Evolutionary process of thoracic jumping ability in Hemiptera

半翅目昆虫における胸部内跳躍機能の進化プロセス

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General Abstract

昆虫が全真核生物の 4 分の 3 に達するほどの圧倒的な種多様性を実現した要因は、現在まで生物学全体の興味の的であり続けてきた。一般的に、適応的な新奇形質の獲得は、生物の適応放散を駆動する大きな原動力と考えられている。昆虫にみられる最も顕著な新規形質である翅の獲得は、効率の良い移動を可能としただけでなく、虫体の保護などを可能にし、適応放散を支えた大きな要因として、広く認識されている。

翅はその誕生以降、飛翔機能の向上や、二次的な新機能の獲得などを経て、多様化を続けてきた。ところが、翅を備える胸部は多くの場合、歩行筋と飛翔筋にその大部分を占有されており変異に乏しい。胸部の多様性の欠如は、多様化した昆虫の頭部や腹部（交尾器）と比較しても顕著である。しかし、なぜ胸部だけが機能的な多様性に乏しいのかという視点は看過されてきた。この疑問に答えるには、胸部機能が多様化している昆虫を材料に、多様化前後の形質変化を検討し、胸部の多様化を妨げている制約が何かを明らかにする必要がある。そこで本学位論文では、極めて例外的に胸部構造が多様性している半翅目の胸部内機能、とりわけ跳躍機能に注目した。

半翅目は、昆虫有数の巨大分類群で、不完全変態群では最大の種多様性を誇る。半翅目の最大の特徴として、発達した飛翔筋を持ちながら、4 亜目全てが跳躍などの新奇機能を胸部内に持つ点が挙げられる。このような半翅目の胸部に見られる特殊化は、昆虫胸部の機能的な多様化を抑える要因と、多様性をもたらす要因の両方を検証できる素晴らしいモデルである。このような特徴を持つ半翅目を用い、新奇機能の獲得前後の形質を比較してその差分を検討することで、胸部に存在する制約と、その制約を打ち消す新奇形質を明らかにできるだろう。

第一章では、強力な跳躍機能を持つ頸吻亜目（Auchenorrhyncha）を対象として形態学的研究を行った。従来、半翅目の代表的な胸部内機能である跳躍については、半翅目の共通祖先で獲得されたとする説と、独立に各グループが獲得したとする説の対立する 2 仮説が提示されていた。しかしいずれの仮説も、跳躍に関わる形質の詳細な解析に基づく検証はなされていない。そこで、上記対立仮説の検証を目的として、頸吻亜目を構成するセミ型下目・ハゴロモ型下目において、跳躍機能を持つ群・持たない群それぞれの内部構造を広範に精査した。その結果、セミ型下目およびハゴロモ型下目では、いずれも構造的に類似した跳躍機能を持つものの、異なる脚制御筋が跳躍筋に変化していることが確認された。このことから、胸部内跳躍機能は頸吻亜目の祖先形質ではなく、二つの下目で独立に獲得されたことが示された。また、脚の筋肉相だけでなく、飛翔筋の縮小状態や内骨格の構造もこの結果を支持した。このことは、頸吻亜目以外の 3 亜目においても独自に胸部内跳躍機能が獲得された可能性を示唆している。

第二章では、半翅目の共通祖先の胸部の祖先形質状態を推測するべく、原始的な形質状態を最も残している腹吻亜目（Sternorrhyncha）を対象とした研究を行った。本亜目に属する全 4 上科のうち、コナジラミおよびキジラミで跳躍行動が知られるが、跳躍に関わる形質の情報は断片的で、その進化系列は未解明あった。そこで、亜目内全てを対象として、形態観察と生態情報の精査を行い、祖先形質を最尤復元した。その結果、腹吻亜目の祖先は跳躍機能を持たず、跳躍を行う 2 上科でそれぞれ独立に跳躍機能が進化したことが示唆された。以上から、頸吻亜目同様、腹吻亜目の共通祖先も跳躍機能を持たず、祖先状態に近い筋肉相を保持しており、跳躍機能は腹吻亜目内で 2 回独立に進化していたと結論付けられた。

以上、第一章・第二章の結果から、半翅目の祖先は、他の跳躍を行わない昆

虫と同様の胸部構造を持っていたにもかかわらず、既存の飛翔や歩行の機能を保ちながら、新奇な跳躍機能の獲得などの改変が繰り返し起こっていることが示された。また、いずれの場合においても飛翔筋の縮小や消失が跳躍機能の獲得とともに観察されたことから、半翅目には、飛翔機能を司るために必要とされる筋肉の容積的な制約を打ち消し、後胸に新しい機能を収納する余地を創出する要因が存在すると考えられた。これを実現する具体的な要因として、中胸への飛翔機能の集約が可能性の一つとして想定された。

そこで第三章では、中胸への飛翔機能の集約を実現した要因として、翅構造、とりわけ前後翅の連結構造に注目して形態学的研究を行った。翅連結構造は、飛翔中に 4 枚の翅を機械的に結合し、2 枚の翅として振る舞わせる構造である。この構造は、半翅目を含む準新翅類に幅広く知られているが、その詳細な進化史については十分に検討されていなかった。そこで本章では、半翅目における翅連結構造の起源を検討するため、半翅目の外群となる咀顎目（チャタテムシ類）の翅連結構造の形態を検討した。その結果、咀顎目は半翅目とは独立に翅連結構造を獲得していたことが明らかになった。同じく半翅目の外群である総翅目（アザミウマ類）や、半翅目各群の既知の形態情報を総合すると、半翅目を含む準新翅類では、それぞれの目に分化してから独立に連結構造が獲得されたことが示唆された。さらに、チャタテムシ・アザミウマではそれぞれの祖先でただ一回連結構造が獲得されていたのに対し、半翅目では腹吻亜目、頸吻亜目、そして異翅亜目＋鞘吻亜目のそれぞれで構造の違いが認められ、連結構造が独立に複数回進化していたことが示唆された。また、多くの場合において、跳躍機能に先立って翅連結構造が進化していたことが示唆されたことから、跳躍機能と翅連結構造の進化には関連性があると考えられた。しかし、跳躍機能を持ちながら翅連結構造を欠くコナジラミのような例もあることから、翅連結構造の獲得は後胸の改変を実現した唯一の要因とまでは言えず、他の形質の関

与が暗示された。

以上から半翅目では、他の昆虫には見られない、著しい後胸機能の改変が少なくとも 4 回、独立に進化したことが確認された。また、同時に後胸内の飛翔筋が縮小または消失していたことから、一般的な昆虫において胸部の機能的多様化を妨げているのは飛翔筋がもたらす胸郭の容積的な制約であることも示唆された。しかし、その一方で、その制約を解消する要因については、まだその全容を解明するに至っていない。今後は、まず胸部内跳躍機能および翅連結構造について、半翅目全体に広げた網羅的な形態精査と進化史の解明が急務である。また、跳躍機能と翅連結構造の両者の因果関係を適切な統計手法を用いて検討するとともに、遠縁の分類群も含めた形態解析を通じ、翅連結構造以外の要因の探索を行うことも必要である。

General Introduction

昆虫の最大の特徴は、全真核生物の 4 分の 3 に達するほどの圧倒的な種多様性である。昆虫がこのような多様化を実現した要因は、現在まで生物学全体の興味の的であり続けてきた。 一般的に、機能・形態を含む適応的な新奇形質の獲得は、生物の適応放散を駆動する大きな原動力である (Hall and Olson, 2003)。昆虫でも、翅や蛹のステージを新奇に獲得したことが、爆発的多様化を実現した最大の要因であると考えられている (Grimardi and Engel, 2005)。特に翅の獲得は、飛翔による効率の良い移動を可能とし、昆虫の適応放散に大きな影響を与えたと考えられている (Brodsky, 1994; Misof et al., 2014a)。また、硬化した前翅が腹部を保護する"鎧"となるなど (Linz et al., 2016)、翅に生じた新たな機能も翅の獲得による多様化の一部といえよう。

翅の出現以降も、トンボやチョウ、カブトムシを比較しても分かるように、翅に生じた新奇形質により、それぞれの昆虫ごとに飛翔能力や翅の形態は多様化している (cf. Reed et al., 2011)。また、頭部や腹部にも、ヤゴが持つマジックハンド状の口器や、雌雄が逆転した交尾器など、数々の新奇な形態や機能の進化が知られており、盛んに研究が進められている (Yoshizawa et al., 2014; Büsse et al., 2017) 。一方、翅を備える胸部を見ると、ほとんどの昆虫が歩行筋と飛翔筋に胸部の大部分を占有されており、その基本構造や機能は変異に乏しい。しかし、なぜ胸部だけが機能的な多様性に乏しいのかという視点は、これまでに全く提示されてこなかった。この疑問に答えるには、胸部機能が多様化している昆虫を材料に、多様化前後の形質変化を検討し、胸部の多様化を妨げている制約が何かを明らかにする必要がある。そこで本学位論文では、胸部構造に顕著な多様性が見られる半翅目の胸部内機能、とりわけ跳躍機能に注目した。

跳躍機能は、動物全体において広く見られる運動機能であり、捕食者からの

逃避・加速・飛翔前の離陸等に有効であると考えられている (Alexander, 1995; Burrows, 2003)。昆虫においても、直翅目、網翅目・咀顎目・半翅目・長翅目・隠翅目・双翅目・甲虫目・膜翅目・脈翅目といった幅広い仲間から報告されており、様々な系統で繰り返し進化してきたと考えられている (Furth and Suzuki, 1992; Picker et al., 2011)。跳躍には、そのエネルギーを生み出すための巨大な跳躍筋が必要である。しかし、胸部の容積の大半は、飛翔力を生み出す間接飛翔筋で占められており (Snodgrass, 1935; Brodsky, 1994; Chapman, 2013)、他の機能が進化する余地はないと考えられる。実際に、跳躍を行う昆虫のほとんどは、バッタに代表されるように、胸郭外部の構造、つまり後腿節を長く大きく発達させ、跳躍脚として進化している (Snodgrass, 1935)。この構造は、飛翔筋に干渉せずして跳躍機能を収納できるという点においては妥当なものであるが、長すぎる脚は跳躍そのものの効率にむしろ負の影響を与え (Burrows and Sutton, 2008)、また本来の機能である歩行に対しても悪影響があると考えられる。

一方、脚の巨大化を伴わない跳躍機構として、胸部内の転節制御筋を跳躍筋として発達させる胸部内跳躍機能が一部の昆虫に知られている。しかし、前述したような問題から、無翅のシリアゲムシやノミなど、既に飛翔機能を失った分類群 (Burrows, 2009a, 2011) や、飛翔とは無関係な前胸にその機能を持つ甲虫 (ミジンキスイ類・ノミヒゲナガゾウ類; Ogawa, 2017, unpubl. data; Yoshida, 2018, pers. comm.) など、飛翔筋との干渉が本質的に起こらない状況下で跳躍機能が獲得されているものが多い。同一胸郭内において飛翔筋と共存した跳躍機能を持つ昆虫は、半翅目以外では甲虫目、膜翅目のごく一部でしか知られておらず、例外的な状態であると言える (Furth and Suzuki, 1992)。

しかし半翅目は、発達した飛翔筋を保持しながら、なおかつ 4 亜目全てで幅広く跳躍機能を胸部内に獲得している点で極めて特異なグループである (Burrows, 2013; Chapman, 2013)。このような半翅目の胸部に見られる特殊化は、

昆虫胸部の機能的な多様性が低い原因と、昆虫胸部に多様性をもたらす要因の両方を検証できる素晴らしいモデルである。このような特徴を持つ半翅目を用い、跳躍機能の獲得前後の形質を比較してその差分を検討することで、胸部に存在する制約と、その制約を打ち消す要因の双方を明らかにできるだろう。そのためには、跳躍機能の進化史を推定し、その進化前後における形態変化から制約の存在を実証する必要がある。また、制約の解消を実現した可能性のある形質についても同時に検討するべきであると考えられる。

第一章では、知られている限りにおいて全昆虫の中で最強の跳躍機能 (Burrows, 2009b) を持つ頸吻亜目 (Auchenorrhyncha) を対象とした形態学的研究を行った。従来、半翅目の代表的な胸部内機能である跳躍については、半翅目の共通祖先で獲得されたとする説と、独立に各グループが獲得したとする説の対立する 2 仮説が提示されていた。しかし、前者は化石の外骨格的特徴を根拠にしたものであり、後者は運動機能の解析を対象とした論文において副次的に示唆されたものであることから、いずれも詳細な形態観察に基づく仮説ではないという問題点があった。そこで、上記対立仮説の検証を目的として、頸吻亜目を構成するセミ型下目・ハゴロモ型下目それぞれに含まれるすべての上科を対象とし、跳躍機能を持つ群・持たない群の胸部筋肉相を精査した。

第二章では、半翅目の胸部の祖先形質状態 (グラウンドプラン) を推測するべく、半翅目の中で最も原始的な形質を保持している腹吻亜目 (Sternorrhyncha) を対象として、同じく形態学的な研究を行った。腹吻亜目はアブラムシ、キジラミ、カイガラムシ、コナジラミの 4 上科からなる亜目である。中でも、英名の "jumping plant lice"が示す通り、キジラミは跳躍行動を示すことでよく知られているが、他の科での跳躍に関する知見は断片的な報告にとどまっていた。例えば、本研究の基礎をなすべき胸部内跳躍行動はキジラミ、コナジラミから知ら

れるが、キジラミ以外ではその跳躍機能の機能解析や進化機序の検討が詳細に行われたことはなかった。そこで、腹吻亜目における跳躍機能の進化を明らかにするため、腹吻亜目に属する全 4 上科を対象に、シンクロトロンを用いた高解像度 μCT による断層画像撮影による 3D モデル構築を実施し、形態の解析に援用した。また、得られた形態データや生態情報をもとに、最尤法を用いた祖先形質復元を行った。

これら第一章・第二章で行った形態観察の結果、半翅目は、昆虫に広く見られる基本的な構造の後胸を持った共通祖先を持ちながら、繰り返しその構造が大きく改変され、跳躍機能の獲得が起こっていたことが示唆された。一方、後胸の改変がある仲間でもその多くが遜色のない飛翔機能を有しており、特に中胸には強大な飛翔筋を有していることが確認された。このことから半翅目では、後胸構造の改変に先立ち、中胸への飛翔機能の集約が起こっていたと予想された。つまり、飛翔機能が持つ容積的な制約から後胸が開放されることで他の機能を付加する余地が創出され、跳躍機能を含む新奇機能の獲得が後胸において実現したという仮説が想定された。

そこで第三章では、中胸への飛翔機能の集約を実現した要因として、翅構造、とりわけ翅連結構造に注目して形態学的研究を行った。翅連結構造は、飛翔中に 4 枚の翅を機械的に結合し、2 枚の翅として振る舞わせる構造である。この構造は、半翅目を含む準新翅類に幅広く知られているが、その詳細な進化史については十分に検討されたことがなかった。第三章では、半翅目がどの段階で翅連結構造を獲得したのかを検討するため、準新翅類に属する咀顎目・総翅目・半翅目のそれぞれについての翅連結構造の形態を詳細に検討した。

Chapter 1

Thoracic musculature associated with jumping in Auchenorrhyncha

Note: This chapter includes a version of an article published in Organisms Diversity & Evolution: Ogawa, N. and Yoshizawa, K. 2017. Morphological dissection of behaviour: thoracic musculature clarifies independent development of jumping mechanisms between sister groups, planthoppers and leafhoppers (Insecta: Hemiptera: Auchenorrhyncha). *Organisms Diversity & Evolution* 17(3): 521-530.

ABSTRACT

Morphological and behavioural characters are frequently examined for comparative studies. Unlike morphology, a single behavioural trait is difficult to subdivide as multiple characters, even when achieved by many evolutionary changes. Therefore, when similar behavioural traits evolved independently among closely retaled taxa, their distinction is difficult. Almost all members of the suborder Auchenorrhyncha (Insecta: Hemiptera) possess a jumping ability that uses metathoracic muscles, and this behavioural trait has been regarded as a synapomorphy. In this study, the anatomical observations of metathoracic muscles revealed that highly elaborated jumping ability was gained independently within the suborder, although the evolution of jumping ability might have been initiated at their common ancestor. My results provide an example of identifying a true evolutionary pathway by dissecting a behavioural character into mechanical elements.

