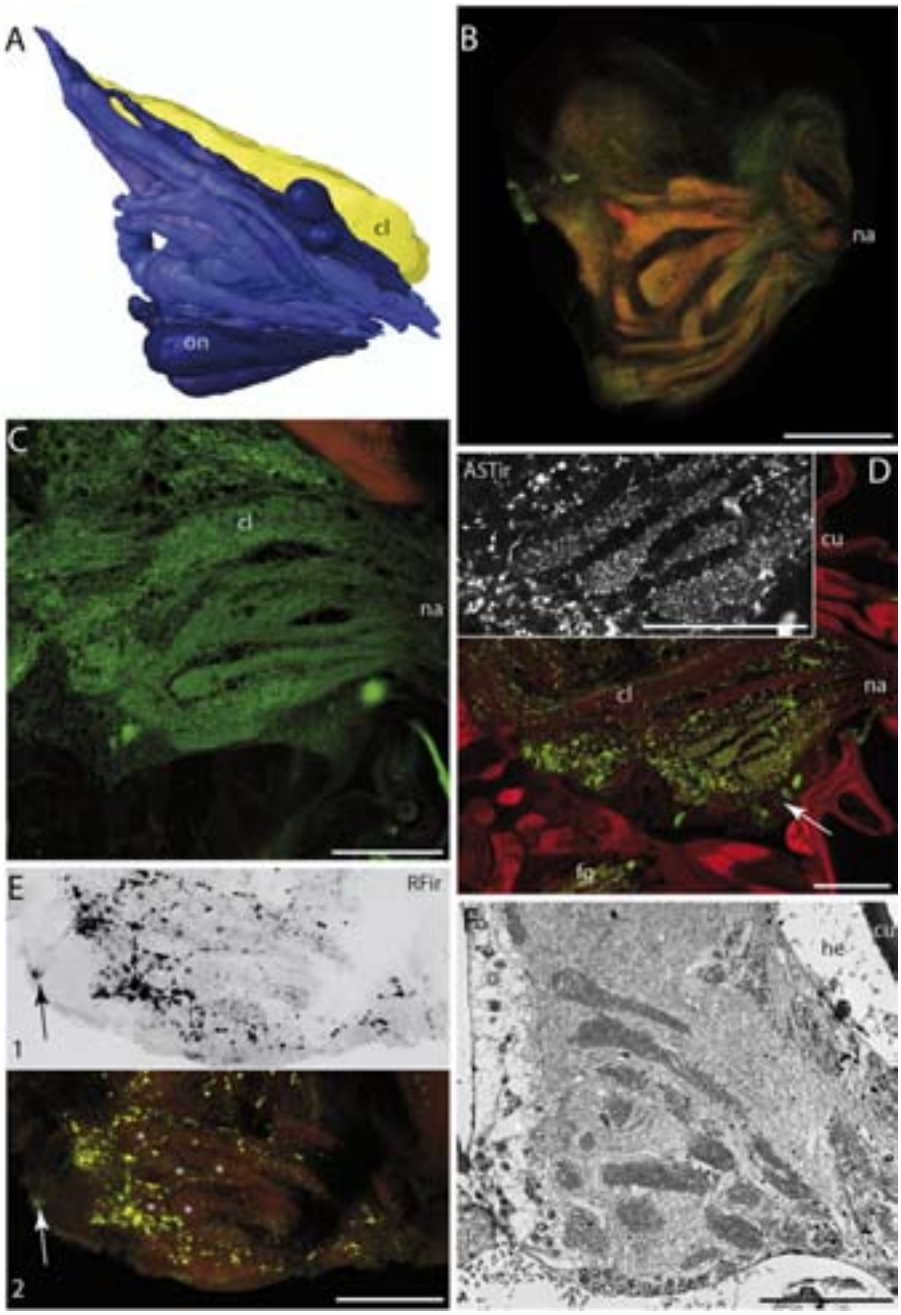

Figure 6 ONs visualized with different methods. **(A)** The 3D reconstruction of the 34 ONs and the corpus lamellosum of one deutocerebral lobe based on a histological section series (frontal view). **(B)** Synapsin immunoreactivity and phalloidin labeling. Medial frontal section of the left antennal lobe. Projection of 43 optical sections covering a thickness of 31.8 μm. **(C)** Single optical section of synapsin immunoreactivity and phalloidin labeling in the left antennal lobe (80 μm horizontal vibratome section). **(D)** Immunolocalization of tubulin and ASTir, single optical section of a 80 μm horizontal vibratome section. Inset shows ASTir in the ONs. ASTir somata are located in front of the ONs (arrow). **(E)** Single optical section of a 80 μm vibratome section of the left antennal lobe (frontal view). 1: Black–white inverted RFir. Arrow identifies 2 medial RFir somata, and asterisks mark single ONs. 2: Synapsin immunoreactivity and RFir. **(F)** Histological cross section (1.5 μm) of the left deutocerebral lobe. Asterisks show selected single ONs. Tangential aspects of projecting tracts are visible in the upper right quadrant. Lower left: tangential section of a frontal connective. cl, corpus lamellosum; cu, cuticle; he, hemolymph; fc, frontal connective; fg, frontal ganglion; na, nervus antennalis; pc, protocerebrum. All scale bars = 100 μm. This figure appears in color in the online version of *Chemical Senses*.

antennal commissure (Figures 3A and 4B, arrow in Figure 7A). Phalloidin histochemistry highlights additional structural features. In a stacked frontal projection of optical sections (Figure 7B), the lamellae are clearly visible. Single sections of this stack (Figure 7C) depict 3 sequential views from anterior to posterior, where the 2 different types of lamellae and the contralateral projections are visible. Phalloidin histochemistry reveals 8 lamellae (Figure 7 asterisks in C2), whereas

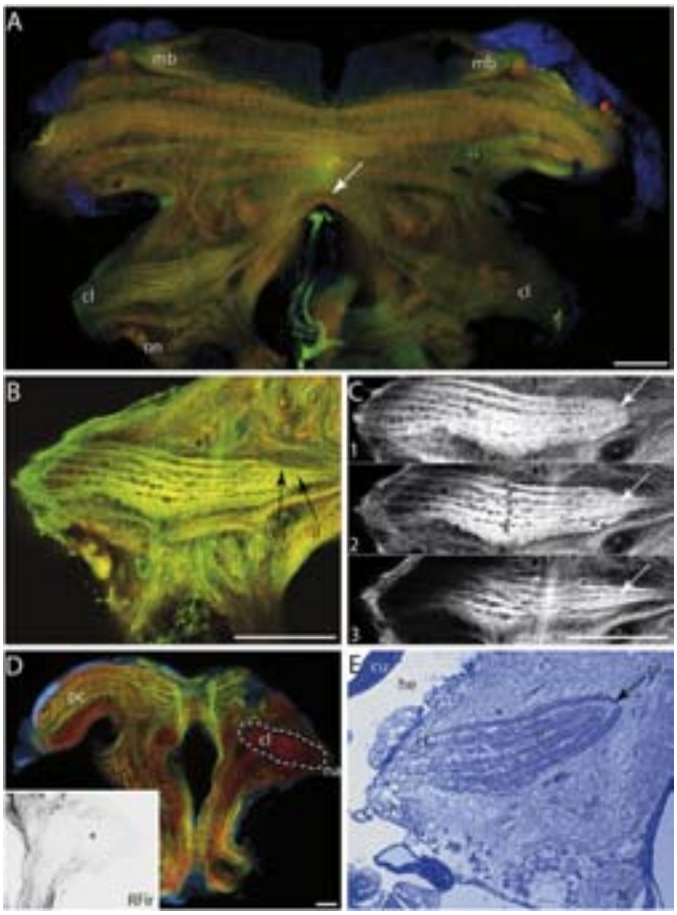

Figure 7 The corpus lamellosum visualized with different methods. **(A)** Epifluorescence microscopic image of the brain (80 μm vibratom cross section) combined with the Zeiss Apotome structured illumination technique for optical sectioning showing synapsin immunoreactivity, phalloidin labeling, and nuclear labeling. Arrow identifies the thin contralateral connection of the neuropil. **(B)** Projection of a confocal stack of 37 single sections covering a thickness of 27.3 μm of the right corpus lamellosum showing synapsin immunoreactivity and phalloidin labeling. The connection of the outer closed lamella is not visible in this view. **(C)** Single optical sections of the projection shown in (B). 1: Anterior section with closed outer lamella. 2: Middle section with outer lamella covered by projecting inner lamellae. In this view, 8 single lamellae are visible (asterisks). 3: Posterior section with projecting inner lamellae. **(D)** Epifluorescence microscopic image of a 80 μm vibratom section combined with the Zeiss Apotome structured illumination technique for optical sectioning showing synapsin immunoreactivity, phalloidin labeling, and nuclear labeling. The region of the corpus lamellosum is marked and no further partitions in the synapsin immunoreactivity are visible. Inset: RFir of the same preparation (black–white inverted). Asterisk marks the position of the corpus lamellosum. **(E)** Histological cross-section (1.5 μm) of the right deutocerebral lobe. The closed outer lamella and inner lamellae are highly contrasted. cl, corpus lamellosum; cu, cuticula; he, hemolymph; il, inner lamellae; mb, mushroom body; ol, outer lamella; on, olfactory neuropils; pc, protocerebrum; tc, tritocerebrum. All scale bars = 100 μm. This figure appears in color in the online version of *Chemical Senses*.

backfilling experiments show 7 lamellae (Figure 4D). Immunolocalization of RFamide-like peptides does not reveal any signal in the CL (inset Figure 7D). However, allatostatin-like immunoreactivity is present in the CL but in a lower intensity as in the ONs (Figure 6D). Only the posterior area was labeled with this antiserum whereas the more anterior area displays only a loose, punctual distribution of label.

Behavioral experiments

When choosing between the containers with live crickets (stimulus) and the empty one (control), individuals of *S. coleoptrata* with a high significance preferred the crickets. The ratio stimulus versus control for all experiments conducted was 36:12 ($n = 48$) (binominal test $P < 0.001$). When presenting the cricket extract, the centipedes also robustly preferred the stimulus over the control (Figure 9). The ratio stimulus versus control for these experiments was 33:14 ($n = 47$) (binominal test $P < 0.005$).

In experiments under controlled field conditions, we could distinguish 2 different behavioral patterns with regard to the samples that we presented: undirected walking over the sample versus arrestment. ''Undirected'' behavior was defined as a rapid movement over the filter paper, whereas for an ''arrestment'' behavior the animals had to halt at the filter paper for at least 3 s. Over 3 nights and duration of nearly 15 h, 39 contacts with the exposed filter papers were observed. Undirected behavior was recorded for 20 contacts, 6 times at the stimulus, 8 times at the control, and 6 times at the water-soaked paper. Arrestment behavior was exclusively recorded at the stimulus and took place 19 times. Overall, 25 visits at the stimulus were recorded. Numbers of contacts on the stimulus were significantly higher than on control and water (Kruskal–Wallis test $P < 0.001$). The Supplementary online video shows a compilation of the 9 contacts that took place during the first night of experiments. In this video, the stimulus is to the left, the control in the middle, and the water-soaked paper to the right. Scenes 1, 3, 5, 6, 8, and 9 show arrestment behavior at the stimulus. It has to be noted that these scenes were recorded in darkness under infrared light illumination. In scenes 5, 6, and 9, the animals can be seen to slowly approach the stimulus in a directed way. Upon reaching the filter paper, the animals abruptly halt and begin to inspect the paper with their mouthparts and anterior limbs. After a few seconds, the animals seem to lose interest and wander off.