Key words functional morphology • behavior • character evolution • evolutionary novelty

INTRODUCTION

For morphology-based phylogenetic analyses and/or studies of morphological evolution, a single structure is usually subdivided into several smaller elements. For example, the insect mandible is composed of a single sclerite, but molar, incisor, and anterior and posterior articulations, among others, are recognized as mandibular elements and coded separately for phylogenetic and/or evolutionary analyses. By contrast, such a subdivision is usually difficult for behavioural characters. Therefore, when a behavioural trait is the subject of phylogenetic comparative analyses, the trait is generally treated as a single character (eg. “eusociality” in Carpenter, 1982; “paratisism” in Johnson et al., 2004; “maternal child care” in Tsai et al., 2015), although a single behavioural change almost always involves multiple morphological, physiological, and/or neural changes.

"Jumping ability" is such an example, and when this trait has been the focus of study, it is treated as a single character (Hennig, 1981). However, this ability was actually achieved by a combination of many structural modifications, including those of muscles, muscle attachments, and associated sclerites and those for energy storage and the locking system, among others (Gorb, 2004). Therefore, the jumping behaviour can be separated into many functional elements morphologically that can clarify the evolutionary pathway of jumping ability. In some simple cases, the independent origins of these functional elements are obvious. For example, locusts jump using muscles in their hind femur, whereas fleas use extrinsic leg muscles (Bennet-Clark and Lucey, 1967; Brown, 1967), and the independent origins are easily recognizable. By contrast, both locusts and jumping cockroaches use hind femur muscles for jumping (Picker et al., 2011); however, distant phylogenetic affinity indicates independent origins of jumping

ability. When similar mechanisms evolved independently in closely related taxa, however, their distinction is far more difficult.

The suborder Auchenorrhyncha (Insecta: Hemiptera) is composed of planthoppers (infraorder Fulgoromorpha) and leafhoppers, treehoppers, froghoppers (or spittle bugs) and cicadas (infraorder Cicadomorpha). Monophyly of Auchenorrhyncha was once debated (Bourgoin and Campbell, 2002; Forero, 2008), but subsequent morphological (Yoshizawa and Saigusa, 2001) and molecular (Cryan and Urban, 2012; Misof et al., 2014a) analyses converged to support its monophyly. The suborder is well known for jumping ability, with the exception of cicadas. The jumping is very fast and strong, and the suborder includes champions among all jumping insects with take-off velocities that reach up to 5.5 m s^{-1} and 719*g* in kicking force (Fulgoroidea: Issidae) (Burrows, 2009b). All auchenorrhynchous insects use the metathoracic muscles for jumping. Therefore, by focusing only on this behavioural character, the most parsimonious interpretation for the evolution of their jumping ability is that it evolved once in their common ancestor and then the ability was lost secondarily in cicadas (Fig. C1-1A), as generally assumed (Kristensen, 1975; Hennig, 1981; Shcherbakov and Popov, 1997). However, the condition of the principal jumping muscle (i.e., trochanter depressor muscle) is different between Cicadomorpha and Fulgoromorpha (Burrows and Bräunig, 2010). In addition, froghoppers (Cicadomorpha: Cercopoidea) have protrusions on the hind coxa and femur that are engaged when the hindleg is cocked for jumping preparation (Burrows, 2009b), whereas the femoral protrusion is completely reduced in planthoppers (Fulgoromorpha) (Burrows, 2009b). Because of these morphological and mechanical differences, jumping ability might have evolved independently in these sister infraorders, which is a less parsimonious interpretation based on the behavioural trait (Fig. C1-1B). Furthermore, when I accept the independent origins of jumping ability,

then independent origins of jumping ability within Cicadomorpha (Fig. C1-1C) or a more complicated evolutionary scenario (Fig. C1-1D) also become equally parsimonious interpretations. To solve this question, detailed morphological observations throughout the auchenorrhynchans and phylogenetic reconstruction of character evolution are required.

In this chapter, I verify these four alternative hypotheses by morphological dissection and observation of auchenorrhynchan jumping muscules. Our examination provides an example assessing the more likely evolutionary pathway by dissecting a behavioural character into mechanical elements. The results also provide useful information for understanding the ancestral traits of morphology and behaviour in Hemiptera.

MATERIALS AND METHODS

Taxa examined

I selected Ricaniidae and Fulgoridae (Fulgoromorpha), Cicadellidae and Membracidae (Membracoidea), Cercopidae, Machaerotidae, Clastopteridae and Aphrophoridae (Cercopoidea) and Cicadidae and Tettigarctidae (Cicadoidea) for examinations. *Stenopsocus nigricellus* (Psocodea: "Psocoptera") was selected as a close outgroup, and the tree was rooted with Zorotypidae (Friedrich and Beutel, 2008). Jumping behavior does not occur in the selected outgroups. The taxa examined are listed in Table C1-1.

Specimen preparation and observation

Specimens fixed in FAA solution (formalin:dehydrated ethanol:acetic acid =

6:16:1) (Ogawa and Miura, 2013) and stored in 80% ethanol were used. A specimen of Tettigarctidae (Cicadoidea) that had originally been dried and later soaked was also examined as a supplemental specimen. Specimens were dissected with a FH-20 razor blade (Feather Safety Razor Co., Ltd., Osaka, Japan) and forceps. Some specimens were macerated in 10% KOH solution to facilitate observations of the skeletons. SZ61 and SZX16 binocular microscopes (Olympus Corporation, Tokyo, Japan) and a Zeiss Axiophoto light microscope (Carl Zeiss, Oberkochen, Germany) were used for observations. The illustrations were drawn using a Cintiq 13HD graphics tablet (Wacom Co., Ltd., Saitama, Japan) and Clip Studio Paint Pro (Celsys, Inc., Tokyo, Japan) and modified in Adobe Photoshop CC and Adobe Illustrator CC. The investigated muscles were homologized based on their origin/insertion points and their positional relation to the circumjacent muscles.

Terminologies of Muscles

Terminologies of individual muscles followed Friedrich and Beutel (2008) because of their systematic nomenclature. The thoracic segments were indicated by roman numerals prefixed to the muscle name (e.g., IIIdvm1: metathoracic dvm1). Correspondence to the traditional nomenclature, abbreviations and their origin and insertion is given in Table C1-2.

Target muscles for observation

I focused on metathoracic indirect flight muscles and trochanter depressor muscles. Meso- and metathoraces of neopteran insects are usually filled with large indirect flight muscles (Fig. C1-2). Whereas some muscles are bifunctional and provide power for flight and walking, the principal indirect flight muscles are usually

monofunctional (Brodsky 1994). They attach to robust sclerites, i.e., tergum, sternum and phragma, and they generate principle flight power (Brodsky, 1994). The indirect flight muscles were classified into three categories: dorsal longitudinal indirect flight muscle (DLM), dorsoventral flight muscle (DVM) and oblique dorsal flight muscle (ODM) (Crossley, 1978; Brodsky, 1994) (Fig. C1-2). DLM, DVM and ODM correspond to dlm1 (phragma-phragmalis muscle), dvm1 (noto-sternalis muscle), and dlm2/3 (noto-phragmalis muscle/scutello-scutellaris muscle) in Friedrich and Beutel (2008), respectively.

The trochanter depressor muscles generate principal power of auchenorrhynchan jumping, which can be subdivided into functional elements (Gorb, 2004; Burrows and Bräunig, 2010). Neopteran insects usually have four bundles of metathoracic trochanter depressor muscles, scm6 (from furca), pcm5 (from metanepisternum and basalare), and dvm7 (from notum) (Friedrich and Beutel, 2008) (Fig. C1-2).

Parsimony Analysis

Phylogenetic relationships of Auchenorrhyncha and outgroups were taken from Urban and Cryan (2007), Cryan and Urban (Cryan and Urban, 2012) and Misof et al. (2014a). Most parsimonious reconstruction of the ancestral character states (jumping behaviour and conditions of jumping/flight muscles) was performed using Mesquite 3.04 (Maddison and Maddison, 2015). Behaviors of leaping over the wither height and/or body length starting from rest position without wing flapping were defined as “Jumping” based on my observation.

Character 1. IIIpcm5: not developed as jumping muscle (0) developed as major jumping muscle (1). State 0 was observed in outgroups and Cicadoidea. State 1 was

observed in all jumping Auchenorrhyncha (Fig. C1-3A-F).

Character 2: Origin of IIIpcm5: restricted to dorsal episternam and basalare (0); expanded to ventral metanepisternum (1). State 0 was observed in outgroups and Fulgoromorpha. State 1 was observed throughout Cicadomorpha including Cicadoidea.

Character 3: Apodeme of basalare: not enlarged (0); enlarged (1). State 1 was only observed in Fulgoromorpha.

Character 4. IIIdvm7: present (0); absent (1). State 1 was only observed in Fulgoromorpha. (Fig. C1-3A, B).

Character 5. Jumping function in IIIdvm7: not developed as jumping muscle (0); developed as jumping muscle (1). State 1 was only observed in jumping cicadomorphans. State of Fulgoromorpha was treated as unknown.

Character 6. DVM (IIIdvm1): present (0); absent (1). State 1 was only observed in Fulgoromorpha (Fig. C1-3A, B). DVM of the other examined taxa was present (State 0).

Character 7. DLM (IIIdlm1): developed (0); reduced or absent (1). A state that occupancy of DLM attachment surface on meso- or metaphragma is below 50% was defined as “reduced”. State 1 was observed in all cicadomorphans (Fig. C1-3C-H). Fulgoromorpha and outgroups possessed developed DLM (State 0)

Character 8. DLM (IIIdvm1): independent from IIIdvm7 (0); surrounded by IIIdvm7 (1). Almost examined taxa have two bundles of DLM (Figs C1-2; C1-3A, B, E, F; Friedrich & Beutel 2008) (State 0). In Aphrophoridae (Fig. C1-3C, D) and Machaerotidae (Cercopoidea), small DLM was surrounded by huge IIIdvm7 (State 1).

Character 9. ODM (IIIdlm2/3): developed (0); reduced or absent (1). A state that occupancy of ODM attachment surface on metaphragma is below 20% was defined as “reduced”. In almost all Cicadomorphans, ODM was reduced (Fig. C1-3C, E, G) (State 1). Fulgoromorpha and outgroups possessed developed ODM (State 0).

Character 10. Hind trochanteral tendon: not sheet shaped (0); sheet-shaped (1). Sheet-shaped tendon (state 1) was observed in Cicadomorphans, although morphologies were more or less different (Fig. C1-4B-D). Fulgoromorpha and Stenopsocidae had a non-sheet-shaped tendon.

Character 11. Mesophragma: occupied by DLM (0); occupied by IIIdvm7 (1); with large empty space (2). The mesophragma of Fulgoromorpha was occupied by DLM (State0: Fig. C1-3A, B). State 1 was observed in jumping Cicadomorpha (Fig. C1-3D, F), and State 2 was only observed in Cicadoidea (Fig. C1-3H).

RESULTS

Metathoracic musculature of Fulgoroidea (Fulgoromorpha:Auchenorrhyncha)

Metathoracic musculature in the Fulgoromorpha is highly modified and rearranged (Fig. C1-3A, B). The lower part of the metathorax is filled with a cylinder-shaped jumping muscle (Fig. C1-3A, B). The jumping muscle originates from a well-developed basalare and small metanepisternum (Character 2:0; 3:1) (Fig. C1-5A), and is inserted into the dorsal region of the robust tendon (Figs C1- 3A, B; C1-4A). The attachment point of the tendon has the form of a funnel (Character 10:0) (Fig. C1-4A). The jumping muscle is homologous to IIIpcm5 (Character 1:1). Completely absent are other trochanter depressor muscles, IIIscm6 and IIIdvm7 (Character 4:1). The DLM and ODM are well developed (Character 7:0; 9:0; 11:0), but the DVM is completely absent (Character 6:1) (Fig. C1-3A, B).

Metathoracic musculature of Membracoidea (Cicadomorpha: Auchenorrhyncha)

As in the Fulgoroidea, the Membracoidea also have jumping muscles in the metathorax. However, their metathoracic musculature differs significantly from that of the fulgoromorphans, as also noted by Burrows and Bräunig (2010) and Burrows (2013) (Fig. C1-3C, D).

Most flight muscles are reduced, and most of the metathoracic space is filled with the jumping muscles composed of two trochanter depressor muscles (Fig. C1-3D) (Burrows 2007; Gorb 2004; Maki 1938). In Membracidae, the tendon is shaped as a shingle sheet with a sclerotized stem. The tendon of Cicadellidae is similar, but the tendon forks basally (Fig. C1-4B) (Character 10:1). Both jumping muscles are inserted on the ventral side of the tendon. The muscle inserted on the ventroproximal side is homologous to IIIdvm7 (Character 4:0; 5:1), and its origin occupies most parts of the metanotum and the entire posterior surface of the mesophragma (Character 11:1). The

muscle inserted on the ventrolateral side of the tendon (including the small branch in Cicadellidae) is IIIpcm5 (Character 1:1), originating from the the anterolateral bulged metanepisternum and the tiny basalare (Character 2:1; 3:0) (Fig. C1-3D). IIIscm6 is absent. Due to the expansion of the attached area of IIIdvm7, DLM is strongly reduced and changes its origin/insertion points to a very narrow area of the dorsal margin of the meso-/metaphragma, respectively (Character 7:1). The DVM is also greatly reduced but retained in all taxa (Character 6:0). The ODM is remained in Cicadellidae, but was completely absent in Membracidae (Character 9:1).

Metathoracic musculature of Cercopoidea (Cicadomorpha)

The metathoracic musculature of Cercopoidea is also highly modified and rearranged (Fig. C1-3E, F). Although the external morphology of the hind leg base is quite different between Cercopoidea and Membracoidea (with narrow and conical hind coxae in Cercopoidea whereas broadely transverse hind coxae in Membracoidea: (Burrows, 2006a, 2007, 2013), the metathoracic musculature of cercopoids is very similar to that of membracoids.

The jumping muscles also comprise two bundles, IIIpcm5 and IIIdvm7 (Fig. C1-3F) (Character 1:1; 4:0; 5:1). The tendon clearly forks basally, and each branch is composed of robust stem and membranous field (Character 10:1), and both surfaces are used for muscle attachment (Fig. C1-4C). The muscle inserted on the inner fork is homologous to IIIdvm7. The muscle is broadly expanded dorsally, and its attachment occupies most area of the metanotum and the entire posterior surface of the mesophragma (Character 11:1). The muscle inserted into the lateral fork is IIIpcm5, originating from anterolateral bulged metanepisternum and small basalare (Character 2:1; 3:0) (Savinov, 1990; Gorb, 2004) (Fig. C1-4F). Absent was IIIscm6. According to

the expansion of the attached area of IIIdvm7, DLM is strongly reduced and changed its origin/insertion points to a very narrow area of the dorsal margin of the meso-/metaphragma, respectively. The right and left bundles of DLM are separated by internal branch of IIIdvm7 (Character 8:1). DLM of Cercopidae and Clastopteridae is completely absent, although that of Aphrophoridae and Machaerotidae remains (Character 7:1). DVM is also greatly reduced but is retained in all taxa (Character 6:0). The ODM is also reduced but is relatively well retained (Fig. C1-3E, F) (Character 9:1).

Metathoracic musculature of Cicadoidea (Cicadomorpha)

The arrangement of the metathoracic muscles in Cicadoidea is distinctly different from the condition observed in the other groups (Snodgrass, 1935; Maki, 1938). DLM is retained but distinctly reduced and placed at the dorsolateral crack formed by the shortened and strongly arched metanotum (Character 7:1). DVM is also reduced in size but retained (Character 6:0). *Graptopsaltria* and *Huechys* (Maki, 1938) retain a reduced ODM, whereas ODM is completely absent in *Meimuna* (Cicadidae) (Character 9:1). The tendon forms a less-sclerotized and expanded dorsal sheet with a small lateral fork (Fig. C1-3B) (Character 10:1). The muscles homologous to the jumping muscles in the other cicadomorphans (IIIdvm7 and IIIpcm5) are weakly developed and composed of three subunits. The lateral subunit originating from the ventral metanepisternum which is homologous to the IIIpcm5 of the jumping cicadomorphans (Character 1:0; 2:0), is inserted on the small fork of the trochanteral tendon (Figs C1-3H; C1-4D). The other two subunits are both inserted on the sheet-like dorsal expansion of the trochanteral tendon (Figs C1-3H; C1-4D) (Character 10:1). One originates from the anterolateral lobe of the scutum along the antecoxal suture (Figs 3H; 4D) and the other from the ventral region of the mesophragma (* in Figs C1-3G, H; C1-4D). Together, these two

subunits correspond to the IIIdvm7 in the jumping cicadomorphans (Character 4:0; 5:0). IIIscm6 is also absent. In Cicadidae, a wide empty area is present on the posterodorsal surface of the mesophragma (Fig. C1-3H) (Character 11:2), unlike the mesophragma of the jumping cicadomorphans, which is completely occupied by IIIdvm7 (Character 11:1) (Fig. C1-3C-F), or unlike the ordinal pterothorax, which is completely occupied by DLM (Character 11:0) (Fig. C1-1). This condition was also observed in the *Tettigarcta* (Tettigarctidae). The specimen was originally dried, and the thoracic musculature was not well preserved, but the condition of the IIIdvm7 and the empty area on the mesophragma were clearly visible.

Parsimony analysis

The ancestral condition of the metathoracic structures potentially associated with the evolution of jumping behaviour was reconstructed parsimoniously using previously estimated trees (Fig. C1-6: characters listed in Appendix 1). The infraorder Fulgoromorpha was implied to share the following states in the common ancestor: enlarged apodeme developed from basalare (Character 3:1), loss of IIIdvm7 (Character 4:1), and loss of DVM (Character 6:1). Expansion of metanepisternum (Character 2:1), development of IIIdvm7 as a jumping muscle (Character 5:1), reduction of DLM (Character 7:1), reduction of ODM (9:1), the sheet-like trochanter tendon (10:1), and occupation of the mesophragma by IIIdvm7 (11:1) were considered to have evolved in the common ancestor of the infraorder Cicadomorpha. The IIIpcm5 develops as a jumping muscle (Character 1:1) in both Cicadomorpha and Fulgoromoprha. The empty area on the mesophragma (Character 11:2) related to the reduction of the jumping muscle IIIdvm7 (Character 5:0) was regarded as an autapomorphy of the Cicadoidea. Jumping function of IIIpcm5 was secondarily reduced in Cicadoidea (Character 1:0).

DISCUSSION

All jumping species of Auchenorrhyncha possess large metathoracic jumping muscles transformed from the trochanter depressors. However, the condition of the jumping muscles is very distinctly different between the two infraorders. Fulgoromorpha have very large jumping muscles developed from only IIIpcm5, which is inserted to the funnel shaped trochanteral tendon and is originated from the expanded basalare (Figs C1-3A, B; C1-4A) (Character 1:1; 2:0; 3:1; 10:0). In contrast, two superfamilies of Cicadomorpha (Membracoidea and Cicadoidea) possess very large jumping muscles composed of modified and enlarged IIIdvm7 and IIIpcm5 inserted on an enlarged trochanteral tendon (Figs C1-3C-F; C1-4B, C) (Character 1:1; 5:1; 10:1). In Cicadoidea, the trochanter depressor muscles originate from the pleura, notum and phragma and are inserted on the trochanteral tendon (Figs C1-3G, H; C1-4D).

The most parsimonious reconstruction of the metathoracic muscular characters suggests that almost all apomorphic conditions associated with the jumping ability evolved independently in Cicadomorpha and Fulgoromorpha (Fig. C1-6). The development of IIIpcm5 as a jumping muscle is shared by Cicadomorpha and Fulgoromorpha (Character 1:1), but its origin (Character 2; Fig. C1-5) and insertion points (Character 3: Fig. C1-4) considerably. Therefore, although the development of jumping ability may have originated in their common ancestor (Kristensen, 1975; Hennig, 1981), it is evident that the highly elaborate jumping ability and mechanism as observed in extant groups of Auchenorrhyncha has been achieved independently between two infraorders, as suggested by Burrows and Bräunig (2010) and Burrows (2013) (Fig. C1-1B, C).

From the present morphological analyses, the evolution of the auchenorrhynchan jumping ability can be explained as follows. In Fulgoromorpha, the muscle originating from the metanepisternum and basalare (IIIpcm5) were transformed into jumping muscles (Character 1:1). The enlarged attachment point was formed by the strong expanded basalare (Character 3:1). The tendon was also modified as an enlarged funnel-shaped structure for expanding the attachment point, which was also strengthened to support the huge contracting power of the jumping muscle. Absence of IIIdvm7 (Character 4:1) and IIIscm6 might be the result of constraint in attachment area in the notum (with the flight muscles) and tendon (with enlarged IIIpcm5), respectively. Because the jumping muscles occupied only the lower half of the metathorax, adequate room for storing the flight muscles could be retained in the upper part. In the jumping Cicadomorpha (Membracoidea and Cercopoidea), IIIpcm5 and IIIdvm7 transformed to the jumping muscles (Character 1:1; 5:1). Attachment points of the jumping muscles were expanded: IIIdvm7 was attached into mesophragma (Character 11:1), in addition to the notum. Reductions of flight muscles and the absence of IIIscm6 (both muscles attached to the notum and phragma) were most likely the result of competition for attachment space for jumping muscles. Whereas the shape of the tendon (a single sheet in Membracoidea vs. birurcated in Cercopoidea), muscle attachiment manner on tendon (ventral side vs. both sides), and location of DLM (Character 8) differ between the two superfamilies, their jumping muscles and related morphologies were considered to be gained in the common ancestor because of the fundamental similarities.

Additionally, under the assumption of independent evolution of the jumping ability in Fulgoromorpha and Cicadomorpha, two equally parsimonious scenarios are also possible, i.e., a secondarily reducted jumping ability in Cicadoidea (Fig. C1-1B) or independent origins of jumping ability in Cicadomorpha (Fig. C1-1C). Of the two

scenarios, the arrangements of the jumping musculatures of cicadomorphans indicates that the secondary reduction hypothesis is more likely. Expansion of the original position of the IIIdvm7 from the notum to notum+mesophragma (Character 11:1) and the reduction of the DLM (Character 7:1) are estimated to have evolved in the common ancestor of Cicadomorpha. Reduction of the DLM is strongly associated with the development of the IIIdvm7 because, in ordinal flying insects, DLM occupies almost the entire surface of the posterior mesophragma (Character 11:0). Although a wide empty space is on the mesophragma of the Cicadoidea (Character 11:2), the cicadoidean DLM occupies only a very narrow dorsal margin of the phragma (Fig. C1-3H), as also observed in the Membracoidea and Cercopoidea (Fig. C1-3D, F). This strongly suggests that the expansion of the jumping muscle to the anterior phragma occurred in the common ancestor of Cicadomorpha, and that the poorly developed jumping muscle in the Cicadoidea represents a secondary reduction. Additionally, the presence of phragma-trochanteralis muscle in Cicadoidea (* in Fig. C1-3G, H) also supports the secondary reduction interpretation. The muscle is never present in insects other than jumping Auchenorrhyncha. Therefore, this muscle in Cicadoidea can be interpreted as a vestige of the jumping muscle after secondary reduction of expanded IIIdvm7 and its absence from the notum.

In summary, the muscle morphology suggests that some fundamental aspects of jumping ability originated in the common ancestor of Auchenorrhyncha, several details of the jumping mechanisms of Cicadomorpha and Fulgoromorpha evolved independently. The lack of the jumping ability in the Cicadoidea represents a secondarily reduced condition. This more complicated evolutionary scenario could not be extracted from a simple maximum parsimony reconstruction of the behavioural character (Fig. C1-1A), and observation of the muscles associated with the jumping

ability was required to reach this conclusion. Our results provide an example that the morphological dissection of a behavioural trait is valid for implicating a more likely evolutionary pathway. Shcherbakov and Popov (1997) claimed that the jumping ability is a synapomorphic trait in the order. However, the implication was proposed based on a fossilized exoskeleton without considering internal morphology. To understand the origins and evolution of the jumping ability in all of groups of Hemiptera, detailed morphological and functional investigations are obligatory.

Table C1-1. Taxa examined for this study. The specimens examined are stored at Systematic Entomology, Hokkaido University (SEHU).

ORDER HEMIPTERA

SUBORDER AUCHENORRHYNCHA

INFRAORDER CICADOMORPHA

SUPERFAMILY CERCOPOIDEA

Family Aphrophoridae

Aphrophora pectoralis

Family Clastopteridae

Clastoptera obtusa

Family Cercopidae

Euscartopsis assimilis

Cosmoscarta sp.

Family Machaerotidae

Machaerota takeuchii

SUPERFAMILY MEMBRACOIDEA

Family Membracoidae

Anthianthe sp.

Archasia sp.

Ophiderma salamandra Fairmaire

Family Cicadellidae

Cicadella viridis

Table C1-1. *Continued.*

SUPERFAMILY CICADOIDEA

Family Tettigardtidae

Tettigarcta crinite

Family Cicadidae

Graptopsaltria nigrofuscata (Motschulsky)

Meimuna iwasakii

INFRAORDER FURGOROMORPHA

Family Delphacidae

Tropidocephala brunneipennis Signoret

Family Fulgoridae

Pyrops candelania

Lycorma delicatula

Family Flattidae

Geisha distinctissima

Family Ricaniidae

Ricania japonica

ORDER ZORAPTERA

Family Zorotypidae

Zorotypus hubbardi (scored from Friedrich & Beutel (2008))

ORDER PSOCODEA

Family Stenopsocidae

Stenopsocus nigricellus

Table C1-2. Correspondence between the generalized thoracic muscle nomenclature and abbreviation (from Friedrich & Beutel 2008, modified).

Category		Abbrev.	Name	Origin	Insertion
Trochanter depressor muscle		scm6	M. furca-trochanteralis	furca	trochanter
		pcm5	M. episterno-trochanteralis	basalare or episternum	
		dvm7	M. noto-trochanteralis	Notum	
Indirect flight muscle	DLM	dlm1	M. phragma-phragmalis	anterior phragma	posterior phragma
	DVM	dvm1	M. noto-sternalis	notum	sternum
	ODM	dlm2	M. noto-phragmalis	notum	phragma
		dlm3	M. scutello-phragmalis	scutal rim	scutellum

Table C1-3. Data matrix used for the parsimonious reconstruction. See text for characters and their states. *Zorotypus hubbardi* were scored from Friedrich and Beutel (2008).

Character No.					5					10	
ZORAPTERA (*Zorotypus hubbardi*)	0	0	0	0	0	0	0	0	0	?	0
PSOCOPTERA (*Stenopsocus nigricellus*)	0	0	0	0	0	0	0	0	0	0	0
Cicadidae (*Graptopsaltria nigrofuscata*)	0	1	0	0	0	0	1	0	1	1	2
Cicadidae (*Meimuna iwasakii*)	0	1	0	0	0	0	1	0	1	1	2
Aphrophoridae (*Aphrophora pectoralis*)	1	1	0	0	1	0	1	1	1	1	1
Clastopteridae (*Clastoptera obtusa*)	1	1	0	0	1	0	1	?	1	1	1
Cercopoidae (*Euscartopsis assimilis*)	1	1	0	0	1	0	1	?	1	1	1
Machaerotidae (*Machaerota takeuchii*)	1	1	0	0	1	0	1	1	1	1	1
Membracidae (*Anthianthe* sp.)	1	1	0	0	1	0	1	0	1	0	1
Cicadellidae (*Cicadella viridis*)	1	1	0	0	1	0	1	0	1	1	1
Delphacidae (*Tropidocephala brunneipennis*)	1	0	1	1	?	1	0	0	0	0	0
Fulgoridae (*Lycorma delicatula*)	1	0	1	1	?	1	0	0	0	0	0
Flattidae (*Geisha distinctissima*)	1	0	1	1	?	1	0	0	0	0	0
Ricaniidae (*Ricania japonica*)	1	0	1	1	?	1	0	0	0	0	0

?, not applicable.

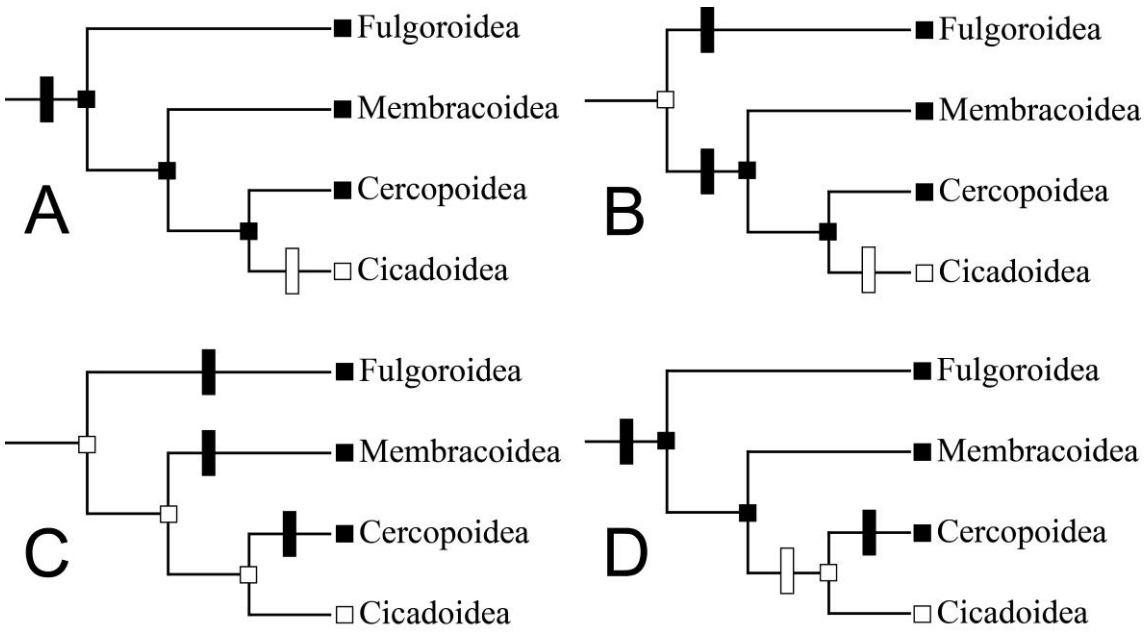


Fig. C1-1. Phylogeny of Auchenorrhyncha and four possible evolutionary scenarios for the evolution of jumping ability. The tree was summarized from Cryan & Urban (2012). The black and white rectangles/squares indicate presence and absence of jumping ability, respectively. A, The most parsimonious interpretation implied from jumping behaviour (2 steps). B. The independent origins hypothesis as suggested by Burrows & Bräunig (2010) and Burrows (2013) (3 steps). C–D, Two additional possibilities with evolutionary steps = 3.