Discussion

The deutocerebrum of *Scutigera coleoptrata*

The nervous system of Scutigeromorpha was first investigated in the late 19th century by Saint-Remy (1887, 1889) and the beginning of the 20th century by Hörberg (1931). Fahlander (1938), in a broad comparative study, described the nervous system of various Chilopoda and also interpreted the results from Saint-Remy and Hörberg. All 3 authors found that the deutocerebrum contains glomerular neuropils that can be divided into 2 different classes. However, although all 3 authors mentioned elongated neuropils

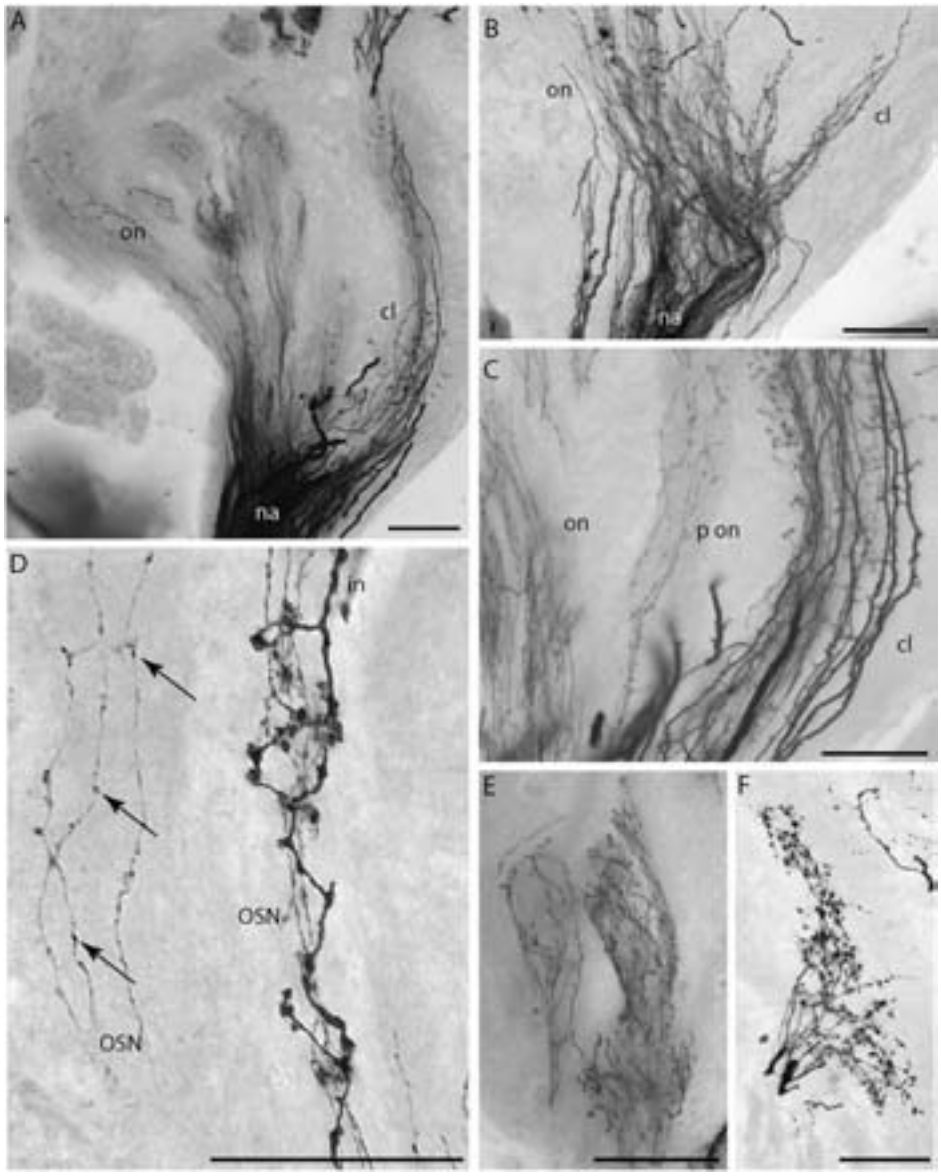

Figure 8 Horizontal sliding mictrotome sections (30 μm) of Golgi impregnated brains of *Scutigera coleoptrata*. **(A)** Penetration of afferent axons into the ONs and the corpus lamellosum. Scale bar = 100 μm. **(B)** Sorting zone of OSNs and mechanosensory axons. Orientation into the more or less parallel arrangement of OSNs. **(C)** Left: parallel axons target the ONs. A posterior ON is innervated by single neurites. Right: Innervation of the corpus lamellosum. Fine lateral and medial branchings are visible. **(D)** Left: thin and parallel OSNs targetting an ON. Arrows mark putative presynaptic buttons. Right: Secondary thicker axon (probably belonging to an interneuron) with putative postsynaptic buttons. **(E)** Arrangement of OSNs in 2 ONs. **(F)** Secondary neuron (presumably interneuron) projects with longitudinal spreading ramifications on an ON. Scale bars in (B–F) = 50 μm.

in the antennal lobe, their number and structural composition remained unclear. Fahlander (1938) described an anterior antennal commissure but overlooked that this structure is actually formed by the extensions of at least 2 neuropils (neuropils A1 and D4 in the present report). By comparing histological section series of different specimens and performing immunohistochemical and tracing experiments, we reconstructed a map of 34 individually identifiable ONs in the antennal lobe of *S. coleoptrata.* This map is fairly invariant between specimens considering number, position and shape of the ONs. Such an invariant arrangement resembles the array of olfactory glomeruli mapped in different hexapod species (e.g., Chambille and Rospars 1981; Rospars 1983; Rospars and Hildebrand 1992; Galizia et al. 1999; Laissure et al. 1999; Berg et al. 2002; Huetteroth and Schachter 2005; Kirschner et al. 2006; Ghaninia et al. 2007; Zube et al. 2008; Dreyer et al. 2010). In Fahlander's (1938) schematic presentation of the *Thereuopoda clunifera* (Scutigeromorpha) brain, he depicted 2–3 elongated neuropils innervated by the antennal nerve and two neuropil masses in the distal region leading to the anterior antennal commissure. Our data reveal that in *S. coleoptrata* there are

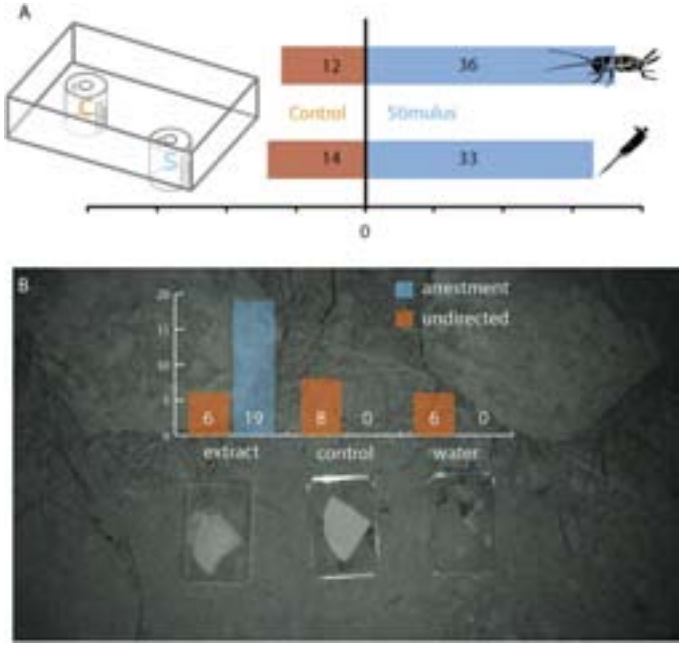

Figure 9 **(A)** Layout of the experimental arena and results of behavioral experiments with juvenile crickets and cricket extract. Single specimens were tested individually in the arena. Left-hand bars indicate the results for the control, and Right-hand bars indicate the results for the stimulus. Upper row: experiments with juveniles of *Acheta domesticus* as stimulus (n = 48 individual experiments); lower row: experiments with cricket extract as stimulus (n = 47 individual experiments). **(B)** Infrared video snapshot of the experimental layout under field conditions with 3 different filter papers in plastic dishes. Left: cricket extract (chemical stimulus), middle: control, and right: water. Video tapes covering 3 experiments that lasted 1 night were analyzed. In each experiment, 5 specimens were tested. Diagram indicates the total number of visits at the plastic dishes and the observed behavior. Left: undirected behavior at the filter paper and Right: arrestment behavior. This figure appears in color in the online version of *Chemical Senses*.

obviously many more elongated neuropils present in this region: A1, A4, A6, D4, D5 (Figure 3A,B).

In the hexapod olfactory system, axons extending from the base of the antennae undergo a sorting process such that smaller bundles are directed to their target glomeruli (Rössler et al. 1999). In malacostracan Crustacea, sensory axons from antenna 1 enter a distinct axon sorting zone, in which thinner axons projecting toward the olfactory lobe separate from thicker axons targeting the lateral antenna 1 neuropil, a mechanosensory processing unit (Schmidt and Ache 1992). Similarly, in *S. coleoptrata*, axons sort out and obtain distinctive trajectories: thinner OSNs and thicker mechanosensory axons are observable (Figure 8B). Inside the glomeruli, the afferents carry numerous boutons that indicate synaptic output. Olfactory glomeruli in honeybees have a concentric organization (Pareto 1972; Arnold et al. 1985; Fonta et al. 1993; Sun et al. 1993; Galizia et al. 1999; Nishino et al. 2005): the periphery is innervated by the axons of OSNs. Projection neurons innervate the periphery and the central core and different populations of local interneurons innervate central and peripheral areas. In those hexapods examined, ASTir neurites in the antennal lobe originate from local interneurons, projections neurons, and centrifugal neurons (reviewed in Schachtner et al. 2005). In the honeybee, ASTir neurites innervate the central core area of the glomeruli (Kreissl et al. 2010). The ASTir neurites form bleb-like boutons, and in addition few ASTir neurites branch in the peripheral glomerulus layers, making occasional synapses possible between OSNs and ASTir local interneurons. In the cockroach, ramifications extend throughout the whole glomerulus (reviewed in Schachtner et al. 2005). Schachtner et al. (2005) assumed that the possession of centrifugal ASTir neurites might be a plesiomorphic character for the Tetraconata but it is not sure from our data if similar centrifugal neurons exist in *S. coleoptrata* too. The architecture of single ONs in *S. coleoptrata* differs in shape and OSN innervation shows no specific pattern to the periphery. ASTir neurons target the whole neuropils and form buttons across its volume (Figure 6D). A longitudinal subdivision of the glomeruli into the cap, subcap, and base regions has been well documented in crayfish, clawed and clawless lobsters, and hermit crabs (Sandeman and Luff 1973; Sandeman and Sandeman 1994; Langworthy et al. 1997; Schmidt and Ache 1997; Wachowiak et al. 1997; Harzsch and Hansson 2008). In histological sections of *S. coleoptrata*, there is not any evidence of further partitions within the ONs, in contrast to layering that hallmarks columns in the olfactory lobe of malacostracan crustaceans (Sandeman et al. 1992; Harzsch and Hansson 2008).

With an average volume of approximately 443 500 μm^3, a single ON of *S. coleoptrata* is quite large in comparison to most other arthropod taxa. Single glomeruli in the olfactory lobe of the largest living land arthropod, the giant robber crab *Birgus latro* have a volume of approximately 280 000 μm^3 (Krieger et al. 2010). The olfactory glomeruli of Hexapoda are smaller and range for example between 1000 and 10 000 μm^3 in the ant *Camponotus japonicus* (Nishikawa et al. 2008) and approximately 4000 μm^3 in *D. melanogaster* (after Pinto et al. 1988). Male-specific macroglomeruli in Lepidoptera can reach a much higher volume, e.g. 300 000 μm^3 in the European corn borer *Ostrinia nubilalis* (Kárpáti et al. 2008) and 3 430 000 μm^3 in the tobacco hawkmoth *M. sexta*, in which also smaller glomeruli are present such as the club glomerulus with 187 323 μm^3 (Huetteroth and Schachter 2005).

The number of glomeruli is often considered a good estimate of the number of olfactory receptor proteins (ORs) expressed in antennal OSNs. For example, *D. melanogaster* with approximately 48 glomeruli expresses 62 functional ORs (Vosshall and Stocker 2007) and *Apis mellifera* with approximately 160–170 glomeruli expresses 170 ORs (Robertson and Wanner 2006). Most other hexapods investigated fall in between these extremes. In *S. coleoptrata*, we found 34 ONs suggesting a similar number of ORs, if the molecular basis in this species resembles that in hexapods. Ants, however, have a much higher number of glomeruli

and might thus express more than 10 times more ORs than *S. coleoptrata* does.

Can centipedes smell?

Our results clearly show that the neuronal substrate of olfaction is present in the deutocerebrum of *S. coleoptrata*. Additional support for the presumptive capability to detect airborne stimuli was obtained in the behavioral experiments. Early ethological experiments with *L. forficatus* (Lithobiomorpha) were conducted by Hennings (1904) and Friedel (1928). In their experimental setup, they presented liquids of meat to the animals, which were able to locate the stimulus or reacted with antennal tremor. Antenna amputated animals did not show any reaction. In contrast, Scharmer (1935) obtained contradictory results for *L. forficatus* with the same experimental setup. Simon (1960) described the key stimuli for *L. forficatus* as tactile or gustatory and characterized the species as a lurking predator. Finally, Meske (1961) again conducted ethological experiments on the olfactory capability of *L. forficatus*. He showed that antennal cleaning behavior was enhanced by presenting high concentrations of butanoic acid and when presenting mashed earthworms ("*Regenwurmsaft*"). Simple choice experiments carried out by Meske (1961) suggested that *L. forficatus* is able to detect the smell of meat and therefore should be able to detect an airborne stimulus. Comparable experiments with *S. coleoptrata* were only conducted by Klingel (1960). He designed an experimental arena consisting of a terrarium equipped with a perforated tube filled with living flies. In his experiments, 10 specimens of *S. coleoptrata* never noticed the presented prey during a period of 1 hour. In addition, experiments with mashed flies ("*Fliegensaft*") absorbed in a cotton ball, also failed to create any reaction. Klingel (1960) concluded that *S. coleoptrata* recognizes prey only by chemotactile stimulation.

Our own behavioral experiments contradict these results, even though we do not know if our experimental setup, the feeding conditions, and the daytime of the experiments match that of Klingel (1960). By testing starved specimens during their activity period at night and providing a longer time period to locate the prey than Klingel (1960) did, we achieved unambiguous results showing that *S. coleoptrata* is indeed able to detect airborne stimuli, both from live prey and from a chemical extract of the prey. These results are in line with the morphological findings concerning the well-developed olfactory centers in the deutocerebrum of this species.