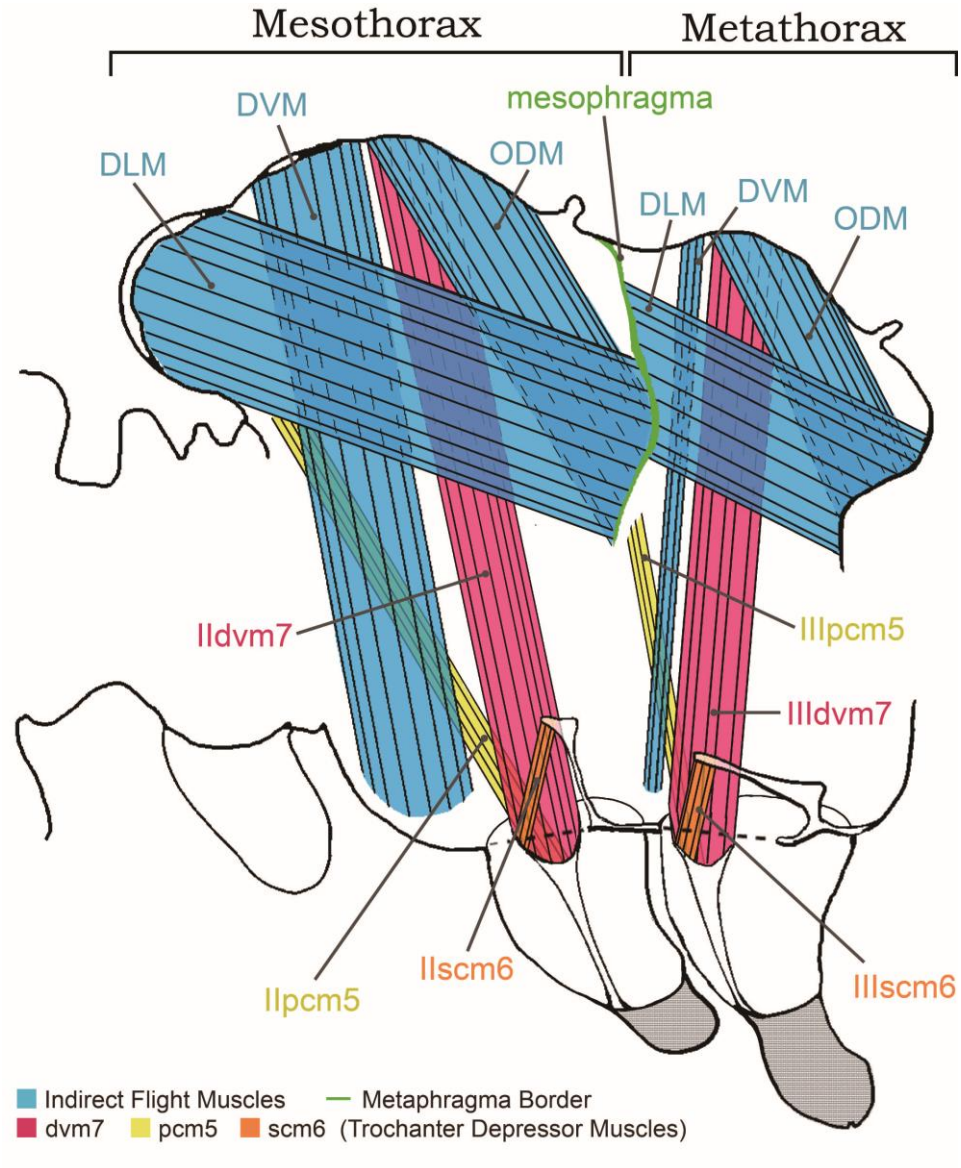


Fig. C1-2. Flight and jumping musculatures of the bark louse genus *Stenopsocus* (Psocodea: “Psocoptera”: Stenopsocidae), lateral view. Redrawn and modified from Badonnel (Badonnel, 1934) and Maki (Maki, 1938). Indirect flight muscles were colored blue: DLM, dorsal longitudinal indirect flight muscle (dlm1); DVM, dorsoventral flight muscle (dvm1); ODM, oblique dorsal flight muscle (dlm2/3). Trochanter depressor muscles were colored by respective muscles. Terminology followed Crossley (1978), Brodsky (1994) and Beutel et al. (2014). The border of mesophragma is highlighted by green line.

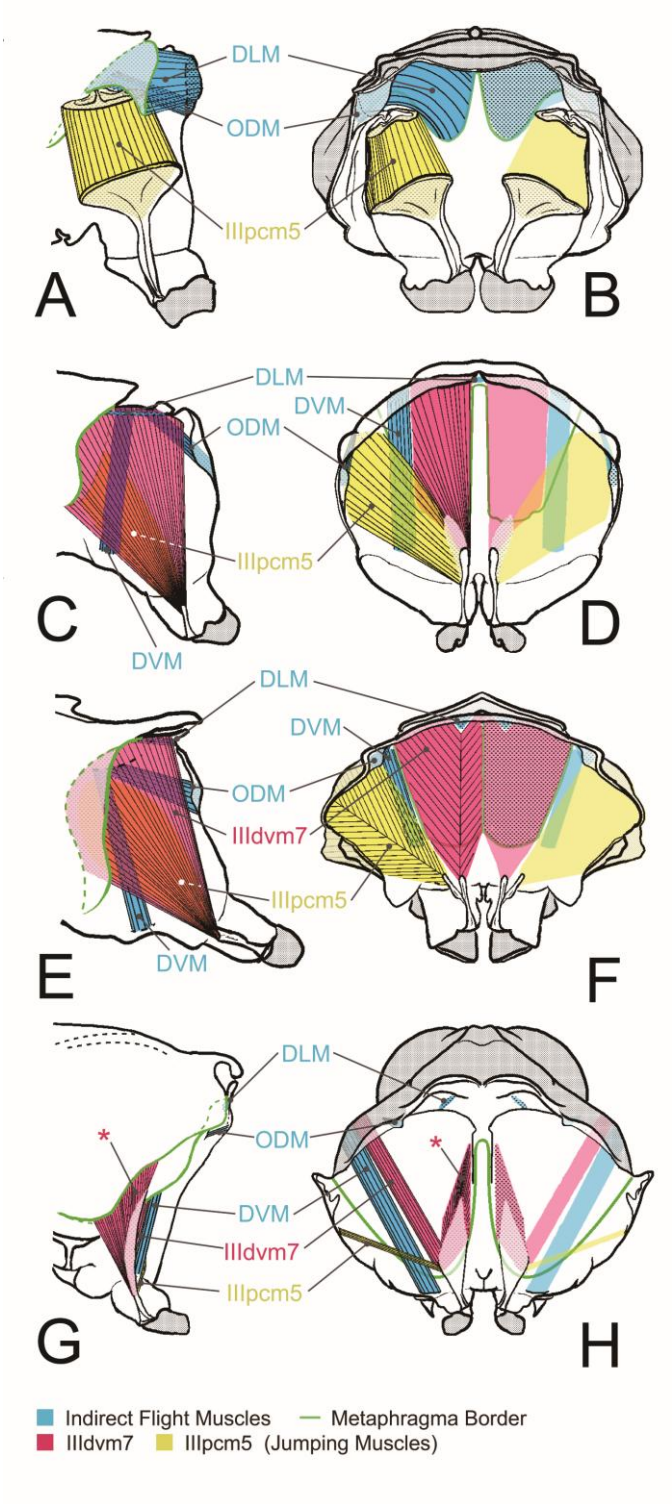


Fig. C1-3. Flight and jumping muscles of Auchenorrhyncha, lateral (left) and posterior (right) views. A, *Ricania japonica* (Fulgoromorpha: Fulgoroidea: Ricaniidae); B, *Cicadella viridis* (Cicadomorpha: Membracoidea: Cicadellidae); C, *Aphrophora pectoralis* (Cercopoidea: Aphrophoridae); D, *Graptopsaltria nigrofuscata* (Cicadidae: Cicadoidea). See Fig. C1-2 for terminology, colours and abbreviations. The right side of posterior images shows muscles (colored shadows) and muscle attachment regions (black dots). The border of mesophragma is highlighted by green line.

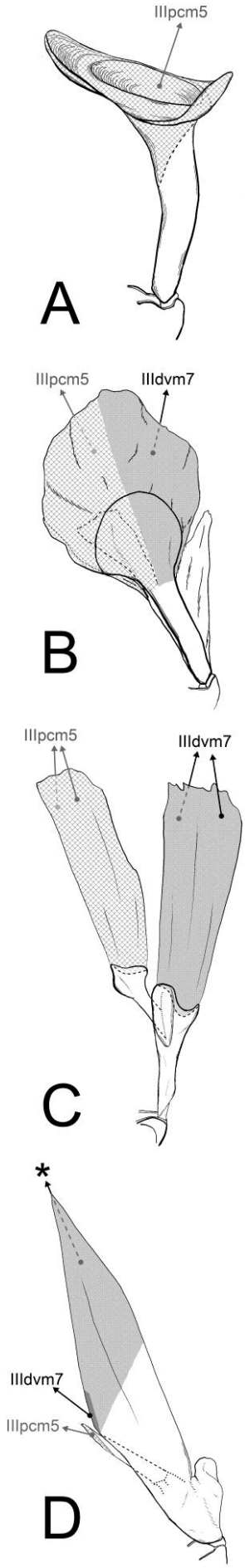


Fig. C1-4. Enlarged view of left metatrocanteral tendons and attachments point of muscles. A, *Ricania japonica* (Fulgoromorpha: Fulgoroidea: Ricaniidae); B, *Cicadella viridis* (Cicadomorpha: Membracoidea: Cicadellidae); C, *Aphrophora pectoralis* (Cercopoidea: Aphrophoridae); D, *Graptopsaltria nigrofuscata* (Cicadoidea: Cicadidae). See Fig. C1-2 for terminology and abbreviations. The painted regions indicate the muscle attachment areas. Deep and light gray indicate the attached area of IIIdvm7 and IIIpcm5, respectively. Arrows with a spot represent muscle bundles. Dashed lines indicate concealed muscles or sclerite borders.

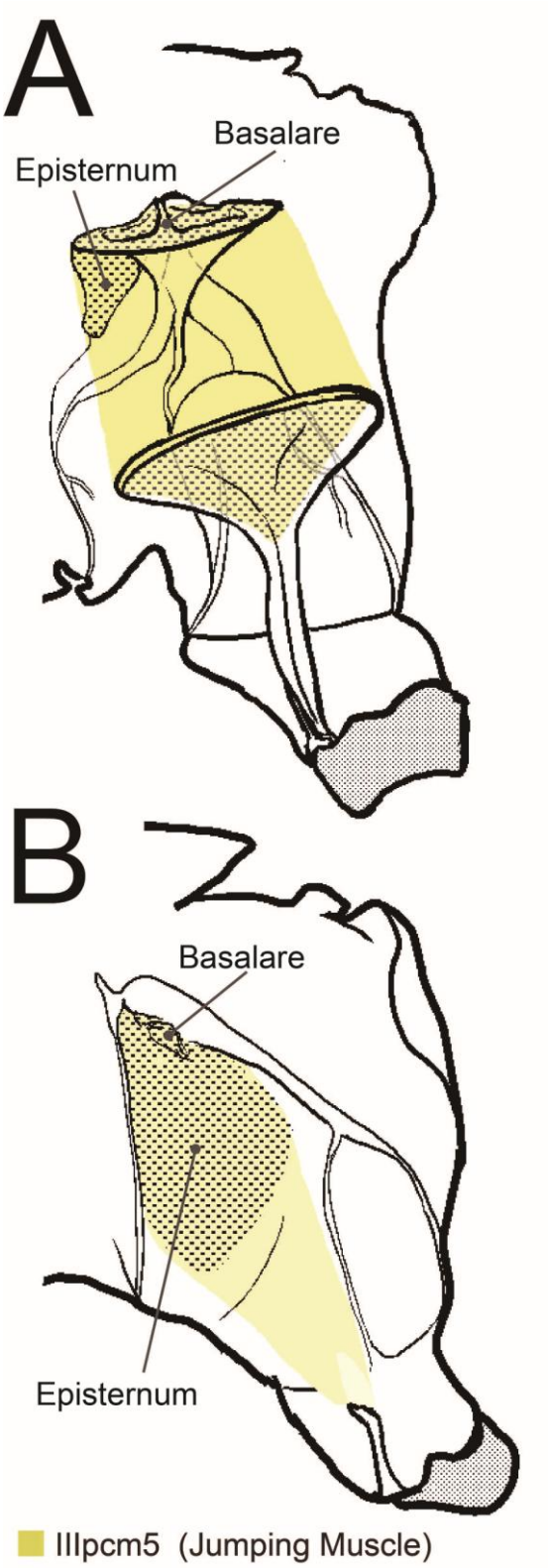


Fig. C1-5. Metathoracic endoskeletons and attachment regions of IIIpcm5, lateral view. See Fig. C1-2 for terminology and abbreviations. A, *Ricania japonica* (Fulgoromorpha: Fulgoroidea: Ricaniidae); B, *Cicadella viridis* (Cicadomorpha: Membracoidea: Cicadellidae). IIIpcm5 is indicated by gray shadow. Dotted areas indicate muscle attachment regions.

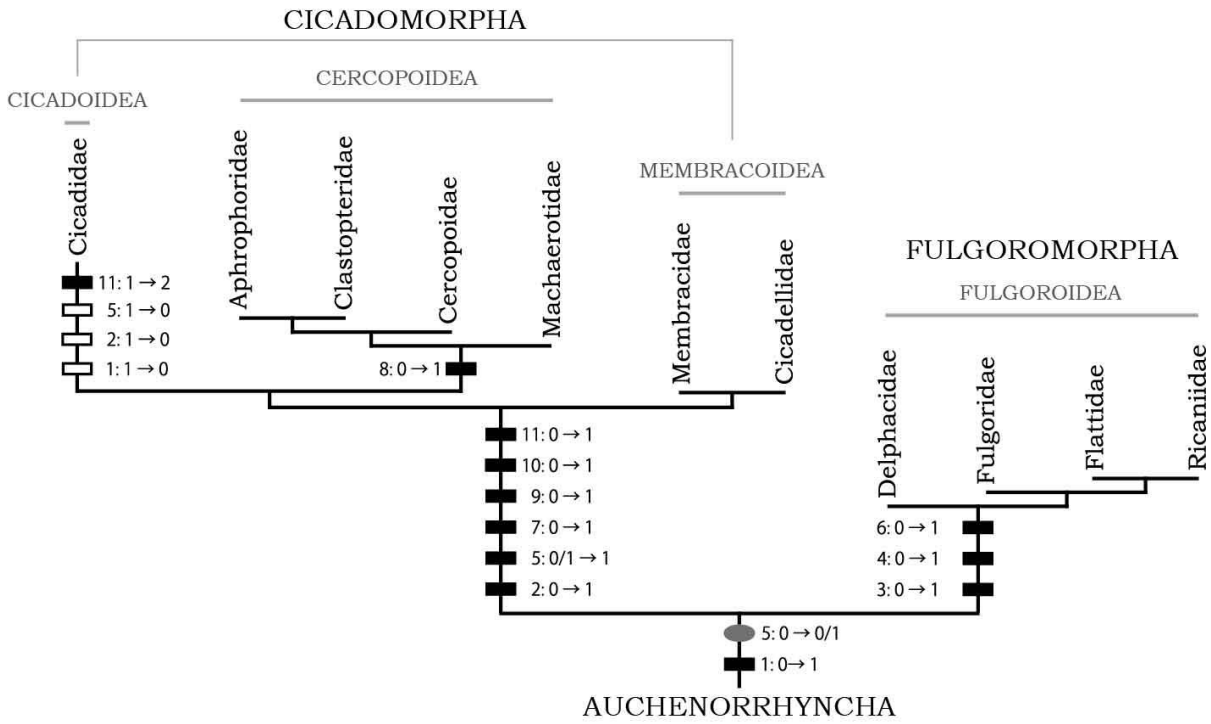


Fig. C1-6. Most parsimonious reconstruction of character states scored in this study (Appendix 1) onto the phylogeny of Auchenorrhyncha estimated by Misof et al. (2014), Cryan and Urban (Cryan and Urban, 2012) and Urban and Cryan (2007). Outgroups are omitted. Character and character state changes reconstructed on the branches are indicated by black (gain) and white bars (reversal). For some characters (e.g., Character 5), an alternative interpretation for character evolution may be possible but, based on the present morphological observation (see text), the interpretation given in the figure is considered to be likely. The result indicated common origin of the jumping ability in Auchenorrhyncha (1:0 –> 1 at the root), but independent evolutionof jumping mechanisms between Cicadomorpha and Fulgoromorpha.

Chapter 2

Evolution of thoracic musculature associated with jumping in Sternorrhyncha

ABSTRACT

As discussed in chapter 1, jumping ability likely originated independently within Auchenorrhyncha, and the common ancestor of Hemiptera probably lacked jumping ability. To elucidate the ground plan of the thoracic structure in Hemiptera, I observed the thoracic musculature of all four sternorrhynchan superfamilies. The suborder Sternorrhyncha is widely accepted as the sister of the rest of Hemiptera and includes two jumping superfamilies, Psylloidea and Aleyrodoidea. The ancestral state reconstruction under likelihood criterion suggested that they gained jumping ability independently. The results from the muscle observation also indicated that the ancestoral sternorrhynchans did not show modified thoracic musculature related to the jumping ability and preserved primitive condition as observed in their outgroup. Based on the implications of Chapter 1 and this chapter, the common ancestor of Hemiptera was estimated to preserve the fundamental musculature of non-jumping insects, and jumping ability was estimated to have evolved at least four times independently within Hemiptera.

INTRODUCTION

As discussed in Chapter 1, I have concluded based on the thoracic musculature that jumping ability evolved twice within Auchenorrhyncha, as also suggested by Burrows and Bräunig (2010) from a kinematic analysis. This result also implies that a more widely accepted hypothesis, regarding the jumping ability as a synapomorphy of Hemiptera (Shcherbakov and Popov, 1997), is questionable. Therefore, the evolution of jumping ability throughout Hemiptera must be reconsidered. To reconstruct the evolutionary history of hemipteran jumping ability, the estimation of their ancestral state of metathorax based on the detailed analyses of skeletal and muscle morphology will provide the most important clue, as discussed in Chapter 1.