Tactile stimuli perceived by the walking appendages have been suggested to play an important role for capturing prey in *S. coleoptrata* but it is unclear whether visual stimuli are also involved in this behavior (Meyer-Rochow et al. 2006; Rosenberg 2009). Considering that many Chilopoda including *S. coleoptrata* are active mostly during night time (Rosenberg 2009) it seems reasonable to argue that not only mechanoreceptive but also chemoreceptive abilities are the major sensory modalities involved in hunting for prey. Our video observations show that in darkness, the animals take notice of the extract and when coming into the vicinity of the extract-impregnated filter paper they immediately show the arrestment behavior suggesting that they perceive chemotactile stimuli with their antennae or walking limbs. It has been well documented that Chilopoda display a distinct cleaning behavior and especially *S. coleoptrata* spends much time in thoroughly cleaning legs and antennae (Rosenberg et al. 2004; Rosenberg 2009). Preliminary data indicate that the antennae of adult specimens are equipped with more than 2000 sensilla that can be divided into 5 different classes based on morphological criteria (Ernst A, Sombke A, Rosenberg J, in preparation). Putative chemosensory sensilla bear terminal pores. It is unclear yet if these sensilla respond to olfactory or gustatory stimuli. However, we interpret the arrestment behavior toward the cricket extract-impregnated filter papers recorded in the behavioral field experiments in total darkness as additional evidence that the animals do also react to volatile substances. The sum of both behaviors recorded on the extract was significantly higher than on the 2 controls, suggesting that an airborne stimulus can be perceived. In closer proximity, contact chemosensory perception (arrestment and probing) is suggested to be the main behavioral feature.

Mechanosensory neuropils in the deutocerebrum of Chilopoda and other Euarthropoda

In a brief description of the deutocerebral neuropils of the centipede *L. forficatus* (Lithobiomorpha), Strausfeld et al. (1995) described that the antennal nerve innervates the antennal lobe and that a lateral strand of the antennal nerve bypasses the glomeruli to project toward a region behind it, which the authors called dorsal lobe in analogy to hexapods. This posterior neuropil in the deutocerebrum was termed *masse lamelleuse* by Saint-Remy (1887) and later latinized by Fahlander (1938) who called it corpus lamellosum.

Mechanosensory neuropils with a general striate or palisade shape are also known from apterygote Hexapoda and Crustacea (reviewed in Strausfeld 1998). For instance, in the firebrat *Thermobia* sp. the neuropil innervated by their antennae is organized in a columnar and striate manner. According to our histochemical labeling against f-actin and dextran-biotin backfills, this corpus lamellosum in *S. coleoptrata* is composed of 7 to 8 parallel lamellae. In Pterygota, the first and second antennomeres supply the AMMC (Rospars 1988) whereas the flagellum is mostly specialized for olfactory perception and supplies the antennal lobe. Although we have a good understanding of the antennal olfactory pathways in hexapods, far less is known about the destination of antennal mechanosensory and gustatory receptor neurons. Unlike olfactory afferents, which project into the olfactory glomeruli, mechanosensory and gustatory

afferents project into the posterior dorsal region of the deutocerebrum and the anterior region of the subesophageal ganglion for example in *Periplaneta americana* (Burdohan and Comer 1996; Nishino et al. 2005), *A. mellifera* (Kloppenburg 1995), *Gryllus bimaculatus* (Staudacher 1998; Staudacher and Schildberger 1999), and *Aedes aegypti* (Ignell and Hansson 2005; Ignell et al. 2005). In these organisms, olfactory afferents are characterized by thin axons, whereas presumptive tactile afferents provide 2 pairs of long branches and several short branches oriented laterally and medially forming a multilayered arrangement in the dorsal lobe. Such an arrangement of short lateral and medial branches is also present in the corpus lamellosum of *S. coleoptrata*. The functional role of 2 centers (dorsal lobe and target site in the subesophageal ganglion) in hexapods could be assessed from their morphological interaction with other neuropils (Nishino et al. 2005). In the cockroach, the proximity to afferents from the maxillary palp allows the integration of signals of a similar quality detected by different peripheral organs. By the location near the antennal olfactory processing center, tactile and gustatory information may be combined with remote airborne sensory signals in the antennal lobe to obtain an integrated picture of the external world (Nishino et al. 2005).

In the present report, we also observed projections of the antennal nerve into the ventrolateral protocerebrum and the subesophageal ganglion. Fahlander (1938) was unsure whether these bundles of neurites were afferents or efferents associated with the antenna. Our data suggest that these afferents may project to gustatory centers in the subesophageal ganglion (as mentioned above in comparison to the cockroach). The projections to the ventrolateral protocerebrum may terminate close to the postantennal organ, which was described to function as a carbon dioxide receptive organ in the Japanese house centipede *Thereuonema hilgendorfi* (Yamana et al. 1986). In the mosquito *A. aegypti* (Ignell and Hansson 2005) an antenno-subesophageal tract contains axons originating from the maxillary palps and extends into the dorsal region of the antennal lobe. Whether a similar tract from the subesophageal ganglia into the antennal lobe occurs in *S. coleoptrata* is unclear.

Malacostracan crustaceans possess 2 pairs of antennae, a first, mostly chemosensory pair associated with the deutocerebrum, and a second, mostly mechanosensory pair associated with the tritocerebrum (overviews in Sandeman and Luff 1973; Blaustein et al. 1988; Sandeman et al. 1992; Schmidt and Ache 1992, 1996a; Mellon and Alones 1993; Schachtner et al. 2005). Mechanosensory and nonolfactory chemosensory input from the first antennae is processed in the lateral antennular neuropil (LAN) and the medial antennular neuropil (MAN) (Schmidt et al. 1992; Schmidt and Ache 1996a; Harzsch and Hansson 2008). Between the lobes of the lateral antenna 1 neuropil, contralateral connections occur in Decapoda. The unpaired medial antenna 1 neuropil receives input from both first antennae. Afferents from the statocysts and other mechanosensory hairs on the antennal base also project into this neuropil (Yoshino et al. 1983; Roye 1986; Schmidt and Ache 1996a). These general architectural features of the mechanosensory neuropils associated with antenna 1 in Crustacea in many respects matches the structural characteristics and connections of the corpus lamellosum in *S. coleoptrata*.

It is well known that the antenna 2 neuropils of those decapods with long second antennae often exhibit a repeated geometrical structure in which the neuropil is transversely segmented (Tautz and Müller-Tautz 1983). Sandeman DC (unpublished results) suggests that there could be a spatiotopic mapping of the antennal mechanoreceptors along the length of the antennal neuropil. Behavioral studies on the attacking behavior that can be released by lightly touching the antennae of blinded crayfish suggest that the animals know where on the antenna they have been stimulated, as they direct the chelae quite precisely to the point of contact (Sandeman in Harzsch et al. forthcoming). It remains to be explored how the architecture of this tritocerebral mechanosensory neuropil relates to that of deutocerebral mechanosensory neuropils in Hexapoda and Chilopoda.

Phylogenetic implications

The fossil record of Myriapoda spans 420 million years (Edgecombe and Giribet 2007; Shear and Edgecombe 2010). Therefore, the adaptation toward an exclusively terrestrial mode of life can be traced back to upper Silurian myriapods. Our results show that the shape of the ONs in *S. coleoptrata* is strikingly different when compared with those in hexapods and malacostracan crustaceans. These differences suggest that upon conquering land, the Myriapoda followed their own distinct pathway in evolving an olfactory system suited for aerial olfaction (compare Sombke et al. 2009). Nevertheless, in our view the presence of distinct neuropils for chemosensory and mechanosensory qualities in *S. coleoptrata*, malacostracan Crustacea, and Hexapoda could indicate a common architectural principle within the Mandibulata. Malacostracan and hexapod olfactory lobes are similar insofar as both serve the homologous pair of appendages, the antennules, equipped with OSNs (Strausfeld 2009). By analogy, a chemosensory function for the ONs and a mechanosensory function for the corpus lamellosum have been suggested (Sombke et al. 2009). In-depth comparisons of species within and across tetraconate taxa (Hexapoda and Crustacea), however, demonstrate that many characters of the organization of tetraconate olfactory centers are shared among distantly related clades but have been modified in various taxon-specific ways (Schachtner et al. 2005). Another similarity between hexapod and malacostracan olfactory systems is that in stomatopod crustaceans, which are classified as basal eumalacostracans (Abele 1991), the antennular lobes comprise discrete glomeruli, not columns. This is also true for marine isopods (Harzsch S, Hansson BS, in preparation). In contrast to

Strausfeld (1998) and Fanenbruck et al. (2004), it can be assumed that a glomerular olfactory (or antennal) lobe is a plesiomorphic character for mandibulates as proposed by Schachtner et al. (2005). This suggests that contra Harzsch (2006, 2007), the absence of olfactory lobes in, for example, the Branchiopoda and certain Maxillopoda is a reduction.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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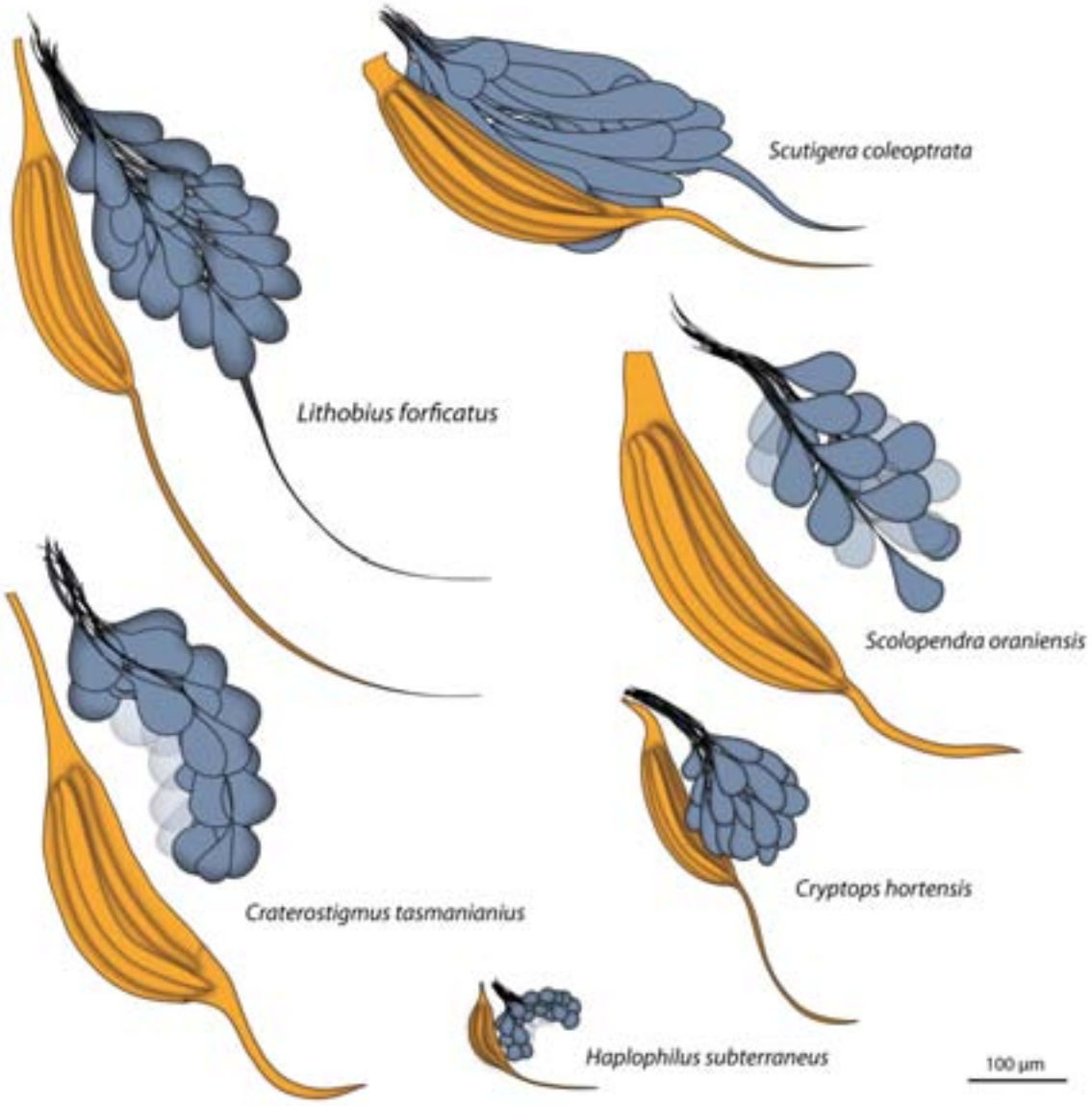
VI

Deutocerebral organization in Chilopoda

Sombke A, Lipke E, Kenning M, Müller CHG, Hansson BS, Harzsch S (subm.) Comparative analysis of deutocerebral neuropils in Chilopoda (Myriapoda): implications for the evolution of the arthropod olfactory system and support for the Mandibulata concept. BMC Neuroscience.



Comparative analysis of deutocerebral neuropils in Chilopoda (Myriapoda): implications for the evolution of the arthropod olfactory system and support for the Mandibulata concept

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Abstract

Background

Originating from a marine ancestor, the myriapods most likely invaded land independently of the hexapods. As these two evolutionary lineages conquered land in parallel but separately, we are interested in comparing the myriapod chemosensory system to that of hexapods to gain insights into possible adaptations for olfaction in air. Our study connects to a previous analysis of the brain and behavior of the chilopod (centipede) *Scutigera coleoptrata* in which we demonstrated that these animals do respond to volatile substances and analyzed the structure of their central olfactory pathway.

Results

Here, we examined the architecture of the deutocerebral brain areas (which process input from the antennae) in seven additional representatives of the Chilopoda, covering all major subtaxa, by histology, confocal laser-scan microscopy, and 3D reconstruction. We found that in all species that we studied the majority of antennal afferents target two separate neuropils, the olfactory lobe (chemosensory) and the corpus lamellosum (mechanosensory). The numbers of olfactory glomeruli in the different chilopod taxa ranged from ca. 35 up to ca. 90 and the shape of the glomeruli ranged from spheroid across ovoid or drop-shape to elongate.

Conclusion

A split of the afferents from the (first) pair of antennae into separate chemosensory and mechanosensory components is also typical for Crustacea and Hexapoda, but this set of characters is absent in Chelicerata. We suggest that this character set strongly supports the Mandibulata hypothesis (Myriapoda + (Crustacea + Hexapoda)) as opposed to the Myriochelata concept (Myriapoda + Chelicerata). The evolutionary implications of our findings, particularly the plasticity of glomerular shape, are discussed.

Background

The Myriapoda represent an arthropod lineage that, originating from a marine arthropod ancestor, most likely conquered land independently from Hexapoda. The successful transition from marine to terrestrial life requires a number of physiological adaptations that are important for survival out of water. The sensory organs of terrestrial species must be able to function in air rather than in water and hence were exposed to new selection pressures that may have reshaped the nervous system (see e.g. [1-4] for examples on terrestrial Crustacea). We are interested in how the structure of the central nervous system mirrors functional adaptations of the olfactory system to a terrestrial life style. Studying the olfactory system in Myriapoda and comparing it to that of Hexapoda may provide insights into how the arthropod nervous system evolved in response to new environmental and ecological challenges.

The Chilopoda together with the Progoneata (Diplopoda + (Symphyla + Pauropoda)) constitute the taxon Myriapoda. The position of monophyletic Myriapoda within the Euarthropoda is still under debate and most of the recent phylogenetic studies either place them as sister group to the Tetraconata (Crustacea + Hexapoda) together forming the taxon Mandibulata (e.g. [5, 6]) or as a sister group to the Chelicerata to form the taxon Myriochelata (e.g. [7]).

Our knowledge of the chilopod nervous system largely relies on studies from the 19th and early 20th century using paraffin sections and light microscopy (e.g. [8-13]). Studies with contemporary neuroanatomical methods are only available for the brain, and specifically for the deutocerebrum (the second brain neuromere) of *Scutigera coleoptrata* [14].

The deutocerebrum in the mandibulate (Myriapoda + (Crustacea + Hexapoda)) brain is associated with the first pair of antennae and is characterized by a unified architecture: it comprises an anterior olfactory lobe that receives the chemosensory afferents from the first antennae, and (at least) a posterior neuropil [14, 15]. This uni- or bipartite posterior neuropil is thought to process mechanosensory stimuli and has a range of different names within the mandibulate taxa: antennal mechanosensory and motor center (AMMC) or dorsal lobe in Hexapoda (e.g. [16]), Corpus lamellosum in Chilopoda [12-14, 17] and lateral antennular neuropil (LAN) plus median antennular neuropil (MAN) in malacostracan Crustacea and Remipedia [2, 18-22]. All of these structures can be unified under the term mechanosensory neuropils.

The chemosensory olfactory lobe (called antennal lobe in Hexapoda) is composed of structural and functional subunits [15], which are called olfactory glomeruli or olfactory neuropils (e.g. [14, 17]). These subunits are clearly demarcated dense neuropils in which the axons of olfactory sensory neurons (OSN) terminate and interact with olfactory interneurons *via* the first synapses of the olfactory pathway [15, 23]. Thus, within the olfactory glomeruli of Hexapoda, malacostracan Crustacea and the House Centipede *Scutigera coleoptrata*, first order integration of olfactory input takes place, which is then relayed to secondary brain centers *via* olfactory projection neurons (e.g. [2, 14, 15]). The glomerular array in hexapods is thought to represent a chemotopic map, which forms the basis of the olfactory code [24-26]. Based on this uniform architecture and several additional synapomorphic characters [15], the olfactory system in general as well as the olfactory glomeruli in particular were suggested to represent homologous structures within the deutocerebrum of the Mandibulata ([14, 15]; but see for additional [27]).

Nevertheless, previous studies have revealed a high degree of plasticity in the shape and arrangement of mandibulate olfactory glomeruli, suggesting a critical evaluation of glomerular. In *Scutigera coleoptrata*, the olfactory glomeruli are elongated and arranged in parallel [14]. On the contrary, in many decapod Crustacea the bilaterally arranged olfactory lobes consist of glomeruli that are cone-like and in the lobe are arranged with their apices pointing inwards (reviews: [15, 20, 21, 28]). In some malacostracan taxa, these glomeruli may be extremely elongated [2, 4], whereas studies on representatives of the basal malacostracan taxon *Nebalia* (Leptostraca) suggest spherical glomeruli to be part of the malacostracan ground pattern (Kenning, Harzsch, Strausfeld; unpublished results). Such spherical glomeruli are also present in marine Isopoda [3]. Furthermore, it has been well documented from crayfish

(Astacidea), spiny lobsters (Palinuroidea) and hermit crabs (Paguroidea) that in the olfactory lobe each glomerulus is stratified and provides an outer cap, a subcap, and a base [2, 4, 28]. Most pterygote insects also feature spherical glomeruli [15], whereas wingless diverge from this pattern [29, 30].

Is the shape of olfactory glomeruli of purely functional significance or does it contain an unexplored phylogenetic signal? Clearly, comparative information on the deutocerebral neuropils in a broad range of myriapods will contribute to this question. This study sets out to analyze the architecture of the central olfactory pathway in Chilopoda in more detail. To that end we analyzed the brains of representatives of eight chilopod species using histology, confocal laser scanning microscopy (cLSM), and 3D reconstruction. Our data are compared and evaluated with regard to the evolution of glomerular shape in Mandibulata.

Results

General morphology of the chilopod brain

Due to the anteriorly projecting antennae in the Chilopoda, the deutocerebrum (DC) is the most anterior part of the brain with regard to the body axis, so that the protocerebrum is always located dorsally and extends into lateral lobes where the optic neuropils are located (Fig. 1-5). In the blind Cryptopidae (Fig. 4F), these lateral lobes are much smaller and are even totally reduced in the Geophilomorpha (Fig. 5C, F). As this study focuses on the organization of deutocerebral neuropils, the protocerebral neuropils will not be further considered here. The morphology of the sensory antennal nerve differs in investigated chilopod species: while Scutigeromorpha, Lithobiomorpha and Craterostigmomorpha exhibit a “solitary” and robust antennal nerve, in Scolopendromorpha and Geophilomorpha it is composed of a bundle of several discrete nerves (Fig. 4E, G; 5C, D, F). Both types of antennal nerves enter the DC at its frontolateral or frontal edges (compare review [31]). Apart from the Geophilomorpha, the anterior part of the deutocerebrum is separated into two discrete hemispheres (Fig. 1D, 2F, 3F, 4E, H; 5C, F). Anterograde backfilling experiments reveal that the antennal nerve targets the deutocerebral neuropils, which therefore are first order processing areas in the brain. In all Chilopoda examined, each deutocerebral hemisphere contains the olfactory lobes (OL) being composed of densely packed olfactory glomeruli (OG) and the Corpus lamellosum (CL). A demarcation between deutocerebrum and tritocerebrum is not clearly apparent, although the stomodeal bridge and frontal connectives indicate the anterior margin of the tritocerebrum (compare [14, 17, 31])

Scutigeromorpha

The organization of deutocerebral neuropils in *Scutigera coleoptrata* (Fig. 1A) was described in detail by Sombke et al. [14] and therefore will be only briefly reviewed here. Due to the roundish head outline, the shape of the brain differs from that of the pleurostigmomorph chilopod taxa. The antennal nerves enter the brain at its frontolateral edges (Fig. 1D) and divide into two branches: an anterior part innervates the olfactory glomeruli whereas the posterior part innervates the Corpus lamellosum.

Histological sections and dextran-biotin backfills reveals that single OG have an elongated shape and are arranged in a parallel array (Fig. 1B, D; and [14]). A 3D reconstruction (Fig. 1D) reveals a bilateral symmetrical pattern with two contralaterally connected glomeruli (anterior deutocerebral commissure *sensu* Fahlander [13]) (Fig. 1C, D: clc). In all histological section series and autofluorescence preparations, a total number of 34 distinct and uniquely identifiable OG per hemisphere in a more or less invariant arrangement is present (Fig. 1D) [14]. The posterior part of the antennal nerve innervates the presumed mechanosensory neuropil called Corpus lamellosum (CL; [12, 14, 17]), which in *S. coleoptrata* is composed of approximately eight parallel neuropilar lamellae [14]. Two different types of lamellae were recognized: the outer lamellae forming a distal connection, and inner lamellae that extend further dorsomedially to project towards the contralateral hemisphere (posterior deutocerebral commissure *sensu* Fahlander [13, 14]). Golgi impregnations shows that axons targeting the CL are much thicker than those targeting the OG and give off short side branches alongside their length [14]. In backfills of the antennal nerve, we found that the dye was also transported along thicker neurites projecting into the ventrolateral protocerebrum and the subesophageal ganglion (Fig. 1C) [14].

Figure 1. A *Scutigera coleoptrata*. **B** Single optical section of a neurobiotin backfill showing an olfactory lobe with distinct olfactory glomeruli. cLSM scan. **C** cLSM scan (maximal projection) of the brain and the subesophageal ganglion. View from ventral. Left antennal nerve was filled with neurobiotin. Antennal neurites project into the seg. **D** 3D reconstruction of the brain of *S. coleoptrata* with deutocerebral neuropils. Blue: olfactory neuropils, yellow: Corpus lamellosum. **Abbreviations: cl** Corpus lamellosum, **clc** contralateral connection, **fg** frontal ganglion, **na** nervus antennalis, **np** neurite projections, **ol** olfactory lobe, **pc** protocerebrum, **seg** subesophageal ganglion. **Scalebars:** A = 10 mm, B, C = 100 µm.

Lithobiomorpha

The lithobiomorph head is flattened, a fact that is mirrored in the shape of the brain (reviewed in [17]). The antennal nerves enter the deutocerebrum at its frontal edges (Fig. 2B-G). The deutocerebrum is organized in an anterior olfactory lobe (OL) with glomeruli (OG) and a posterior Corpus lamellosum (CL) (Fig. 2B-G). In contrast to *S. coleoptrata,* the OL extends in a slightly dorsomedian direction. Antennal afferents were revealed by neurobiotin backfills which, in addition to the terminations in the OL and CL, also show a bundle of neurites projecting from the antennal nerve through the tritocerebrum deep into the subesophageal ganglion (Fig. 2C, F: np). Within the OL, two OG feature a contralateral connection (Fig. 2C, F: clc, D: arrow). Histological sections and neurobiotin backfills reveal that single OG have a drop-like to elongated shape that narrows to their anteriodistal edges (Fig. 2D, G). All OG appear compact without any subcompartments. The 3D reconstruction reveals a bilateral symmetrical pattern with a total number of 43 OG per hemisphere (Fig. 2G). The CL is located posteriorly to the OG and extends a small contralateral connection (not shown). The neuropil is composed of at least four lamellae (Fig. 2C, E asterisks). However, the lamellae are more densely packed than in *S. coleoptrata* so that a correct count was not possible.