The suborder Sternorrhyncha, which includes jumping plant lice, whiteflies, aphids and scale insects, is generally regarded as the sister taxon of the rest of Hemiptera (Cryan and Urban 2012; Johnosn et al., 2018). This is a big group including four superfamilies and more than 16,000 species (Forero, 2008). As suggested from a common name, "jumping plant lice", Psylloidea are well known jumpers in this suborder. From former studies of their internal structures (Crawford, 1914; Weber, 1929; Maki, 1938) and kinematic analyses (Burrows, 2012), the jumping ability of plant lice is known to be driven by furcal trochanter depressor muscle and highly modified metathoracic structure. Their jumping ability is very strong, being comparable with auchenorrhynchans (Burrows, 2003, 2006b, 2009b, 2012). However, jumping ability in the other superfamilies of Sternorrhyncha is only poorly investigated.

In Aphidoidea (aphids), the jumping ability has been reported from *Yamatocallis,* in which jumping by using their thickened front femur, is known (Akimoto, 2014, pers. comm.; Ogawa, 2018, pers. obs.). Although the detailed jumping

mechanics and internal morphology of *Yamatocallis* have not been reported, their jumping ability is apparently non-homologous with the inner metathoracic jumping systems of Psylloidea. In contrast, jumping behavior of Aleyroidea (whiteflies) by using metathoracic muscles has been reported by Weber (1935). Although he provided detailed information of their morphology including musculature, the evolutionary history of this behavior and mechanics has never been discussed. Therefore, the evolutionary origin of the metathoracic jumping ability in Psylloidea and Aleyroidea is still ambiguous.

In this chapter, I discuss the evolutionary process of jumping system in Sternorrhyncha. To assess their minute morphology, I reconstructed their 3D models by using a high-resolution synchrotron micro computed tomography (HR-μCT), which provides useful information for understanding the ancestral traits of morphology and behavior in Hemiptera. I also provide basic 3D model for revealing kinematic computing simulation on hemipteran jumping.

MATERIALS AND METHODS

Taxa examined (Table C2-1)

I selected *Trialeurodes vaporariorum* (Aleyrodoidea: Aleyrodidae), *Glycaspis brimblecombei* (Psylloidea: Aphalaridae), *Heteropsylla cubana* (Psylloidea: Psyllidae), *Diaphorina citri* (Psylloidea: Liviidae), *Trioza nigra* (Psylloidea: Triozidae), *Mindarus japonicas,Tuberolachnus salignus* (Aphidoidea: Aphididae), Margarodidae sp. (Coccoidea: “Archaecoccoidea”: Margarodidae) and *Planococcus citri* (Coccoidea: “Neococcoidea”: Pseudococcidae) as ingroups. *Sternopsocus nigricellus* (Psocodea: "Psocoptera": Stenopsocidae) and *Zorotypus hubbardi* (Zoraptera: Zorotypidae;

Friedrich and Beutel, 2008) were selected as outgroups. The µCT data of *Mindarus japonicas* and Margarodidae sp. was supplementary used for checking and verifying the character coding for these groups, but their detailed 3D model was not provided in this study.

Reconstruction of ancestral characters in Hemiptera

Phylogenetic relationships of Sternorrhyncha and outgroups were taken from Johnson et al. (2018). Character state selected from *Trioza nigra* and *Sternopsocus nigricellus* were adopted for *Trioza urticae* (Trizidae) and *Graphopsocus cruciatus* (Stenopsocidae) in Johnson et al. (2018), respectively. Maximum likelihood reconstruction (ML) of ancestral behavioral and morphological characters were performed using Mesquite 3.51 (Maddison and Maddison, 2018). The behavioral information was referred from Burrows (2012), Weber (1935), Yoshizawa (pers. comm. 2018), Akimoto (pers. comm., 2014) and Ogawa (pers. obs. 2018).

Specimen preparation for 3D reconstruction

To understand the musculature including minute species accurately, samples were scanned using synchrotron microcomputed tomography (µCT) and, based on the obtained data, 3D models were reconstructed. The drying and scanning methods were previously described in Yoshizawa et al. (2018). In brief, the samples were desiccated using CO_2 critical point dryer (EM CPD300, Leica, Wetzlar, Germany) after dehydration series and scanned using BL47XU beamline at the Super Photon ring-8 GeV (SPring-8, Hyogo, Japan). Configured pixel size was 0.48 or 0.52 µm/pix, respectively. The image sequence were trimmed, modified (bit number, size, contrast and angle) and stacked in the software FIJI (Schindelin et al., 2012). 3D models were

segmented in the software ITK-SNAP (Yushkevich et al., 2016) following Yoshizawa et al. (2018). The investigated muscles were homologized as in Chapter 1. The terminologies were followed Beutel et al. (2014). The segmented muscles were colorized on ITK-SNAP as R:G:B = 0:255:50 (skeleton), 0:120:255 (indirect flight muscles), 255:0:120 (IIIdvm7), 250:255:0 (IIIpcm5), 255:150:0 (IIIscm6) and 255:0:255 (IIIspm2), respectively.

Specimen preparation for microscopy observation

Microscopy observation was also performed to check the musculature. Specimen preparation and observation method were already described in Chapter 1.

Character Coding

Character state changes were reconstructed on the phylogenomic tree estimated by Johnson et al. (2018), which included all sternorrhynchan superfamilies and outgroup. Unsampled families were omitted from the tree, and a coded character matrix (Table C2-2) was reconstructed by the maximum likelihood criterion using Mesquite 3.51 (Maddison and Maddison 2018). Criteria for jumping/non-jumping behavior was shown in Chapter 1.

Character 0. Metathoracic jumping behavior: unknown (0) known (1). State 1 was reported in Aleyroidea and Psylloidea (Weber, 1935; Burrows, 2012).

Character 1. IIIdvm7: present (0); absent (1). State 1 was only observed in Psylloidea and Aphidoidea. (Fig. C2-3, C2-4).

Character 2. IIIpcm5: present (0); absent (1). State 1 was also only observed in Psylloidea and Aphidoidea. (Fig. C2-3, C2-4).

Character 3. IIIscm6: present (0); absent (1). State 1 was only observed in Coccoidea. (Fig. C2-5). IIIscm6 of Psylloidea was extremely developed and filled with most room of metathorax (Fig. C2-3).

Character 4. IIIspm2: present (0); absent (1). The groups with jumping ability (Aleyrodoidea and Psylloidea) and Stenopsocidae lost this muscle (State 1) (Fig. C2-2, C2-3).

Character 5. ODM (IIIdlm2): present (0); absent (1). State 1 was confirmed in Psylloidea (Fig. C2-3) and Coccoidea (C2-5A, B).

RESULTS

Metathoracic musculature of Aleyrodoidea

The whitefly examined here (*Trialeurodes vaporariorum*) preserves the fundamental elements of metathoracic musculature (Fig. C2-2), although some modifications of IIIdvm7 and furcal structure were reported by Weber (1935). The trochanter depressor muscles originate from metanotum (IIIdvm7; Character1), developed basalare (IIIpcm5; Character 2:0) and furca (IIIscm6; Character 3:0) (Fig. C2-1A, B, C). The notal trochanter depressor presents, and the muscle (IIIdvm7; Character 1:0) attaches to mesophragma. The furca is medially expanded (Fig. C2-2A,

B), and metafurca-pleuralis muscle (IIIspm2) is absent (Character 4:1). All the indirect flight muscles, DLM, DVM and ODM are preserved (Character 5:0), but the ODM is very small (Fig. C2-2A).

Metathoracic musculature of Psylloidea

The metathorax in jumping plant lice is highly modified from the the condition as observed in the outgroups (Fig. C2-3) as already reported in Weber (1929) and Crawford (1914). The dorsal (IIIdvm7) and plural (IIIpcm5) trochanter depressor muscles are completely absent (Fig. C2-3C) (Character 1:1; 2:1). A large furca-trochanteral muscle (IIIscm6), that occupies most room of metathorax, is the only trochanter depressor muscle (Character 3:0) (Fig. C2-3A, B, C). The muscle is inserted to the deformed furca that is modified as an extremely huge Y-shaped arm. The associated tendon is also arranged as a half spiral wide slope with a huge stem (Fig. C2-3C). DLM and DVM are preserved but reduced. ODM is absent (Character 5:1) (Fig. C2-3A, B).

Metathoracic musculature of Aphidoidea

The metathorax of aphids possesses reduced and simplified musculature as reported by Weber (1928). The furcal trochanter depressor muscle (IIIscm6) is only preserved (Character 3:0) among the three trochanter depressors (Fig. C2-4C), and plural (IIIpcm5) and dorsal (IIIdvm7) trochanter depressor muscles are absent (Character 1:1; 3:1). The furca is not modified, and IIIspm2 is present (Character 4:0) (Fig. C2-4A). All the indirect flight muscles, DLM, DVM and ODM are present, although they are reduced in volume (Character 5:0) (Fig. C2-4B).

Metathoracic musculature of Coccoidea

The metathorax of scale insects are also simplified from the condition as observed in the outgroups. Contrary to aphids, the furcal trochanter depressor muscle (IIIscm6) is absent, and plural (IIIpcm5) and dorsal (IIIdvm7) trochanter depressor muscles are preserved (Character 1:0; 2:0; 3:1) (Fig. C2-5A, C). The furca is not specialized, and IIIspm2 is retained (Character 4:0) (Fig. C2-5A). The reduced DLM and DVM are present, but ODM is absent (Character 5:0) (Fig. C2-5A, B).

Maximum likelihood analysis

To imply the ancestral condition of behavioral and morphological characters, the maximum likelihood ancestral state estimation was conducted using the phylogenomic tree estimated by Johnson et al. (2018). Jumping behavior was estimated to have evolved independently in the superfamilies Psylloidea and Aleyroidea (Character 0:1) (Fig. C2-3). Presence of all three trochanter depressor muscles (IIIdvm7, IIIpcm5, IIIscm6; Character 1-3), metafurca-pleuralis muscle (IIIspm2; Character 4), and all flight muscles (Character 5) was implied as the ancestral condition. Absence of IIIdvm7, IIIpcm5, IIIspm2 and ODM (Character 1:1; 2:1; 4:1; 5:1) was estimated to be derived in Psylloidea. Of them, absence of IIIspm2 (Character 4:1) was identified to have independently evolved also in Aleyrodoidea, and absence of IIIspm2 and ODM (Character3:1; 5:1) was estimated to be derived independently also in Coccoidea. Absence of IIIdvm7 and IIIpcm5 (Character 1:1; 2:1) were estimated to be derived in Aphidoidea.

DISCUSSION

From the results of the likelihood ancestral state reconstruction, three trochanter depressor muscles were estimated to be retained in the common ancestor of Sternorrhyncha, as seen in the outgroups (Character 1:0; 2:0; 3:0). Aleyrodoidea is the sister clade of the rest of sternorrhynchans (Johnson et al., 2018), in which all three trochanter depressor muscles are preserved (Fig. C2-2C). Different thoracic musculatures observed between sister taxa, Coccoidea and Aphidoidea, also suggested the muscle modifications evolved independently between these superfamilies. Of three trochanter depressors, Coccoidea possess IIIpcm5 and IIIdvm7 (Fig. C2-5C), whereas Aphidoidea only have IIIscm6 (Fig. C2-4C). These contradictory states observed between sister taxa can explained only by independent loss of different muscles from the possession of full set of the trochanter depressors (Character 1:0; 2:0; 3:0).

In Psylloidea, a huge furcal trochanter depressor muscle was observed (Character 3:0) (Fig. C2-3A), and it was considered to be the main jumping muscle. The origin of the jumping muscle from the furcal trochanter depressor muscle was also pointed out by Weber (1929) and Crawford (1914). The present observation revealed that the muscle is inserted to the highly modified and robust furca, and the other trochanter depressor muscles (IIIpcm5 and IIIdvm7) are completely absent in Psylloidea (Character 1:1; 2:1) (Fig. C2-3C). The longitudinal indirect flight muscles were reduced, especially ODM was lost (Fig. C2-3B). The musculature apparently shows that the psyllid metathorax is highly modified from the condition as seen in the outgroups, and the function of flight power generator is probably weakened in exchange for gaining strong jumping ability. In contrast, aleyroid metathorax keeps the plesiomorphic condition because all three trochanter depressor muscles are preserved and not so modified (Fig. C3-2-2C). The flight muscles are also well preserved and are apparently functional (Fig. C2-2A).

However, the skeletal morphology of Aleyrodoidea is somewhat modified from the plesiomorphic condition: the furca is expanded medially (Fig. C2-2A), and IIIdvm7 is additionally inserted into mesophragma, which probably improved the jumping ability of Aleyrodoidea by increasing the attachment area of the furcal and notal trochanter depressors (IIIscm6 and IIIdvm7). These modifications were never observed in the other groups of Sternorrhyncha. These differences observed between Psylloidea and Aleyrodoidea strongly suggest that the jumping ability evolved independently between these superfamilies. The expansion of the attachment area of IIIdvm7 on mesophragma was also observed in Auchenorrhyncha (Chapter 1) and thus can be regarded as a homoplasic character, convergently evolved according to the enlargement of the jumping muscles.

The character states of the metafurca-pleuralis muscle (IIIspm2) also supports the independent modification of furca in Aleyroidea and Psylloidea. Although IIIspm2 is preserved in non-jumping Aphidoidae, Coccoidea and outgroup Psocodea (Character 4:0) (Fig. C2-4A, 5A), this muscle is absent in jumping Psylloidea and Aleyroidea (Character 4:1) (Fig. C2-2A, 3A). By the likelihood ancestral state reconstruction, the absence of IIIspm2 was estimated to have evolved independently in two jumping superfamilies and Psocodea (Character 4:1). Although Psocodea was estimated to lose the muscle regardless of jumping ability, the musculature suggests that the two jumping sternorrhynchan superfamilies independently gained the modified furca, as well as the loss of furcal muscle, according to the gain of jumping ability.

The likelihood ancestral state reconstruction of a behavioral character (Character 0) suggested that the sternorrhynchan metathoracic jumping ability was independently originated twice, once in Aleyrodoidea and once in Psylloidea (Fig. C2-1). The muscle condition supports that the common ancestor of Sternorrhyncha did

not have jumping ability and preserved musculature as seen in the outgroups (e.g., Psocodea). Therefore, the jumping ability, as observed in Psylloidea and Aleyrodoidea, can be interpreted to have evolved within Sternorrhyncha.

In conclusion, the ancestoral Sternorrhyncha was estimated to have no jumping ability and possess a full set of trochanter depressor muscles as seen in outgroup. Together with the conclusion provided in Chapter 1, this also implies that the common ancestor of Hemiptera did not have jumping ability and preserved full set of the trochanter depressors as seen in Psocodea, contradicting the generally accepted hypothesis that the jumping ability is a synapomorphy of Hemiptera (Shcherbakov and Popov, 1997).

The results also suggest that Aleyrododidea and Psylloidea independently gained their thoracic jumping ability (Fig. C2-1) with modification of the muscles and skeleton. The results of Chapter 1 and 2 provided another case of multiple evolution of thoracic jumping ability in Hemiptera, and Hemiptera have been estimated to be gained thoracic jumping ability at least four times (Aleyrodoidea, Psylloidea, Auchenorrhyncha and Fulgoromorpha) (In General Discussion Chapter; Fig. D-1). The coexistence of thoracic jumping ability and flight ability is very rare among insects and, apart from Hemiptera, similar condition have only been reported from a limited species of Coleoptera and Hymenoptera (Furth and Suzuki, 1992). This strongly suggests that Hemiptera may have a special character that facilitates the thoracic jumping ability. To reveal it, more detailed and comprehensive observation of thoracic morphology related to jumping and flight is required.

Table C2-1. Taxa examined for this study. The specimens examined are stored at Systematic Entomology, Hokkaido University (SEHU).