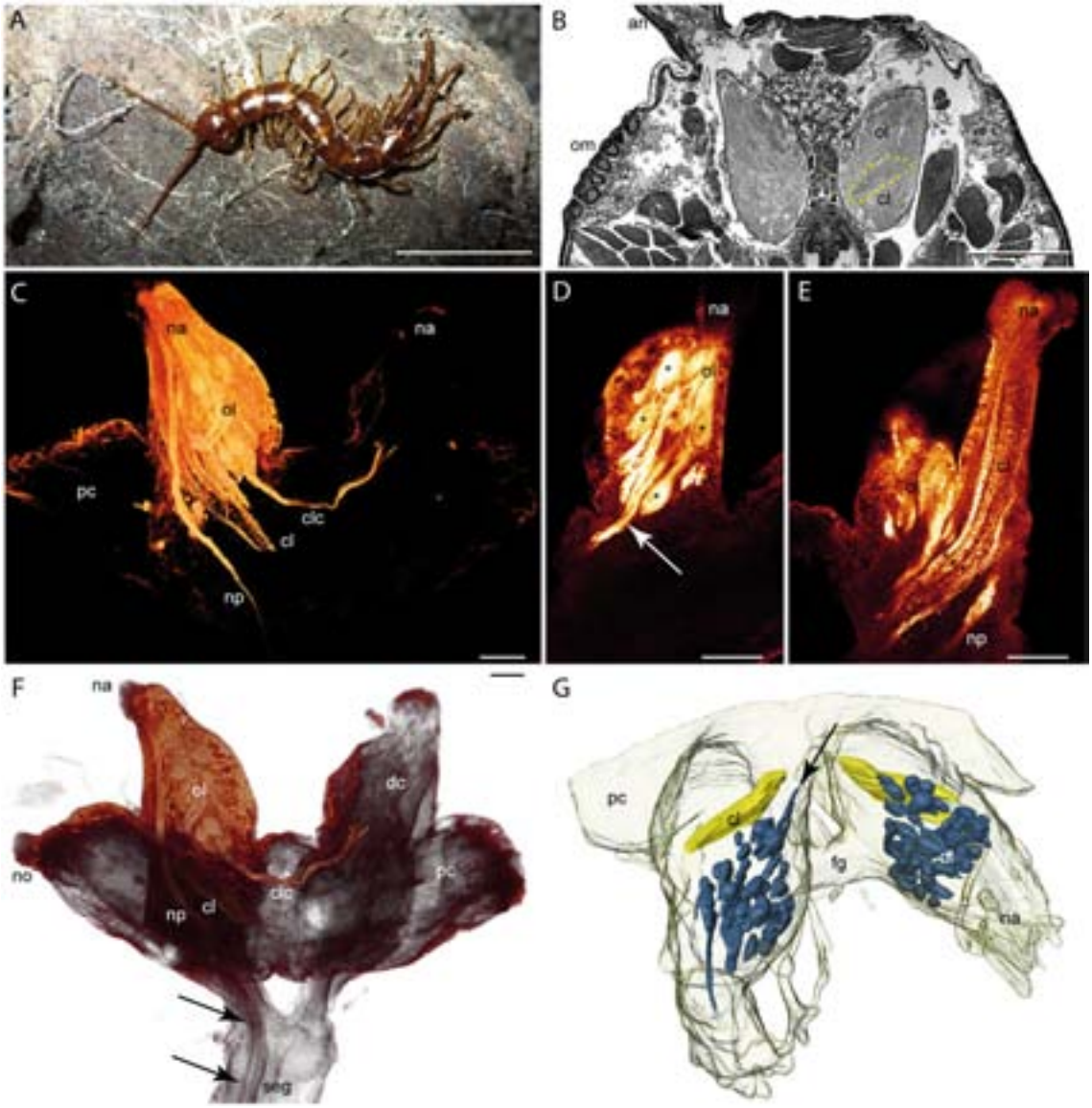

Figure 2. A *Lithobius forficatus***. B** Histological horizontal section of the head showing the deutocerebral lobes with olfactory glomeruli and Corpus lamellosum (dashed line) as well as the ommatidia. **C** Voltexrendering (Amira) of a neurobiotin backfill showing deutocerebral neuropils and neurite projections. **D** Horizontal optical section (cLSM scan) of a neurobiotin backfill (dorsal deutocerebrum) showing single olfactory glomeruli (asterisks). The arrow points to the contralateral connection of the olfactory lobe. **E** Horizontal optical section (cLSM scan) of a neurobiotin backfill (ventral deutocerebrum) showing the Corpus lamellosum. Asterisks mark single lamellae of the neuropil. **F** Voltexrendering (Amira) of a neurobiotin backfill showing the brain with deutocerebral neuropils and projections. Arrows point to antennal neurites projecting into the subesophageal ganglion. **G** 3D reconstruction of the brain with deutocerebral neuropils. Blue = olfactory neuropils, yellow = Corpus lamellosum. Contralateral connection of OG and CL is not shown. **Abbreviations: an** antenna, **cl** Corpus lamellosum, **clc** contralateral connection, **dc** deutocerebrum, **fg** frontal ganglion, **na** nervus antennalis, **no** nervus opticus, **np** neurite projections, **ol** olfactory lobe, **om** ommatidia, **pc** protocerebrum, **seg** subesophageal ganglion. **Scalebars:** A = 10 mm, B = 500 µm, C-E = 100 µm**.**

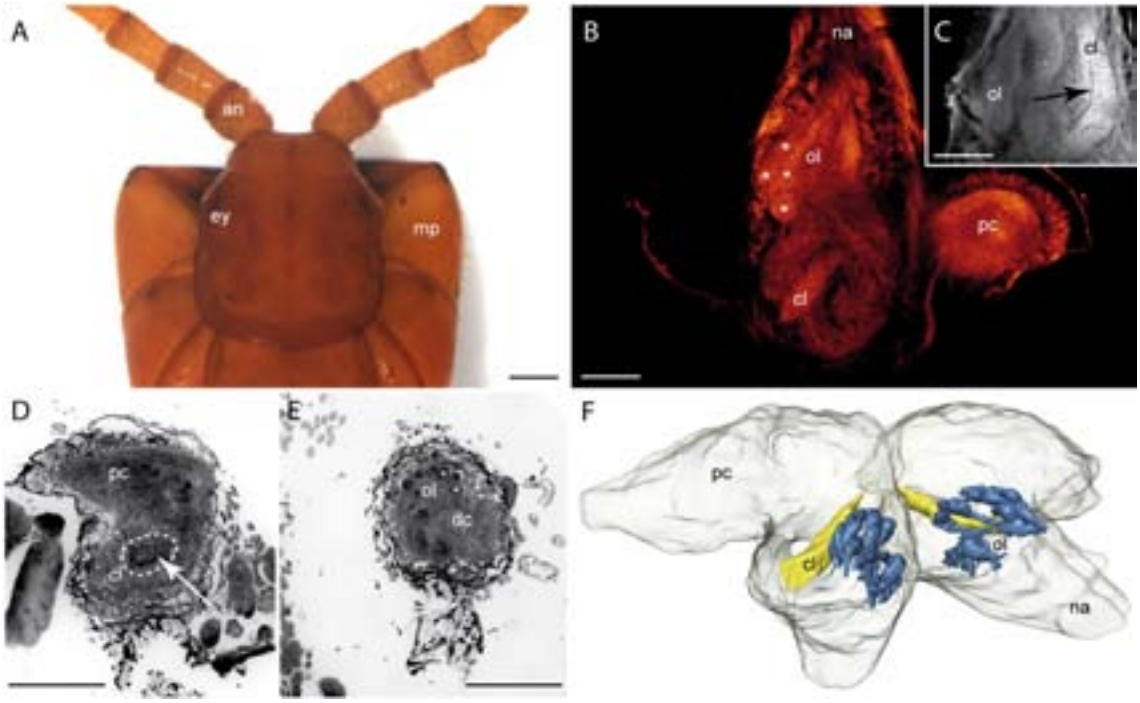

Figure 3. A Head and maxillipedes of *Craterostigmus tasmanianus* from dorsal. **B** Horizontal optical section of an autofluorescence preparation (cLSM stack). Single olfactory glomeruli (asterisks) are weakly detectable. **C** Different horizontal section of the same preparation as in B. The arrow marks the structural composition of the Corpus lamellosum. **D** Histological cross section of the right brain hemisphere showing the proto- and deutocerebrum with the Corpus lamellosum (dashed line). **E** Histological cross section of the left deutocerebrum showing the dorsomedian located olfactory glomeruli. **F** 3D-reconstruction of the brain with deutocerebral neuropils. Blue = olfactory glomeruli, yellow = Corpus lamellosum. Contralateral connection of the CL is not shown. **Abbreviations: an** antenna, **cl** Corpus lamellosum, **dc** deutocerebrum, **ey** eye, **mp** maxillipede, **na** nervus antennalis, **ol** olfactory lobe, **pc** protocerebrum. **Scalebars:** A = 1mm, B-E = 100 µm.

Craterostigmomorpha

This is the first investigation of the nervous system of *Craterostigmus tasmanianus* (Fig. 3A). Like in the Lithobiomorpha, the head of the Craterostigmomorpha is flattened, which is reflected in the shape of the brain (Fig. 3F). The robust antennal nerves enter the deutocerebrum at its frontal edges (Fig. 3B, F). In principle, the

deutocerebrum is organized in an anteriormedian OL and a posterior CL (Fig. 3B-F). Histological sections and autofluorescence preparations reveal that single OG have a drop-like to elongated shape with a nearly circular profile and a smaller diameter distally (Fig. 3B, E, F). The OG are arranged in an anterioposterior direction (Fig. 3B, F). A contralateral connection of the OLs was not found. The 3D reconstruction shows a total number of 36 OG per hemisphere (Fig. 3F). The CL is located posteriorly to the OL and features a thin contralateral connection (not illustrated). Due to the fixation, a lamellar organization was not clearly recognizable. However, a partition into discrete lamellae is likely (Fig. 3C, D arrow).

Scolopendromorpha

Similar to the Lithobiomorpha and Craterostigmomorpha, the head and also the brain of the Scolopendromorpha are flattened. The DC is innervated by several antennal nerve bundles (Fig. 4E, G) at its frontal edges and extends posteriorly. The DC is composed of anteriorly located olfactory glomeruli and a posteriorly located CL (Fig. 4B-E, G, H). The OG are arranged in an anterioposterior direction. In the three investigated scolopendromorph species, the shape of the OG appears spheroid to drop-like elongated (Fig. 4B-E, G, H). In *Scolopendra oraniensis* and *Cryptops hortensis*, one ventral glomerulus is much bigger (Fig. 4C, G: vog). A contralateral connection of the OLs is absent. Based on histological sections, backfill experiments, and autofluorescence preparations, all OG appear compact without any subcompartments and are arranged in a bilateral symmetrical pattern. Numbers of OG range from 51 in *Scolopendra subspinipes* (Fig. 4E), across 56 in *Cryptops hortensis* (Fig. 4H) to 58 in *Scolopendra oraniensis* per hemisphere. The CL is located posterioventrally to the OL. A contralateral connection is always present, although it varies in thickness in the three investigates species. In *Scolopendra subspinipes,* it appears as a thick connection (Fig. 4E), while in *Cryptops hortensis* it appears very thin (not shown in Fig. 4H). Single lamellae are not clearly detectable. However, backfill experiments reveal an alternating texture within the neuropil (Fig. 4B, C arrow, G). Neurobiotin backfills revealed an additional neurite bundle projecting from the antennal nerve through the tritocerebrum into the subesophageal ganglion (Fig. 4B, C, G: np).