ORDER HEMIPTERA
- SUBORDER STERNORRHYNCHA
  - SUPERFMILY ALEYRODOIDEA
    - Family Aleyrodidae
      - *Trialeurodes vaporariorum*
  - SUPERFAMILY PSYLLOIDEA
    - Family Aphalaridae
      - *Glycaspis brimblecombei*
    - Family Psyllidae
      - *Heteropsylla cubana*
    - Family Liviidae
      - *Diaphorina citri*
    - Family Triozidae
      - *Trioza nigra*
  - SUPERFAMILY APHIDOIDEA
    - Family Aphididae
      - *Mindarus japonicas*
      - *Tuberolachnus salignus*
  - SUPERFAMILY COCCOIDEA
    - GROUP "ARCHAEOCOCCOIDEA"
      - Family Margarodidae
        - Margarodidae sp.
    - GROUP "NEOCOCCOIDEA"
      - Family Pseudococcidae
        - *Planococcus citri*

ORDER PSOCODEA
- Family Stenopsocidae
  - *Stenopsocus nigricellus*

ORDER ZORAPTERA (Scored from Friedrich and Beutel, 2008)
- Family Zorotypidae
  - *Zorotypus hubbardi*

Table C2-2. Data matrix used for the most likelihood reconstruction. See text for characters and their states. Character states of *Zorotypus hubbardi* were scored from Friedrich and Beutel (2008).

	0	1	2	3	4	5
Planococcus citri (Coccoidea: Pseudococcidae)	0	0	0	1	0	1
Tuberolachnus salignus (Aphidoidea: Aphididae)	0	1	1	0	0	0
Trioza nigra (Psylloidea: Triozidae)	1	1	1	0	1	1
Diaphorina citri (Psylloidea: Liviidae)	1	1	1	0	1	1
Heteropsylla cubana (Psylloidea: Psyllidae)	1	1	1	0	1	1
Glycaspis brimblecombei (Psylloidea: Apharalaridae)	1	1	1	0	1	1
Trialeurodes vaporariorum (Aleyrodoidea: Aleyrodidae)	1	0	0	0	1	0
Stenopsocus nigricellus (PSOCODEA: Stenopsocidae)	0	0	0	0	1	0
Zorotypus hubbardi (ZORAPTERA: Zorotypidae)	0	0	0	0	0	0

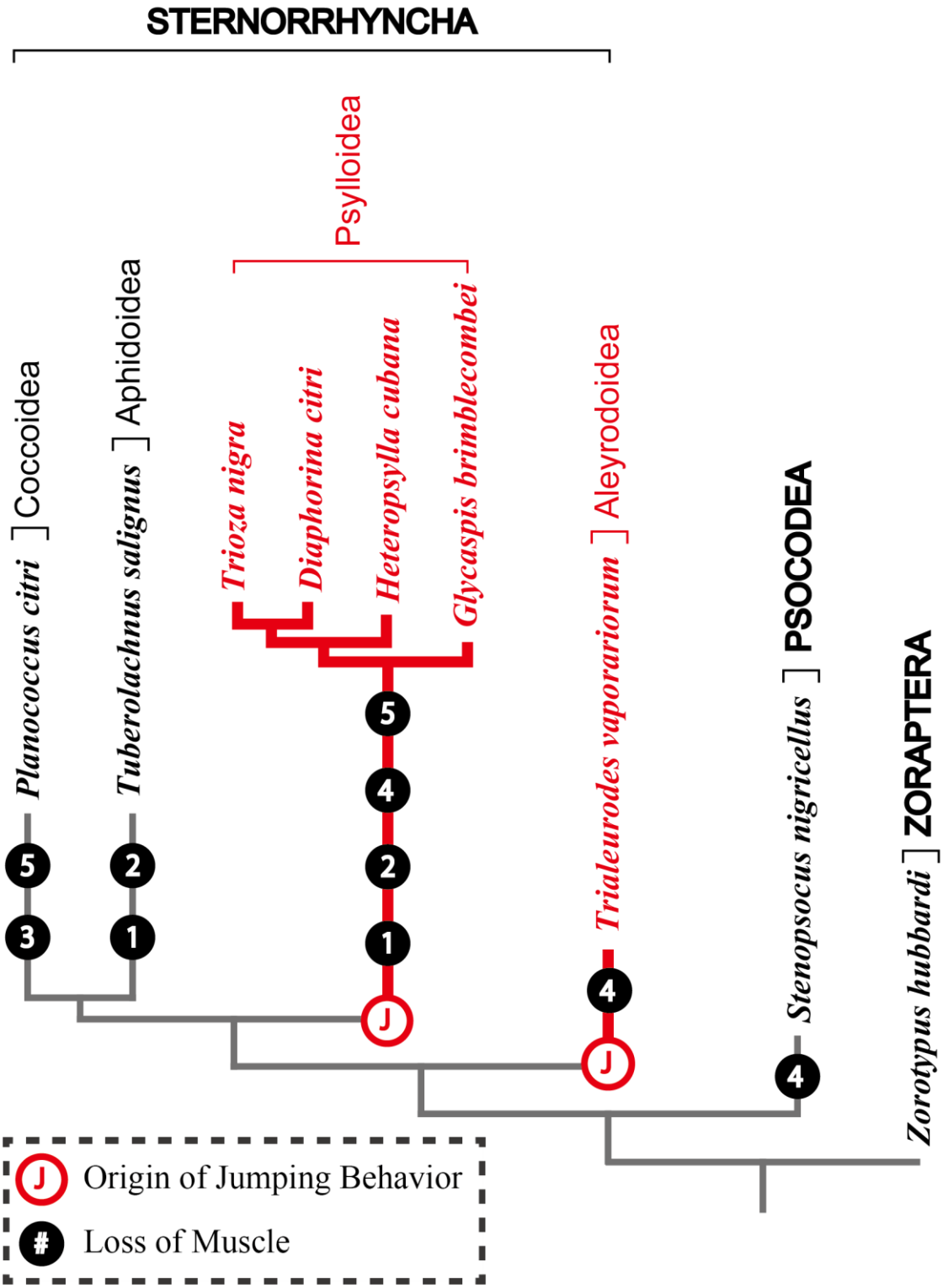


Fig. C2-1. ML reconstruction of jumping behavior and related muscles in Sternorrhyncha. The phylogenetic relationship was cited from Johnson et al. (2018). The numbers correspond with coded characters. 1, IIIdvm7. 2, IIIpcm5. 3, IIIscm6. 4, IIIspm2. 5, ODM (IIIdlm2). J, jumping behavior.

Fig. C2-2. 3D reconstruction images of right half metathorax of *Trialeurodes vaporariorum* (Aleyrodidae: Aleyrodoidea). A, lateral view. B, anterial view. C, hind coxa and trochanter with trochanter depressor muscles.

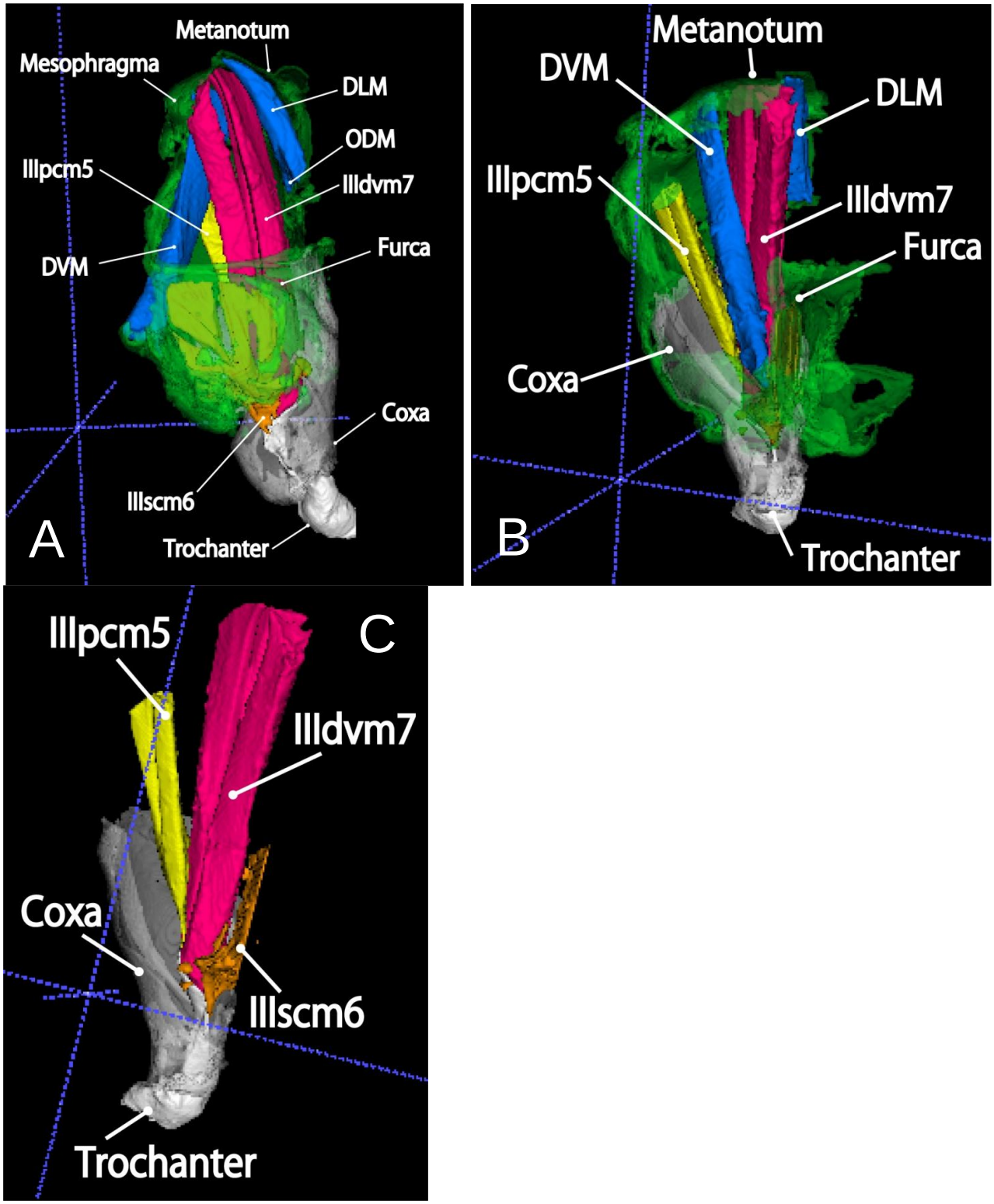


Fig. C2-3. 3D reconstruction images of right half metathorax of *Heteropsylla cubana* (Psyllidae: Psylloidea). A, lateral view. B, anterial view. C, hind coxa and trochanter with a trochanter depressor muscle.

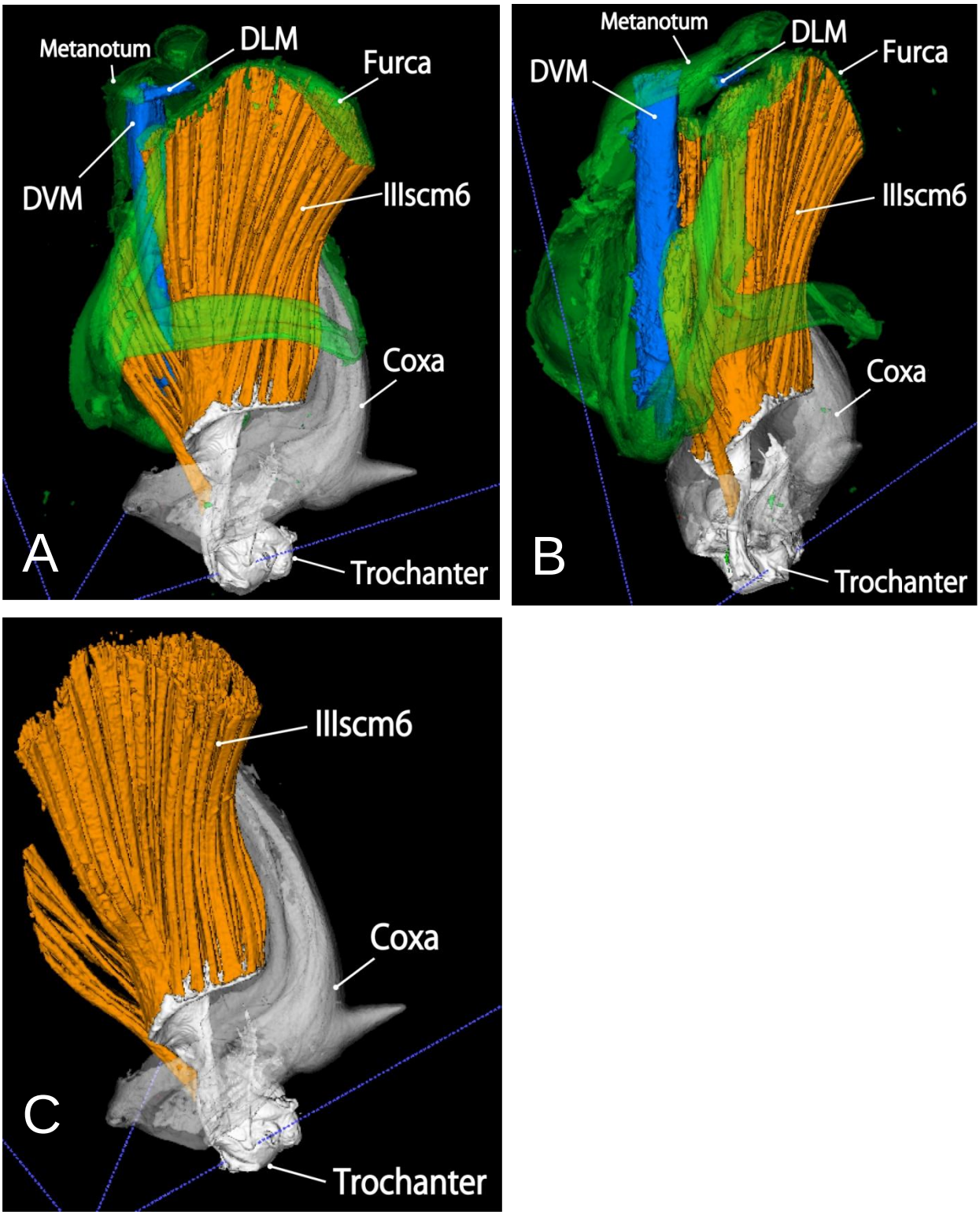


Fig. C2-4. 3D reconstruction images of left half metathorax of *Mindarus japonicas* (Aphididae: Aphidoidea). A, posterolateral view. B, anterial view. C, hind coxa and trochanter with a trochanter depressor muscle.