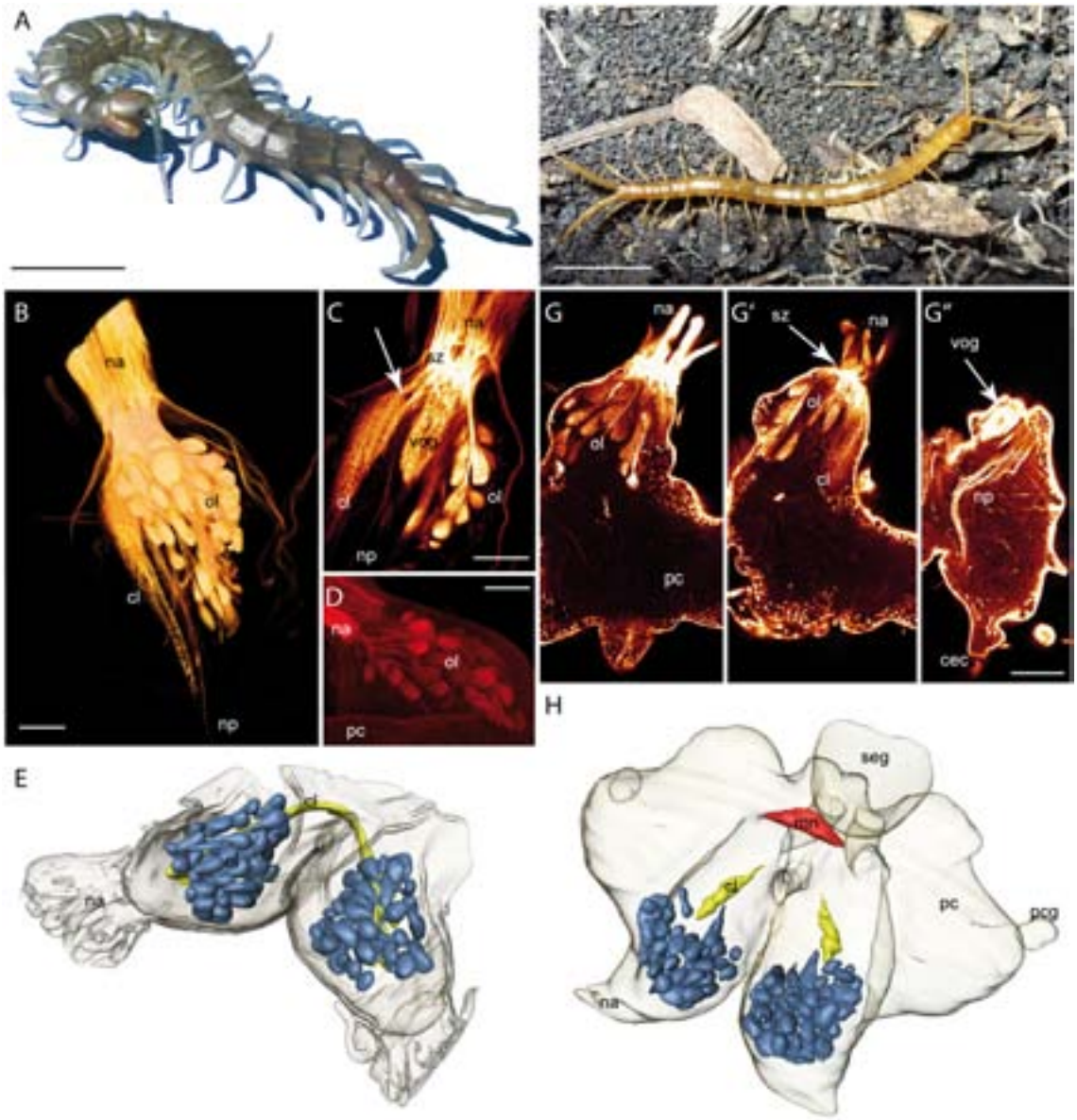

Figure 4. A *Scolopendra oraniensis*. **B** Neurobiotin backfill of the antennal nerve in *S. oraniensis* showing the olfactory lobe, the Corpus lamellosum, and neurite projections (horizontal maximal projection, cLSM scan). **C** Single optical horizontal section of a Lucifer yellow backfill in *S. oraniensis* (cLSM scan). Antennal neurites cross each other in a sorting zone and project into different neuropils. The arrow marks the structural composition of the Corpus lamellosum in which single lamellae are weakly noticeable. The large ventral OG is visible in this section. Single olfactory glomeruli in the olfactory lobe are arranged like in a grape. **D** Neurobiotin backfill of the antennal nerve of *S. oraniensis.* Only a subpopulation of the antennal neurites and olfactory glomeruli is labeled (horizontal maximal projection, cLSM scan). **E** 3D reconstruction of the brain of *Scolopendra subspinipes* (dorsal protocerebrum is not shown) with deutocerebral neuropils. Blue = olfactory glomeruli, yellow = Corpus lamellosum. **F** *Cryptops hortensis*. **G** Single horizontal optical sections (cLSM) of a neurobiotin backfill of the right antennal nerve in *C. hortensis* from dorsal to ventral. Antennal nerve bundles and innervation of single olfactory glomeruli. **G'** Sorting zone (arrow) of antennal neurites and Corpus lamellosum. **G''** Larger ventral olfactory glomerulus (arrow) and neurite projections. **H** 3D reconstruction of the brain of *C. hortensis* with deutocerebral neuropils and midline neuropil. Contralateral connection of the CL is not shown. Blue = olfactory glomeruli, yellow = Corpus lamellosum, red = midline neuropil. **Abbreviations: cec** circumesophageal connectives, **cl** Corpus lamellosum, **mn** midline neuropil, **na** nervus antennalis, **np** neurite projections, **ol** olfactory lobe, **pc** protocerebrum, **pcg** protocerebral gland, **seg** subesophageal ganglion, **sz** sorting zone, **vog** ventral olfactory glomerulus. **Scalebars:** A and F = 10 mm, B-D, G = 100 µm.

Geophilomorpha

The brain of obligatory blind Geophilomorpha is spherical in shape and also the most modified within the Chilopoda [17, 31]. The dominant component of the geophilid brain is the deutocerebrum and a clear demarcation between proto- and deutocerebrum is not detectable (Fig. 5C, F). In the investigated species, a distinct, unpaired midline neuropil is present (Fig. 5C, F). The antennal nerve comprises 10-15 bundles of sensory neurons and innervates the DC at its frontal edges (Fig. 5B - F). The deutocerebral hemispheres are fused posteromedially (Fig. 5B - F). In both investigated species, the OL is composed of spheroid to slightly ovoid OG. In *Stigmatogaster dimidiatus*, the OL appears slightly invaginated posteriorly (Fig. 5D, E: arrow) thus resulting in a cup-like shape. The number of OG is 49 in *Haplophilus subterraneus* and 97 in *Stigmatogaster dimidiatus* per hemisphere. A conspicuous contralateral connection features the OL of *S. dimidiatus*, where two elongated glomeruli extend a thin clc (Fig. 5E: arrow, F: asterisks). However, in *H. subterraneus,* a clc of the OL is not present. The OG appear compact without any subcompartments and a bilateral symmetry seems to be present (Fig. 5B - F). In both investigated species, an enlarged ventral glomerulus is found (Fig. 5F: vog; not shown in the reconstruction of *H. subterraneus*). The CL is located posterioventrally to the OL (Fig. 5B - F) and features a thin contralateral connection (Fig. 5F). In the autofluorescence preparations, this thin connection could not be depicted clearly. In most of the preparations, the CL appears lamellar (Fig. 5B, D inset; arrows). Posteriorly directed antennal neurite projections were revealed by neurobiotin backfills, which showed a bundle of neurites projecting from the antennal nerve, through the tritocerebrum (asterisk in inset Fig. 5D) and probably into the subesophageal ganglion *via* the circumesophageal connectives (Fig. 5D: cec). Interestingly, several somata were filled by neurobiotin in the ventral brain (Fig. 5D left: arrow) branching intensively in an anteroposterior direction. Whether these somata belong to projection- or local interneurons remains unknown.

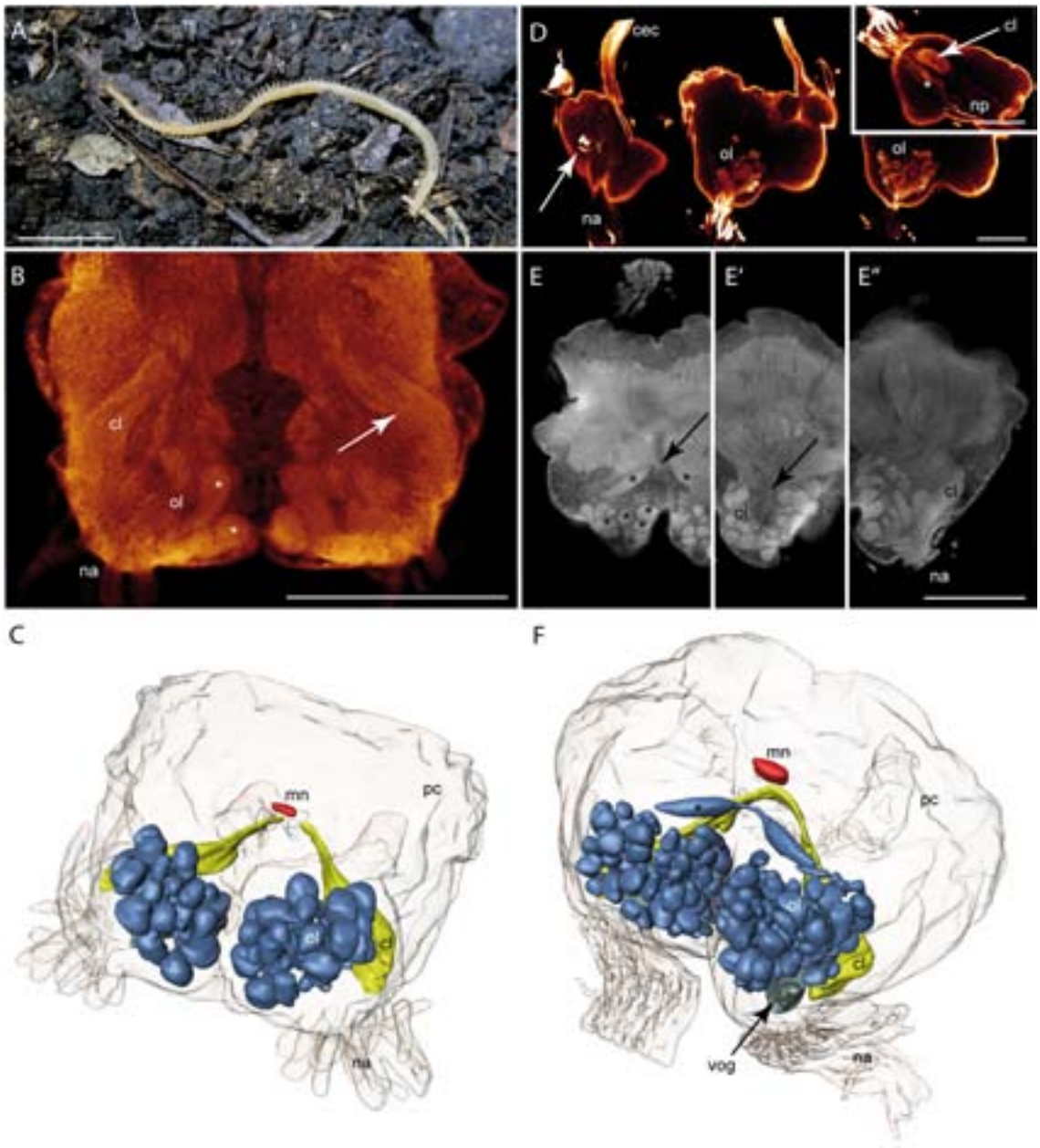

Figure 5. A *Geophilus carpophagus*. **B** Single horizontal optical section (cLSM) of an autofluorescence preparation of the brain of *Haplophilus subterraneus* showing olfactory glomeruli (asterisks) and the structural composition of the Corpus lamellosum (arrow). **C** 3D reconstruction of the brain of *H. subterraneus* with deutocerebral neuropils and midline neuropil. Blue = olfactory glomeruli, yellow = Corpus lamellosum, red = midline neuropil. Contralateral connection of the CL is not shown. **D** Single horizontal optical sections (cLSM) of a neurobiotin backfill of the right antennal nerve in *Stigmatogaster dimidiatus* from ventral to dorsal. Left: several somata stained by the neurobiotin backfill (arrow) and neurite projections into a circumesophageal connective. Middle: Antennal nerves and olfactory glomeruli. Right: The slightly concave appearance of the olfactory lobe. Inset: Sagittal optical section of the same preparation showing the structural composition of the Corpus lamellosum (arrow) and neurite projections (asterisk). **E** Single horizontal optical sections (cLSM) of an autofluorescence preparation of the brain of *S. dimidiatus* from dorsal to ventral. Olfactory glomeruli (asterisks) and the contralateral connection between the posterioventral OG (arrow). **E'** Concave appearance of the olfactory lobe (arrow). **E''** ventrolateral position of the Corpus lamellosum. **F** 3D reconstruction of the brain of *S. dimidiatus* with deutocerebral neuropils and midline neuropil. Blue = olfactory glomeruli, gray = bigger ventral olfactory glomerulus, yellow = Corpus lamellosum, red = midline neuropil. **Abbreviations: cec** circumesophageal connective, **cl** Corpus lamellosum, **mn** midline neuropil, **na** nervus antennalis, **np** neurite projections, **ol** olfactory lobe, **pc** protocerebrum, **vog** ventral olfactory glomerulus. **Scalebars:** A = 5 mm, B, D, E = 100 µm.

Discussion

Chilopoda: two separate deutocerebral neuropils

Fahlander [13] described the nervous system of various Chilopoda and also interpreted the results from Saint-Rémy [8] and Hörberg [12] in a broad comparative study. Although previous authors mentioned a glomerular organized antennal lobe for the Chilopoda (e.g. [10-13, 32]), the number, organization, and structural composition remained unclear. Sombke et al. [14] reinvestigated the deutocerebral neuropils in *Scutigera coleoptrata* using a variety of histological and immunhistochemical methods. Similar to *Scutigera coleoptrata* [14], the deutocerebrum of the Chilopoda investigated here is organized into structured neuropils that can be divided into two different regions: olfactory lobe and Corpus lamellosum (Fig. 6, 7). Moreover, a similar organization of deutocerebral neuropils may be present in representatives of the Diplopoda ([33], Seefluth and Sombke unpublished data.) as well as in representatives of the Hexapoda and Crustacea.

The chilopod olfactory lobes and olfactory glomeruli

In principle, the olfactory lobe (OL) extends from the entrance of the antennal nerve into the brain on towards the dorsomedian brain and is composed of olfactory glomeruli (OG) which are located in the anterior part of the deutocerebrum. The innervation of the OG reaches it from its distal borders. As a result of different innervation angles of the antennal nerves, the overall orientation of the olfactory lobes differs in the Chilopoda (Fig. 7). In *Scutigera coleoptrata,* the bilateral OLs are arranged in an angle of 180° to each other, while, for example in *Lithobius forficatus,* this angle is approximately 90°. In *Cryptops hortensis* and the investigated Geophilomorpha, the two OLs are arranged more or less in parallel. The alignment of the OG also differs in investigated taxa: while in *S. coleoptrata* the OG form more or less parallel layers, the drop-like shape in *L. forficatus*, *C. tasmanianus* and the representatives of the Scolopendromorpha results in a more compact arrangement. A central coarse neuropil in the OL in these taxa is not present. In contrast to the remaining chilopod taxa, the OL of the Geophilomorpha appears globular and slightly invaginated (Fig. 6, 7). The presence of a central coarse neuropil in the OL of Geophilomorpha is uncertain. In many hexapod taxa, the glomeruli surround a coarse neuropil e.g. Dictyoptera [34], Hymenoptera [35], Lepidoptera and Diptera (reviewed in [15]). Contrary, in Archaeognatha the OL is composed of elongated OG without a central neuropil [30]. In malacostracan Crustacea, the OG are arranged in a peripheral radial array that surrounds a loose core of neuronal processes (reviewed in [15, 28]). The innervation of single glomeruli in these animals is from the periphery [15].