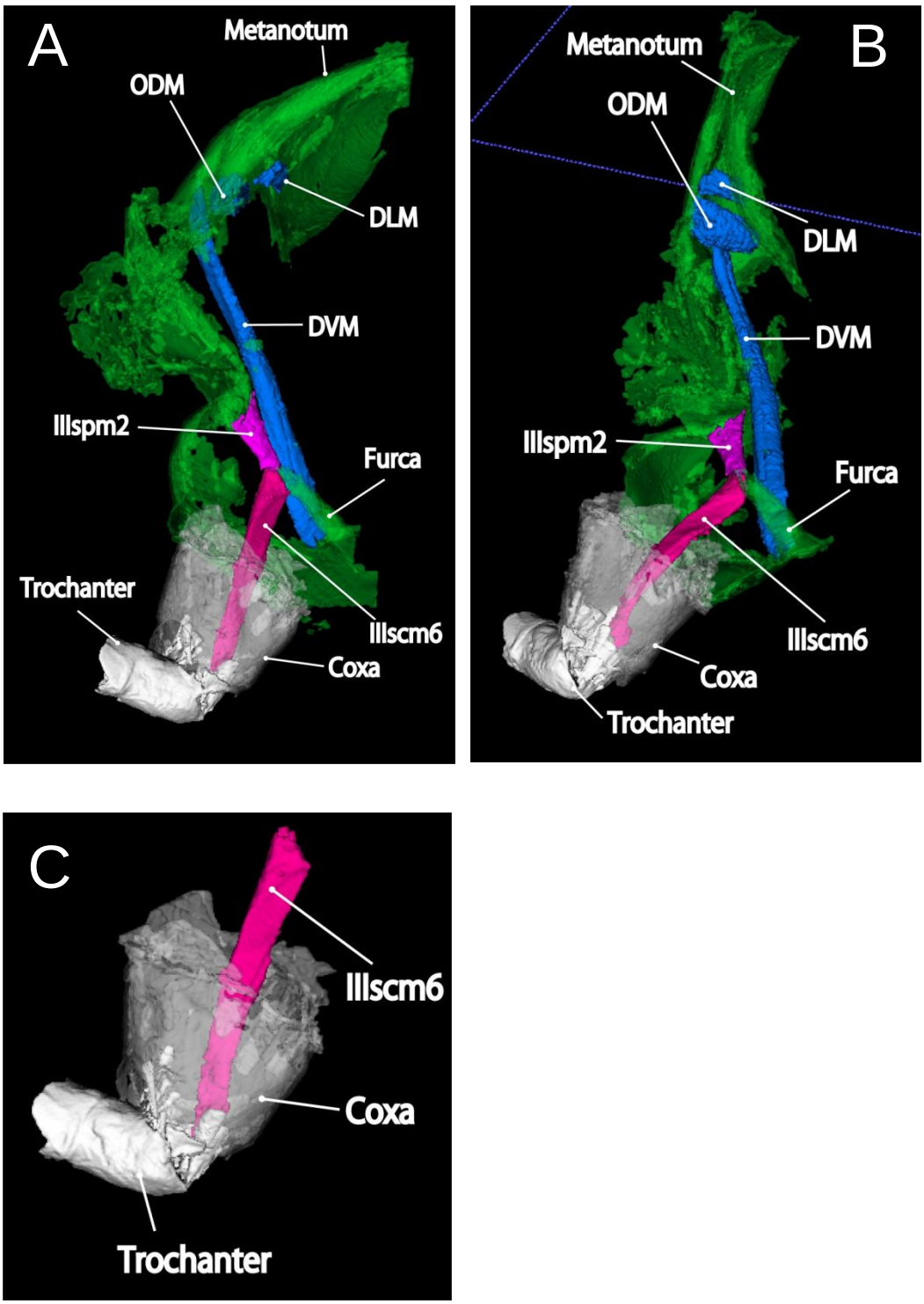
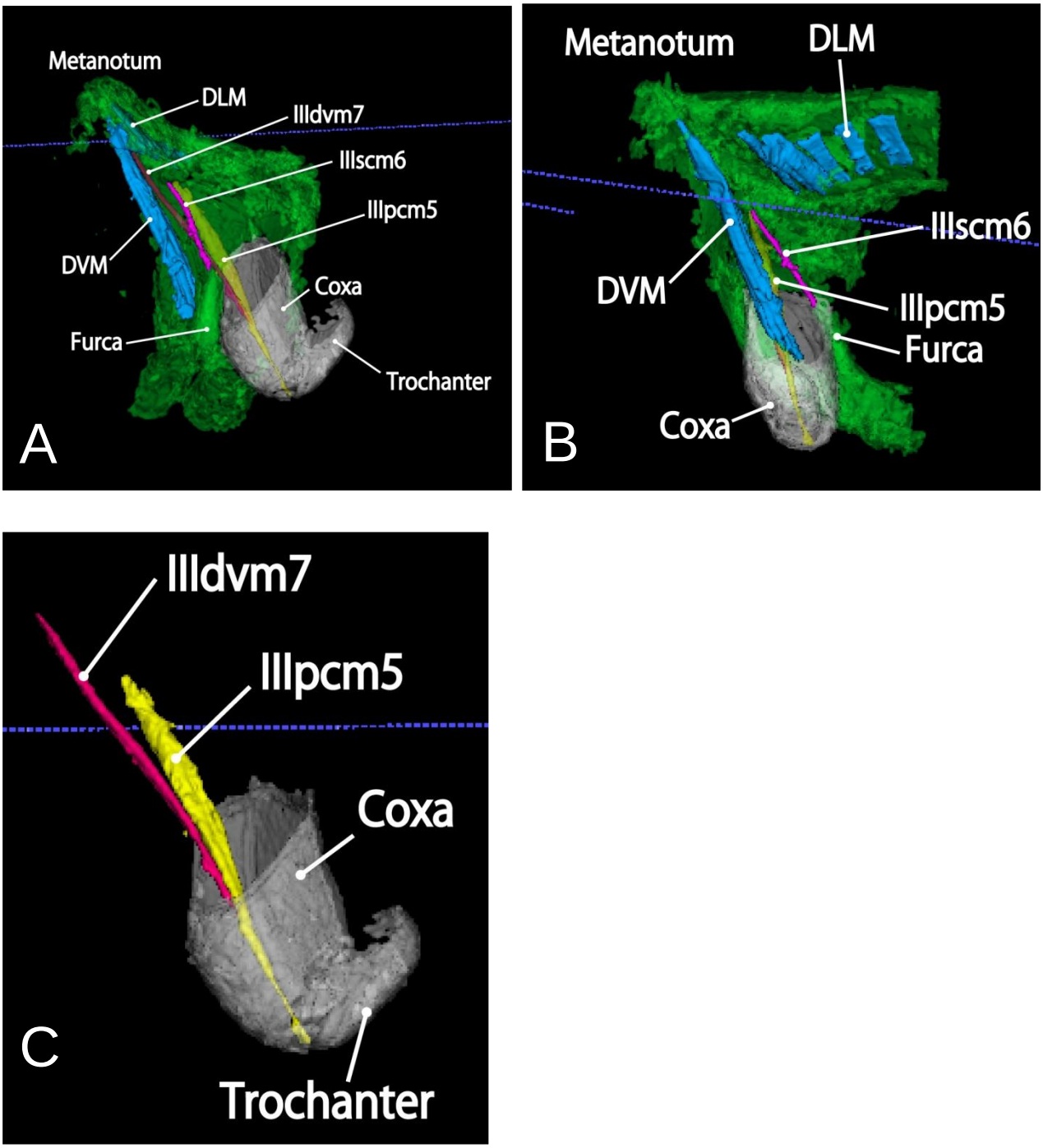


Fig. C2-5. 3D reconstruction images of right half metathorax of Margarodidae sp. (Coccoidea). A, posterial view. B, anterial view. C, hind coxa and trochanter with trochanter depressor muscles.



Chapter 3

Origin of wing coupling structure in Paraneoptera

Note: This chapter includes a version of an article published in Journal of Morphology: Ogawa, N., Yoshizawa, K. 2017. Origin and transformation of the in-flight wing-coupling structure in Psocodea (Insecta: Paraneoptera). Journal of Morphology 279 (4): 517-530. doi:10.1002/jmor.20785

ABSTRACT

Many four‐winged insects have mechanisms that unite the forewings and hindwings in a single plane. Such an in‐flight wing coupling apparatus may improve flight performance in four‐winged insects, but its structure is variable among different insect groups. The wings of bark lice (Insecta: Psocodea:“Psocoptera”) also have an in‐flight wing coupling apparatus, but to date, its morphology has not been studied in detail. In this study, we examined the wing‐coupling structure in representative species of the three suborders of bark lice (Trogiomorpha, Troctomorpha, and Psocomorpha) and inferred its origin and transformation. We conclude that the main component of the psocodean wing coupling apparatus evolved once in the common ancestor via modification of cuticular structures at the apex of the forewing CuP vein. Morphological differences in components of the coupling structures are phylogenetically informative at the intraorder level and include an autapomorphy that characterizes Troctomorpha and a synapomorphy that supports a sister relationship between Troctomorpha and Psocomorpha.

INTRODUCTION

The evolution of insect wings and powered flight are cited as epochal events in their history (Brodsky, 1994), and insect flight mechanisms have attracted significant interest from functional and comparative biologists (Wootton, 1992; Brodsky, 1994; Grodnitsky, 1995, 1999). Winged insects usually have two pairs of (i.e., four) wings. Some four-winged insects, such as dragonflies (Odonata) and locusts (Orthoptera), flap the fore- and hindwings independently (Chapman, 2013), but this condition is rather exceptional among insects, possibly because this mode is less efficient and less stable (Pope, 1994). Loss of the thrust-generating function in either the fore- or hindwing pair (i.e., dipterous flight) is more frequent; this occurs, for example, in Diptera (true flies) and Strepsiptera (the twisted wing parasites). The forewings of Coleoptera (beetles), the elytra, function to protect the wings from external damage (Linz et al., 2016). The elytra are sclerotized and held away from the body during flight, and they do not generate thrust. Complete loss of the capacity to generate thrust in either the fore- or hindwing pair occurs in mayflies (Ephemeroptera), earwigs (Dermaptera), crickets (Orthoptera), stick insects (Phasmida), scale insects (Hemiptera), wasps (Hymenoptera), lacewings (Neuroptera), and moths (Lepidoptera) (Grodnitsky, 1995).

The predominant flight mode in four-winged insects is functionally two-winged flight (Grodnitsky, 1999). In such insects, the fore- and hindwings are connected by an in-flight coupling apparatus. Grodnitsky (1999) defined two coupling modes in the functionally two-winged insects: wings that are coupled during the downstroke only (part-time coupling) and wings that are mechanically connected and fully synchronized during flight (full-time coupling). The wing coupling apparatus of “full-time” coupled flyers has been reported in Paraneoptera [bark lice (Psocodea:

Weber, 1936), thrips (Pesson, 1951a; Thysanoptera: Moritz, 1997), aphids, cicadas and true bugs (Pesson, 1951b; Hemiptera: Bohne and Schneider, 1979; D'Urso and Ippolito, 1994; Ni et al., 2002)] and Holometabola [wasps (Hymenoptera: Basibuyuk and Quicke, 1997), moths and butterflies (Lepidoptera: Tillyard, 1918; Braun, 1924), and caddisflies (Trichoptera: Stocks 2010a; Tillyard 1918)]. Such full-time wing coupling apparatuses are one of two primary types: 1) setae of one wing that interact with setae or other structures on the other wing, such as in Thysanoptera (Pesson, 1951a; Moritz, 1997), Hymenoptera (Basibuyuk and Quicke, 1997), and Trichoptera (Tillyard, 1918; Stocks, 2008, 2010b, 2010a); 2) non-setal cuticular structures, often in the form of grooves, such as in various Hemiptera (Pesson, 1951b; D'Urso and Ippolito, 1994) and the jugum in Lepidoptera and Trichoptera (Tillyard, 1918; Stocks, 2010a, 2010b).

The in-flight wing coupling apparatus of the free-living Psocodea, or "Psocoptera", is located on the distal end of the CuP vein (Fig. C3-1A, "fli") and clasps the hindwing at all times during wing flapping. The psocopterans also have a repose-coupling apparatus located on the R or Sc vein that engages the costa of the hindwing at rest (Mockford, 1967; New, 1974) (Fig. C3-1A, "rep"), but this structure is not further examined here. The psocopteran in-flight wing coupling structure is formed from non-setal cuticles and consists of a "hook" that engages the anterior margin of the hindwing (Weber, 1936; Günther, 1974). Although there are some scanning electron microscopy (SEM) studies on the psocopteran in-flight coupling structures (Lawson and Chu, 1974; New, 1974), the structures have not been studied comprehensively. Therefore, their evolutionary pattern and phylogenetic value are as yet unclear. Furthermore, there is also uncertainty about the homology of the structures with those of other paraneopteran orders (i.e., Thysanoptera and Hemiptera: Lawson and Chu, 1974).

Currently, three suborders of 'Psocoptera' are recognized: Trogiomorpha, Troctomorpha (including the parasitic lice) and Psocomorpha (Yoshizawa et al., 2006). Mockford (1967) and Yoshizawa (2002, 2005) recognized that in-flight wing coupling structures in psocids are phylogenetically informative characters, having a 'hook' composed of truncated 'spines' fused at their bases as either a synapomorphy supporting the clade Troctomorpha + Psocomorpha (Mockford, 1967) or an autapomorphy of Psocomorpha (Yoshizawa, 2002, 2005). Molecular phylogenetic approaches support Troctomorpha and Psocomorpha as sister taxa (Yoshizawa et al., 2006; Yoshizawa and Johnson, 2014), but few morphological characters that might support this relationship are known. I expanded on the previous wing characters surveys that were mostly based on light microscopy by including additional taxa and using SEM to examine structures at higher magnification and resolution. I evaluated wing coupling characters based on the phylogenetic hypotheses presented by Yoshizawa & Johnson (2010, 2014), Friedemann et al. (Friedemann et al., 2014) and Yoshizawa & Lienhard (2016). I examined the homology and character state transformations of the wing coupling apparatus.

MATERIALS & METHODS

Taxon selection (Table C3-1)

The taxa examined were as follows: Trogiomorpha, 5 species representing 4 families; Troctomorpha, 7 species representing 9 families; and 16 species representing the 23 families of Psocomorpha. I included a species of Psocomorpha: Calopsocidae, recently synonymized with Pseudocaeciliidae (Yoshizawa and Johnson, 2014), because of the highly modified, elytra-like wings. *Aeolothrips kurosawai* (Thysanoptera:

Aeolothripidae) and *Cinara* sp. (Hemiptera: Aphididae) were selected as outgroups.

Treatment of Specimens

Specimens examined were stored in 80% or 99% ethanol. Forewings were removed and dehydrated in 100% ethanol for 1 hour. Wing cuticle was hardened by soaking with 1,1,1,3,3,3-hexamethyldisilazane for 1 hour and air drying prior to mounting on 10mm aluminum stubs. Wings were held in place with sticky urethane sheets (Kokuyo Hittsuki Sheet, Kokuyo Co. Ltd., Tokyo) and coated with Au-Pd in a Hitachi E101 ion sputter-coater (Hitachi High Technologies Corp., Tokyo) for 120 sec. I used a Jeol JSM-5310LV scanning electron microscope (Jeol Ltd., Tokyo) and photographed screen images with Jeol Digi Capture SUP-7707 Version 1.0.11. Figures were arranged with Adobe Photoshop CC 2014 and Adobe Illustrator CC 2014. In order to make comparisons easier, pseudo-colorization of homologous structures, change of contrast intensity, uniting multiple images as a single image, and/or flipping of images were applied to the figures.

Character Coding

Character state changes were reconstructed on the composite phylogenetic tree (Fig. C3-2 in Yoshizawa and Johnson, 2014), which included almost all psocid families and was used as a backbone tree. Placement of Pachytroctidae and Liposcelididae was based on Yoshizawa & Johnson (2010); those families were not sampled in Yoshizawa & Johnson (2014). The outgroup taxa were based on Friedemann et al. (Friedemann et al., 2014) and Yoshizawa & Lienhard (2016). The composite tree were trimmed as in chapter 2, and a coded character matrix was reconstructed by the parsimony criterion using Mesquite version 3.04 (Maddison and Maddison, 2015).

Terminology

Different terms have been used for the wing structures in Psocoptera, but the terms used here are based on Weber (1936) and Günther (1974).

RESULTS

Summary of general morphology (Fig. C3-1)

Structures forming the forewing in-flight coupling apparatus are near the apical-most region of the CuP vein and are termed the **retinaculum**, **CuP-tip**, and **retainer** (Fig. C3-1A, B). The term "nodulus" indicates the region where the CuP and A1 veins join near the posterior wing margin (Fig. C3-1A, B, junction of two green lines). **Rib-like** structures (Fig. C3-1B, r) are arranged at almost equal intervals along the CuP vein (Günther, 1974; Fig. C3-1B) generally with each structure arranged transverse to the vein. The rib-like structures occur on other veins, but their presence is taxonomically variable.The "**retinaculum**" (Fig. C3-1B, Rc) is composed of many spine-like cuticles ("**retinacular spines**") that as a unit form a hook-like structure. The "**CuP-tip**" (Fig. C3-1B, Ct) refers to the apical-most section of the CuP vein between the Rc and the posterior wing margin that bears modified **rib-like structures**.

The "**retainer**" (Fig. C3-1B, Rtr) is a highly thickened and bent structure on the posterior wing margin that bears a large number of fine stud-like projections. The retainer is taxonomically variable in the degree of swelling and the morphology of the projections. The retinaculum and retainer together form a 'clip-like' functional unit that engages the costal margin of the hindwing.

The counterpart of the in-flight wing coupling apparatus in hindwings (i.e., the anterior margin) is not specialized among the taxa examined, although the wing

coupling apparatus of forewings is diversified. The anterior margin of the hindwing engages in both types of wing coupling (repose and in-flight) and is bent and rolled inwardly (Fig. C3-1C). At rest, the repose-coupling apparatus (Fig. C3-1A, “rep”) fits into the bend (white arrow, Fig. C3-1C) and supports the hindwing. During flight, the ‘clip-like’ unit formed by the retinaculum and retainer catches the hindwing margin.

Trogiomorpha

Venation and the rib-like structure (Fig. C3-3)

The A1 and CuP veins do not join (i.e., the nodulus is absent), although they terminate closely. *Psoquilla* sp. (Psoquillidae) lacks A1. The CuP is thickened as in other veins. The rib-like structure is distributed on all veins, but the ribs on the CuP are more prominent.