In *Scutigera coleoptrata*, 34 individually identifiable OG per olfactory lobe were detected repeatedly in several specimens and these glomeruli form a fixed array, so that individual OG are identifiable [14]. In the present study, glomerular numbers were only determined in few specimens, so that the numbers have to be viewed with caution. Nevertheless, we speculate that the determined numbers are taxon-specific within the Chilopoda. In *Lithobius forficatus*, 43 OG were detected, and 36 in *Craterostigmus tasmanianus*. In the investigated Scolopendromorpha, the number of OG ranges around 50-60, while in the Geophilomorpha, some variation was encountered (49 in *H. subterraneus*, 97 in *S. dimidiatus*). In hexapods, the number of

olfactory glomeruli ranges from about 20 in Collembola to approx. 250 in ants (reviewed in [15, 29]) and seems to be invariant within species (e.g. [35-45]). In Crustacea the number of OG varies from approximately 150 to 1300 (reviewed in [4, 15, 46]) but it is uncertain if crustaceans have a fixed set of OGs [46, 47].

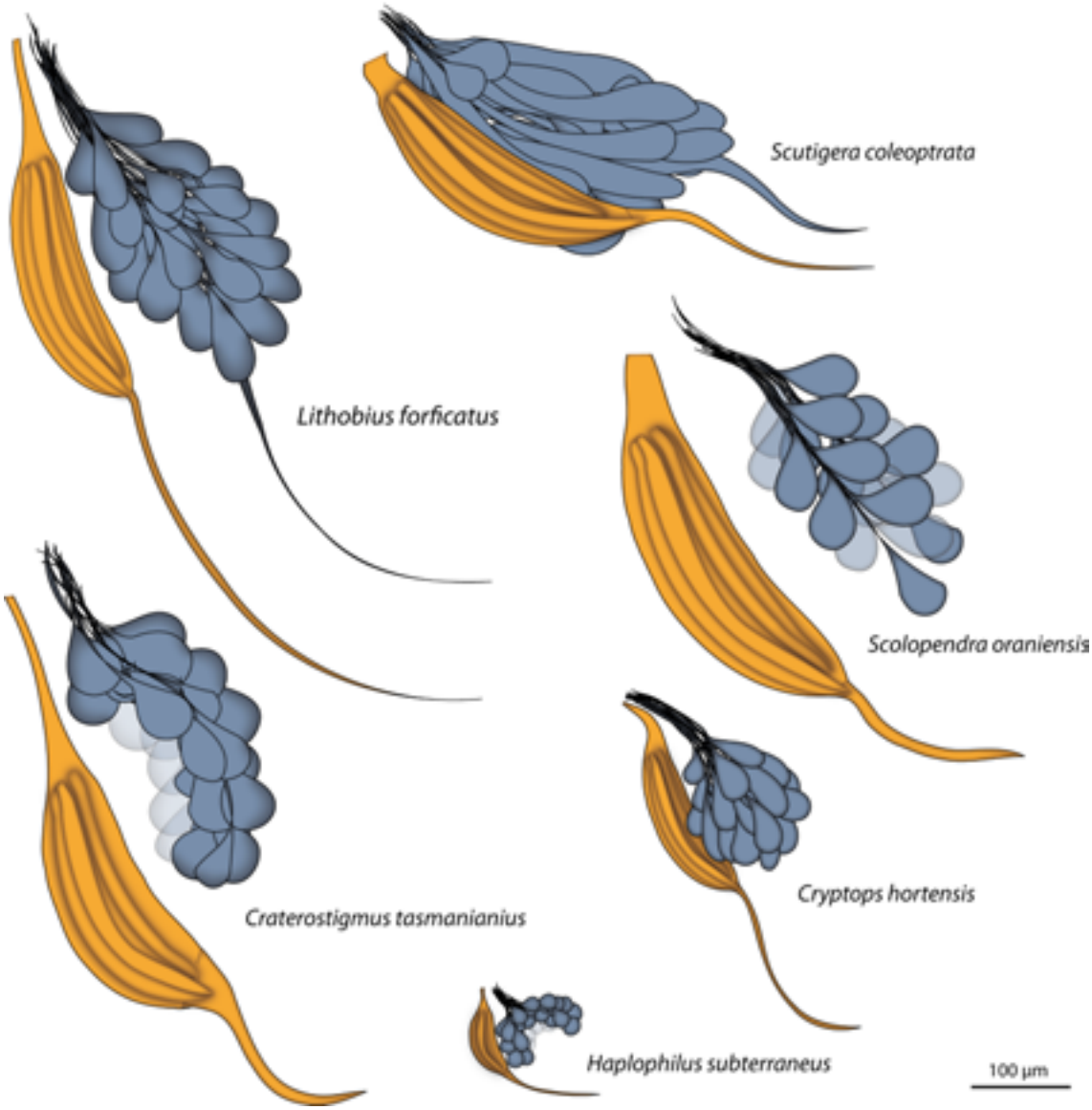

Figure 6. Schematic representation of the deutocerebral neuropils in representatives of the Chilopoda (left hemisphere). Horizontal view with equal scaling.

The number of glomeruli is generally thought to provide a good indication regarding how many different olfactory receptor proteins (OR) are expressed in the antenna. One OSN typically expresses a single OR, and all OSNs expressing a specific receptor project their axons to the same glomerulus. Odor input thus paints a map of activation over the glomerular array.

The size of OG is more or less taxon-specific and constant within the investigated chilopods (Fig. 6). The only exceptions are the ventrally located enlarged OG in

Scolopendra oraniensis, *Cryptops hortensis*, and *Stigmatogaster dimidiatus*. In general glomeruli of increased fitness-related importance tend to increase in size. Sex-specific enlargement ("macroglomeruli") are known from various hexapods e.g. moths [48], cockroaches [49] or honeybees [35]. Other enlargments have been found to be associated with trail pheromones and with specific food cues [50]. In this study a functional correlation was not conducted, and no conclusions regarding the functional significance of macroglomeruli in Chilopoda can be drawn.

In histological sections, backfills, and autofluorescence preparations, there is not any evidence of further compartmentalization of the OG in the investigated Chilopoda as it is known from hexapods and malacostracan crustaceans. In honeybees (Hexapoda), olfactory glomeruli have a concentric organization [35, 51-55], where only the periphery is innervated by axons of sensory neurons. A longitudinal subdivision of the OG into cap, subcap, and base has been well documented in crayfish, clawed and clawless lobsters, and hermit crabs (Crustacea) [2, 4, 56-60].

The shape of the OG in the investigated chilopods displays a considerable plasticity (Fig. 6, 7). OG in the Scutigeromorpha have an elongated shape and in some, the distal end is often thickened and/or bent posteriorly [14]. According to Fahlander [13], the internal organization of deutocerebral neuropils in *Lithobius forficatus* strongly resembles those of the Scutigeromorpha, but here we show that the shape of the actually OG differs. In *Lithobius forficatus* and *Craterostigmus tasmanianus*, the overall shape of OG ranges from elongated (more than two times longer than wide) to drop-shaped, with a smaller anterior diameter. In the Scolopendromorpha, the shape of OG is mostly drop-like to spheroid and in the Geophilomorpha, the OG have a spheroid shape. In most pterygote Hexapoda, the antennal lobe (or olfactory lobe) is organized into numerous roughly spheroid OG. However, in Archaeognatha the OG have an elongated shape [30]. The shape of crustacean olfactory glomeruli differs considerably in the investigated taxa (reviewed in [15]) ranging from slightly elongated in Leptostraca [11], over spheroid in Isopoda and Euphausiacea [3, 61]to cone-shaped. In many decapod Crustacea, the cone-like glomeruli are arranged in a radial array with their apices pointing inwards (reviews: [15, 20, 21, 28]). In some malacostracan taxa, these glomeruli may be extremely elongated [2, 4]. Within several taxa of the Chelicerata, spheroid neuropil units have been reported but these are not associated with the second brain neuromere [62-67].

In this context, the question arises if the shape of olfactory glomeruli is of purely functional significance or if it does contain an unexplored phylogenetic signal. Clearly, considering glomerular shape alone is not sufficient to answer these questions. Looking in more detail at the projection patterns and connectivity of the neuronal key players that form the glomeruli may be imperative to explore this aspect: afferents of the OSNs, connectivity with local interneurons, and morphology of olfactory projection neurons. For the Chilopoda, it is unclear if elongated or spheroid glomeruli represent the ancestral shape of the OG this group. As laid out above, both morphs occur in Chilopoda and transitional forms exist. Here, we take benefit from the fact that the debate on the internal phylogeny of Chilopoda rather unequivocally gravitated into accepting the Pleurostigmophora concept of Verhoeff [68] in the past four decades so that we can map our results on a stable phylogeny of Chilopoda. Based on the number and position of stigmata, this phylogenetic concept separates the Scutigeromorpha (= Notostigmophora) as sister group to all other Chilopoda (Pleurostigmophora). This

phylogenetic concept has received substantial support from the analysis of morphological, molecular and combined morphological-molecular data sets (e.g. [69-74]). Based on this phylogeny, it seems legitimate to assume that elongated OG mark the plesiomorphic state in Chilopoda, perhaps retained from the myriapod, or even the mandibulate ground pattern. The exclusive occurrence of spheroid OG has to be considered an additional apomorphy of the Geophilomorpha.

The Corpus lamellosum

In a brief description of deutocerebral neuropils in *Lithobius variegatus*, Strausfeld et al. [32] described that the antennal nerve innervates the olfactory lobe and that a lateral strand projects to a region behind it, which the authors called dorsal lobe in analogy to the hexapod mechanosensory neuropil. This posterior deutocerebral neuropil in Chilopoda had already been termed "*masse lamelleuse"* by Saint-Remy [9] and latinized by Fahlander [13] who called it Corpus lamellosum (CL). Because it reflects the characteristics of this structured neuropil, we suggest maintaining this nomination. As mentioned above, in *Scutigera coleoptrata* the posterior partition of the antennal nerve innervates the CL in which approximately eight parallel lamellae were found [14]. Golgi impregnations showed that sensory neurites innervating the CL appear much thicker than those innervating the olfactory glomeruli and give off short side branches along their length [14]. Although similar Golgi experiments on other chilopod taxa have not been conducted yet, it appears to us that the architecture of the CL in the other chilopod taxa investigated here is similar to that of *S. coleoptrata*. In *S. coleoptrata*, the parallel lamellae project dorsomedially and extend into the posterior deutocerebral commissure [13, 14]. By backfilling the antennal nerve in *Lithobius forficatus*, at least 4 single lamellae are visible. The report of Fahlander [13] that in *Lithobius forficatus* the CL is not composed of distinct lamellae can therefore be rejected. In *Craterostigmus tasmanianus*, a lamellation is only partially visible. In the Scolopendromorpha, backfill experiments also show an arrangement of parallel fibers suggesting a lamellation in the investigated genera. In the Geophilomorpha, the CL also appears lamellar. Possibly, due to a higher degree of condensation, single lamellae could not be detected. In summary, all the investigated chilopods exhibit a CL, which is composed of parallel lamellae and features a contralateral connection.

In pterygote Hexapoda, the first and second antennomeres of the antenna supply the dorsal lobe (mechanosensory neuropil), whereas the flagellar sensilla are mostly specialized for olfactory perception and their neurites project into the olfactory lobe [49]. Mechanosensory neuropils with a general striate or palisade shape are also known from apterygote hexapods and various crustaceans (reviewed in [75, 76]). For instance, in *Thermobia* sp. (Hexapoda: Zygentoma) the mechanosensory neuropil is organized in a columnar and striated manner [75]. Examples where mechanosensory and gustatory afferents project into the posterior region of the deutocerebrum and the anterior subesophageal ganglion are e.g. *Periplaneta americana* [55, 77], *Apis mellifera* [78], *Gryllus bimaculatus* [79, 80], and *Aedes aegypti* [26, 81]. In these organisms, presumptive tactile antennal afferents provide two pairs of long branches and several short branches are orientated laterally and form a multilayered arrangement medially in the dorsal lobe. This arrangement exhibits a similarity to the branching pattern of sensory axons in the CL of *Scutigera coleoptrata* [14]. In

malacostracan crustaceans, the first pair of antennae, which are mostly chemosensory, is associated with the deutocerebrum. The second, mostly mechanosensory pair of antennae is associated with the tritocerebrum [15, 18-20, 28, 47, 56, 82]. Mechanosensory and non-aesthetasc chemosensory input from the first antennae is processed in the lateral and median antennular neuropil (LAN and MAN) [18-21, 28]. Between the lobes of the LAN, contralateral connections occur in Decapoda. The general organization of the LAN and MAN in many respects matches the innervation and connections of the CL. However, in decapod crustaceans with long second antennae, only the tritocerebral antenna 2 neuropil (AnN) exhibits a serial transverse arrangement of synaptic fields [76].

To summarize, the posterior deutocerebrum in Chilopoda, Hexapoda, and Crustacea is characterized by at least one neuropil (Corpus lamellosum, dorsal lobe, lateral and median antennular neuropil) that presumably processes mechanosensory input from the first pair of antennae. Such a neuropil is absent in Chelicerata.

Posterior neurite projections

In all investigated Chilopoda (except for *C. tasmanianus*), antennal afferents also project into the subesophageal ganglion. These neurites project ipsilaterally and bypass the deutocerebral neuropils. In addition, in *S. coleoptrata* a second projection to the ventrolateral protocerebrum was found [14]. We speculate that these posterior neurites may project to a gustatory or motoric center in the subesophageal ganglion. In Crustacea, these characteristic posterior neurite projections have not yet been described. However, in Hexapoda, certain neurites from the antennal nerve also project to the subesophageal ganglion and the thoracic ganglia [55, 83-85]. Barrozo et al. [85] suggested that these neurite projections with a characteristic larger diameter might serve to insure a rapid neuronal transmission of sensory inputs towards centers responsible for controlling motor activities and physiological processes.

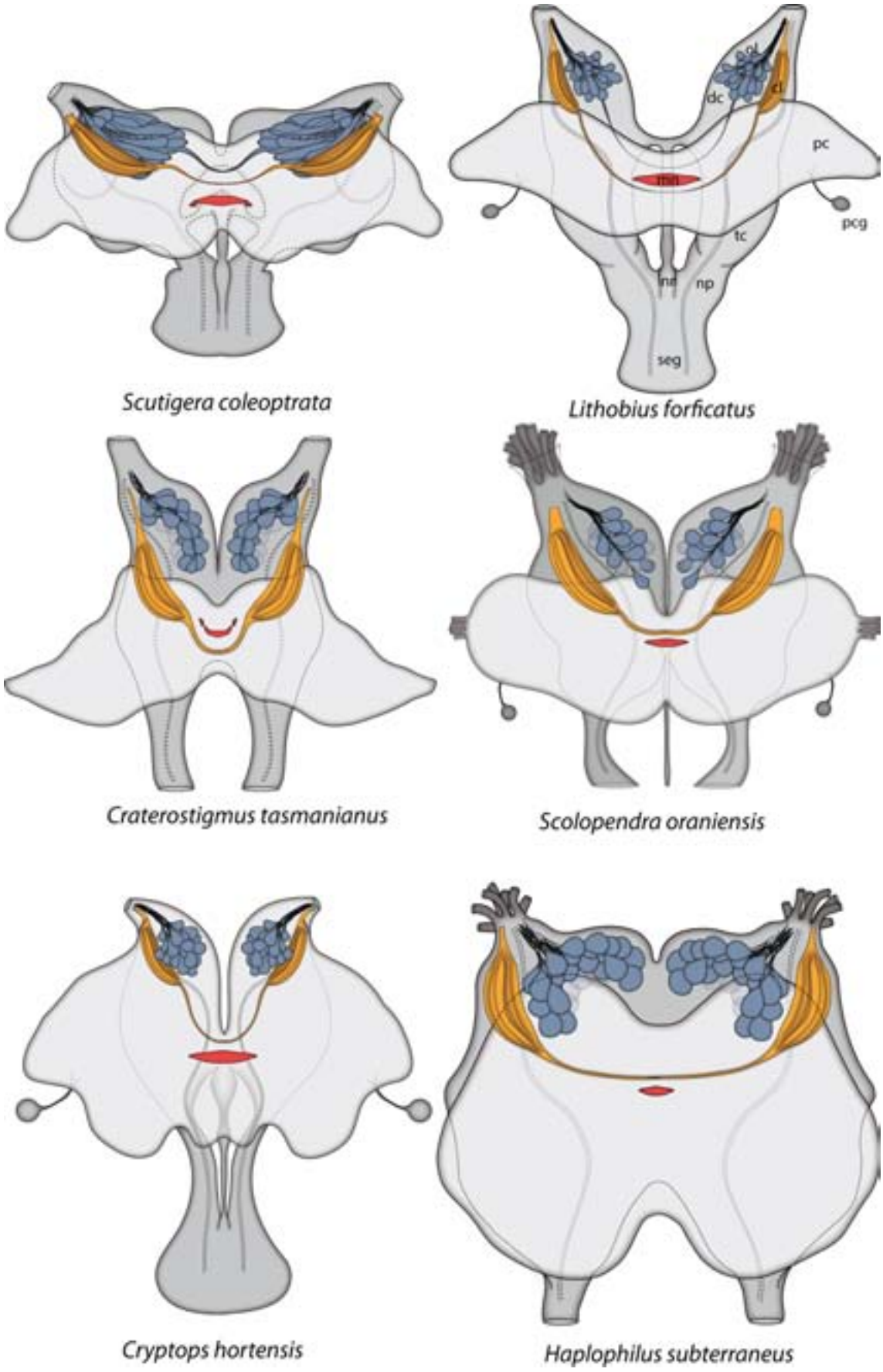

Figure 7. Schematic representation of the brains of selected Chilopoda with illustration of the olfactory glomeruli (blue), Corpus lamellosum (yellow), Midline neuropil (red) and neurite projections (dashed lines). View from dorsal. The protocerebrum appears brighter. Protocerebral glands are not illustrated for all taxa.

Abbreviations: cl Corpus lamellosum, **dc** deutocerebrum, **mn** midline neuropil, **np** neurite projection, **nr** nervus recurrens, **ol** olfactory lobe, **pc** protocerebrum, **pcg** protocerebral gland, **seg** subesophageal ganglion, **tc** tritocerebrum.

The deutocerebrum and olfactory lobes of Euarthropoda in an evolutionary context: support for the Mandibulata concept

In Chilopoda, Crustacea, and Hexapoda, distinct neuropils for processing sensory information of the (first pair of) antennae are located in the deutocerebrum. According to *Hox*-gene expression patterns and morphological investigations, the deutocerebrum and the deutocerebral antennae are homolog within Mandibulata and correspond to the chelicere neuromere in Chelicerata [86-88]. Although in some Chelicerata glomerular chemosensory processing areas associated with a sensory appendage are located in the trunk ganglia (e.g. [66]), distinct neuropils for processing of chemo- and mechanosensory information have not yet been reported for their second brain neuromere. Moreover, a characteristic divergence of sensory neurites and the presence of a mechanosensory neuropil are not realized in Chelicerata.

In the Chilopoda, the deutocerebrum is characterized by two distinct neuropil regions, which are innervated by antennal sensory afferents. The olfactory glomeruli are bilaterally and symmetrically arranged and appear presumably in a taxon-specific fixed number. Contralateral connections occur in some species. The Corpus lamellosum is a structured neuropil and exhibits a contralateral connection. In the Chilopoda, antennal neurite projections transit the deutocerebrum and project into the subesophageal ganglion. The deutocerebrum of representatives of the Diplopoda is organized in a similar way ([33], Seefluth and Sombke, unpublished data), and above we have laid out that this myriapod pattern of organization displays many similarities to malacostracan Crustacea and Hexapoda.

Our most important conclusion is that the presence of a bifunctional deutocerebrum composed of distinct neuropils for chemo- and mechanosensory qualities is homologous in Chilopoda, Diplopoda, Hexapoda and Crustacea and can therefore be postulated as an apomorphic character complex for the Mandibulata. In this view, the absence of olfactory lobes in various Crustacea (Branchiopoda and certain “Maxillopoda”) and Hexapoda (Odonata, certain Hemiptera and Coleoptera) as well as the absence of the mechanosensory neuropils in Cephalocarida (Crustacea; [89]) can be interpreted as a reduction. Thus, neuroanatomical data strongly contradict a sistergroup relationship of Myriapoda and Chelicerata (“Myriochelata; [7]), but instead support the Mandibulata concept (e.g. [5, 6]).

Methods

Experimental animals

Specimens were collected on the Balearic Island Ibiza (Spain) mainly in pine forests or in Germany mainly in litter and soil. Specimens of *Craterostigmus tasmanianus* were collected by Robert Mesibov in Tasmania. If not fixed directly after capture, individuals were kept in plastic tubes (50 ml; Carl Roth, Germany) at room temperature. For keeping of animals, they were transferred into plastic boxes supplied with bark and water. They were fed with *Drosophila melanogaster* or juveniles of *Achaeta domestica*.

Representatives of all five chilopod subtaxa were investigated: (1) *Scutigera coleoptrata* (Linnaeus, 1758), Scutigeromorpha: Scutigeridae; collected in Spain: Ibiza. (2) *Lithobius forficatus* (Linnaeus, 1758), Lithobiomorpha: Lithobiidae; collected in Germany: Aachen, Greifswald. (3) *Craterostigmus tasmanianus* Pocock, 1902, Craterostigmomorpha; collected in Australia: Tasmania. (4) *Cryptops hortensis* (Donovan, 1810), Scolopendromorpha: Cryptopidae; collected in Germany: Aachen, Greifswald. (5) *Scolopendra oraniensis* Lucas, 1846, Scolopendromorpha: Scolopendridae; collected in Spain: Ibiza. (6) *Scolopendra subspinipes* Leach, 1815, Scolopendromorpha: Scolopendridae; ordered from btbe Insektenzucht GmbH, Germany (http://www.futtertiere24.de/). (7) *Haplophilus subterraneus* (Shaw, 1794), Geophilomorpha: Himantariidae; collected in Germany: Aachen, Greifswald. (8) *Stigmatogaster dimidiatus* (Meinert, 1870), Geophilomorpha: Himantariidae; collected in Spain: Ibiza.

Histology

For section series, several individuals were anesthetized, decapitated and prefixed for 24 h in a solution of 80 % ethanol, 37 % formaldehyde and 100 % acetic acid (10:4:1). After washing in sodium hydrogen phosphate buffer (PBS, pH 7.4), specimens were postfixed for 1 h in 2 % OsO_4 solution (same buffer) at room temperature and, following dehydration in a graded series of acetone, embedded in Araldite (Araldite epoxy resin kit, Agar Scientific). Serial semithin sections (1 – 1.5 µm) were prepared with a Microm HM 355 S rotary microtome and stained using 1 % toluidine blue and Pyronin G in a solution of 1 % sodium tetraborate.

Autofluorescence preparation

For autofluorescence analysis, specimens were anesthetized and decapitated. Dissected brains were fixed in a solution of 4 % paraformaldehyde and 4 % glutaraldehyde (1:1) for at least one week at 4 °C. After several washing steps in PBS, brains were dehydrated in a graded series of ethanol and embedded in methyl salycilate. For cLSM, an excitation of 488 nm was used to detect autofluorescence from the nervous tissue.

Antennal Backfilling

For antennal backfills, specimens were anesthetized and mounted in plastic Petri-dishes. One antenna was cut and the antennal nerve was exposed. For neurobiotin backfills, the antennal nerve stump was isolated in petroleum jelly, covered by aqua dest. for two minutes, and subsequently exposed to 5 % neurobiotin (Vector Laboratories) being dissolved in aqua dest. Preparations were incubated at 4 °C for 1 day. After final dissection and fixation in 4 % paraformaldehyde for 24 hours, the preparations were washed in several changes of PBS and incubated in streptavidin conjugated to Cy3 (1:2000, Jackson Immunoresearch) for 24 hours. After washing in several changes of PBS, the preparations were dehydrated in a graded series of ethanol and mounted in methyl salycilate. In controls the brains of which were not subjected to backfills, incubation in streptavidin alone resulted in an absence of all labeling. For Lucifer yellow backfills, the treatment of the antennal nerve stump was the same as for neurobiotin backfills, but instead of washing and incubating, the preparations were directly dehydrated in a graded series of ethanol after fixation and mounted in methyl salycilate.

Microscopy, 3D reconstruction, and terminology

Wholemounts and brain sections were examined with a Nikon eclipse 90i microscope and a Leica SP 5 II confocal laser scanning microscope (cLSM). All images were processed with Adobe Photoshop using global contrast and brightness adjustment features.

The alignment and 3D reconstruction was made using AMIRA 5.1 (Visage Imaging) operated on a FS Celsius work station. In each section, contours of the nervous system and neuropilar regions were demarcated and a 3D reconstruction was generated. The 3D reconstruction of the brain of *Craterostigmus tasmanianus* was generated by merging two reconstructions of single brain hemispheres of the same specimen.

The neuroanatomical terminology is according to Richter et al. [23].

Authors' contributions

AS and EL conducted the sampling, preparation and fixation of brains, backfill experiments and the 3D reconstructions. MK prepared the schematic representations of the chilopod brains. AS drafted the main part of the manuscript and all other authors assisted in drafting the manuscript. All authors read and approved the final manuscript.

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