Morphology of the retinaculum (Fig. C3-4)

The retinaculum is present in all examined trogiomorphan taxa, and all are composed of several separated spines (Fig. C3-4; Character 3:1; 4:0) that are continuous with the row of ribs on the CuP. In *Prionoglaris* (Prionoglarididae; Fig. C3-4A), the retinaculum is composed of approximately nine nearly straight spines. *Echmepteryx* (Lepidopsocidae) also possesses a simple retinaculum composed of approximately 10 straight spines (Fig. C3-4E). In all other species, the retinaculum is composed of curled spines (Fig. C3-4C, D). *Psyllipsocus* (Psyllipsocidae) has a simple retinaculum composed of only four bent spines (Fig. C3-4C). The retinaculum of *Psoquilla* consists of approximately 10 densely arranged and strongly curled and twisted spines (Fig. C3-4D). The spines of *Psyllipsocus*, *Echmepteryx* and *Psoquilla* are apically fringed

(Fig. C3-4A, C, D; Character 5:1).

The retinaculum of *Neotrogla* (Prionoglaridídae; Fig. C3-4B) shows an extremely different structure from that of the other trogiomorphan species, including a species of the family (*Prionoglaris*). The rib-like structure on the CuP vein becomes gradually recumbent and merges into the vein toward the retinaculum (Character 6:1). The retinaculum is composed of one short and three long, sharp spines projecting posteriorly.

Morphology of the CuP-tip (Fig. C3-4)

The CuP-tips in *Prionoglaris*, *Psyllipsocus*, *Psoquilla* sp. and *Echmepteryx* are continuous from the basal CuP without remarkable modification (Fig. C3-4A, C–E). The ribs on the CuP-tips are transversely arranged without detectable modifications from those on the CuP vein (Character 8:0). The CuP-tip of *Neotrogla* retains only one transversely arranged rib near the base of the retinaculum (Fig. C3-4B).

Morphology of the retainer (Fig. C3-4)

The retainer of Trogiomorpha tends to be thickened and bent (Character 11:1), but no further remarkable deformation compared to the unmodified posterior wing margin was detected. The surface is uniformly covered with scaly studs (Characters 14:0; 15:0), but the studs of *Neotrogla* are recumbent and mostly merge into the vein (Fig. C3-4B). In *Echmepteryx*, the surface studs distal to the retainer are gradually reduced (Fig. C3-3E; Character 15:2).

<u>Troctomorpha</u>

Venation and rib-like structure (Fig. C3-5)

The A1 and CuP veins closely approximate at the distal ends. They are clearly joined (= nodulus: Fig. C3-5C, D, E), separated (Fig. C3-5B), or are intermediate in condition (Fig. C3-5A). The rib-like structures are distributed on all veins, but they are more prominent on the CuP.

The species in two families of Nanopsocetae have somewhat simplified forewings. The forewing of *Embidopsocus* (Liposcelididae) lacks a rib, a coupling structure and most veins (Character 2:1; Fig. C3-6A). The forewing of *Tapinella* (Pachytroctidae) lacks the coupling structure and nodulus (Fig. C3-6C), but all principal veins and the reduced ribs (Character 1:1) are retained (Fig. C3-6B).

Morphology of the retinaculum (Fig. C3-5)

The retinaculum is composed of curled, separated spines (Character 3:1: 4:0). The number of spines and their condition are variable: they are numerous and in contact with each other in *Stimulopalpus* (Fig. C3-5A); they are 5–10 in number and closely approximated in Troctopsocidae Gen. (Troctopsocidae; Fig. C3-5B), *Selenopsocus* (Troctopsocidae; Fig. C3-7C) and *Manicapsocus* (Electrentomidae; Fig. C3-7E); and there are five well-separated spines in *Musapsocus* (Musapsocidae; Fig. C3-5D). The spines are apically fringed (Fig. C3-7A–C, E) except for *Musapsocus* with simple spines (Fig. C3-7D).

Morphology of the CuP-tips (Fig. C3-7)

The ribs on the CuP-tip of troctomorphan species are arranged diagonally or vertically against the CuP (Character 8:1) and are thinner than those on the basal CuP vein (Fig. C3-7A–E). In particular, the ribs on the CuP-tips of *Musapsocus*,

Stimulopalpus, *Selenopsocus* and *Manicapsocus* are highly modified; much smaller ribs are arranged densely and vertically in multiple rows (Character 9:1; Fig. C3-7A, C–E), although the ribs on the CuP-tips of Troctopsocidae Gen. are not reduced in size and are arranged in a row (Character 9:0; Fig. C3-7B).

Morphology of the retainer (Fig. C3-5)

The retainer is inwardly rolled (Character 10:1) and uniformly ornamented with scale-like studs (Characters 14:0; 15:0). However, the retainers of some troctomophan taxa are more modified. *Musapsocus* has a slightly broadened retainer (Character 12:1), and the studs are more densely arranged on the counterpart of the retinaculum (Fig. C3-5D). The retainer of *Selenopsocus* is also swollen (Character 12:1; Fig. C3-5C). *Stimulopalpus* has a wide swelling (Character 12:1) with a narrow dent (Character 13:1; Fig. C3-5A). The retainer of *Manicapsocus* is also swollen (Character 12:1), and the retainer is fully expanded and rolled inwardly (Character 10:1; Fig. C3-5E). The retainer of Troctopsocidae Gen. is only slightly bent (Character 11:1; Fig. C3-5B) and lacks a swelling (Character 12:0).

<u>Psocomorpha</u>

Venation and the rib-like structure (Fig. C3-8)

The CuP and A1 veins are joined distally, consistently forming the nodulus. The CuP vein has well developed ribs, but ribs are not present on the other veins.

Morphology of the retinaculum (Fig. C3-9)

The morphology of the retinaculum is quite stable throughout the suborder; it

consists of completely fused curled spines (Character 3:1; 4:1). Judging from the numbers and condition of the slits, the retinaculum appears to be composed of many twisted spines in most psocomorphans. In *Archipsocus* (Archipsocidae), the retinaculum is simplified and apically pointed, and it appears to be composed of three spines (Fig. C3-9A). The retinacular spines are not fringed in the Psocomorpha in general, but the retinacular spines of *Matsumuraiella* (Dasydemellidae; Fig. C3-9B), *Amphipsocus* (Amphipsocidae; Fig. C3-9D), *Valenzuela* (Caeciliusidae; Fig. C3-9E), *Aaroniella* (Philotarsidae; Fig. C3-9I), *Goja* (Epipsocidae; Fig. C3-9M) and *Psilopsocus* (Psilopsocidae; Fig. C3-9O) have apparent to obscure apical fringes.

Morphology of the CuP-tips (Fig. C3-9)

In *Archipsocus*, the CuP-tip becomes obscure with only a reduced rib (Fig. C3-9A). In the other infraorders, the morphology of the CuP-tip is stable. The ribs are arranged diagonally (Character 8:1), and the anterior ribs are continuous with the retinaculum (Character 7:1; cf. Fig. C3-9B). The CuP-tip of *Calopsocus* (Calopsocidae; Fig. C3-9K) lacks the rib (Character 9:2), making the surface of the CuP-tip smooth. The ribs of *Heterocaecilius* (Pseudocaeciliidae) are also reduced and are only represented by some diagonal slits (Fig. C3-9L).

Morphology of the retainer (Fig. C3-8)

The retainer is generally bent and rolled inwardly (Characters 10:1, 11:1). Further deformation of its shape also occurs. In *Archipsocus* (Fig. C3-8A), the spiny area is restricted to a position in front of the retinaculum, and the other areas lack any ornamentation (Characters 14:2, 15:2). In *Valenzuela* (Caeciliusidae; Fig. C3-8E), *Peripsocus* (Peripsocidae; Fig. C3-8F), *Aaroniella* (Philotarsidae; Fig. C3-8I),

Trichopsocus (Trichopsocidae; Fig. C3-8J) and *Calopsocus* (Calopsocidae; Fig. C3-8K), the retainer has a swelling (Character 12:1), which is particularly prominent in *Calopsocus* and *Heterocaecilius* (Character 12:2).

The retainer surface is generally ornamented with fine scale-like studs. However, the retainer decoration of some species (*Valenzuela*; Fig. C3-8E, *Aaroniella*; Fig. C3-8I, *Trichopsocus*; Fig. C3-8J, *Calopsocus*; Fig. C3-8K) and *Heterocaecilius* (Fig. C3-8L)) is modified to fine spines (Characters 14:1, 15:1). The shapes of the surface studs of *Amphipsocus* (Amphipsocidae; Fig. C3-8D) and *Matsumuraiella* (Dasydemellidae; Fig. C3-8B) differ significantly between the proximal and distal regions with the retinaculum as the boundary; the proximal surface is covered with scale-like studs (Character 14:0), but the distal surface is arranged with fine spines (Character 15:0). *Goja* (Epipsocidae; Fig. C3-8M) has scale-like studs only on the proximal surface, and the studs disappear distally (Character 15:2).

Outgroups (Fig. C3-10)

Wing coupling apparatuses are found consistently in the outgroups (Hemiptera and Thysanoptera), but their conditions are completely different from those of the psocopterans. Aphids (Hemiptera) possess hamuli (= hooked hairs) along the costal margin of the hindwing (Ni et al., 2002). The coupling apparatus of thrips (Thysanoptera) consists of the marginal setae of the fore- and hindwings (Ellington, 1980). Their vein surfaces are decorated occasionally similar to those of Psocoptera (Fig. C3-10A, C). *Cinara* (Aphididae: Hemiptera) possesses ribs on all of the veins (Character 1:1; Fig. C3-10B). The veins of *Aeolothrips* (Aeolothripidae: Thysanoptera) are covered with embossed tiles, each bearing a microtrichium (Fig. C3-10D; Character 1:0). In the outgroups, the nodulus is not formed.

Character coding and phylogenetic reconstruction

Based on the observations, 15 characters that may be relevant to psocid phylogeny were selected and coded from the wing coupling structure (Table C3-2). Only qualitative or discontinuous quantitative characters were coded, although some continuous characters were also mentioned in the above morphological descriptions. The result of the parsimonious reconstruction of these characters is shown in Fig. C3-11. The character indices are as follows: Consistency Index = 0.41; Retention Index = 0.65.

Character 1. Decoration of CuP: tile pattern with microtrichia (0); rib-like structure (1). State 0 was only observed in *Aeolothrips* (Thysanoptera) (Fig. C3-10C, D). State 1 was observed in *Cinara* sp. (Hemiptera; Fig. C3-10A, B) and all Psocoptera (Figs. C3-3A–E; C3-5A–E; C3-8A–P) except for the veinless *Embidopsocus* (Liposcelididae; Fig. C3-6A).

Character 2. Venation. Present (0); reduced (1). Almost all examined taxa possessed venation (State 0), but the venation is reduced in *Embidopsocus* (Liposcelididae: Troctomorpha; Fig. C3-6A), which almost entirely lacks veins (state 1).

Character 3. Retinaculum on CuP. Absent (0); present (1). This is identified as an autapomorphy of Psocodea (state 1), but its secondary absence was detected in *Embidopsocus* and *Tapinella* (state 0).

Character 4. Arrangement of retinacular spines. Clearly, separated from each other (0); fused with each other (1). State 1 was identified as an autapomorphy of Psocomorpha (Fig. C3-8A–P; State 1).

Character 5. Tip of the retinacular spines. Not divided (0); fringed (1). State 1 was

observed in some taxa of all three suborders (Fig. C3-7B–E) and was identified as a highly homoplasious condition.

Character 6. Retinacular spines. Standing (0); laying (1). State 1 was only observed in *Neotrogla* (Prionoglarididae; Fig. C3-4B).

Character 7. Retinaculum and ribs on CuP-tip. Separated (0); anterior rib merged into the retinaculum (1). State 1 was identified as an autapomorphy of Psocomorpha (Fig. C3-9A–P).

Character 8. Angle of the ribs against the CuP-tip. Transversal (0); diagonal to vertical (1). State 1 was identified as a synapomorphy of Troctomorpha and Psocomorpha.

Character 9. Number of rows of ribs on CuP-tip. 1 (0); 2 or more (1); absent (2). State 1 was observed in almost all troctomorpha except for Troctopsocidae Gen. (Fig. C3-7A, B, D, E). The CuP-tip of *Calopsocus* completely lacks ribs, and this was coded as state 2.

Character 10. Retainer. No inward rolling (0); rolling inwardly (1). State 1 was detected in all Psocoptera (Fig. C3-3A–E; 5A–E; 8A–P) except for the species lacking the wing coupling structure (*Tapinella* and *Embidopsocus*). Although the outgroups lack the retainer, state 0 was adopted due to it having a non-rolling forewing hind margin (Figs.C3- 6A, B; C3-10A, C)

Character 11. Posterior margin of the retainer. Not bent (0); bent (1). Almost all examined taxa have a bent retainer (State 1), but the retainer of *Stimulopalpus* (Amphientomidae), *Tapinella* (Pachytroctidae), *Embidopsocus* (Liposcelididae), *Heterocaecilius* (Pseudocaeciliidae), *Hemipsocus* (Hemipsocidae), and *Psilopsocus* (Psilopsocidae) is not bent (Figs. C3-5B; C3-6A, B; C3-8L, N, O; State 0).

Character 12. Retainer. No swelling (0); with swelling (1); strongly swelling and forming a protrusion (2). State 1 was observed in several scattered taxa (Figs. C3-5A, B, D; C3-8E–G, I, J, M, O), and *Heterocaecilius* (Pseudocaeciliidae) and *Calopsocus* (Calopsocidae) have a huge protrusion on the retainer (State 2; Fig. C3-8K, L).

Character 13. Surface of the retainer swelling. No dent (0); with dent (1). State 1 was only observed in *Stimulopalpus* (Amphientomidae; Fig. C3-5B).

Character 14. Surface of the proximal region of the retainer. Covered with scale-like studs (0); with trichomes (1); bare (2). The proximal region of the retainer surface of *Cinara* sp. and almost all psocopterans is covered with tiny scale-like studs (State 0). The surface of several psocopteran and thysanopteran taxa is covered by fine trichomes (Fig. C3-8E, I–L). The proximal retainer surface of *Archipsocus* sp. is bare (Fig. C3-8A; State 2).

Character 15. The surface of the distal region of the retainer. Covered with scale-like studs (0); with trichomes (1); bare (2). The distal retainer surface of *Cinara* sp. and almost all psocopterans is also covered with tiny scale-like studs (State 0). The distal surface ornamentations of Thysanoptera and psocopteran taxa are trichomes (Fig. C3-8B, D, E, I–L; State 1). This region is bare in *Echmepteryx* (Lepidopsocidae), *Archipsocus* (Archipsocidae) and *Goja* (Epipsocidae) (Figs. C3-3E; C3-8A, M; State 2).

DISCUSSION

Origin and homology

The psocopteran wing coupling system is composed of three functional units: two on the forewing, the retinaculum and retainer (Fig. C3-1B), and the costal margin of the hindwing, in which the retinaculum and retainer engage the anterior margin of the hindwing during flight.

Scanning electron micrographs observations clearly suggest that the retinaculum is composed of highly modified rib-like structures. Their homology is most clearly indicated in *Prionoglaris* (Trogiomorpha: Prionoglarididae; Fig. C3-3A), in which the retinacular spines and normal ribs differ only by the degree to which their apexes are extended.

Wing veins in other insects are often arrayed with rows of microtrichia (Fig. C3-10), and the microtrichia are probably homologous with the ribs. The rib-like structures are thin and semi-circular projections that give the CuP vein a rasp-like appearance. Similar rib-like structures occur in *Cinara* (Hemiptera: Aphididae; Fig. C3-10B) but are not part of a wing-coupling system. Therefore, the presence of the retinaculum is apparently an autapomorphic condition for Psocodea.

The retainer is formed by various but relatively simple modifications of the cuticle on the surface of the posterior wing margin. The retainers in all specimens examined curved inward to some degree (Fig. C3-1) with additional bends and/or protrusions in some species of Troctomorpha and Psocomorpha.

I conclude that the psocopteran wing coupling system is unique. The morphology of the components is distinct from those of outgroup taxa, although Lawson and Chu (1974) suggested the homology of the structure between Psocoptera and Hemiptera. The wing coupling system in Thysanoptera is formed by setae located on the fore- and hindwing margins (Ellington, 1980). In Hemiptera: Sternorrhyncha it is composed of ‘hamuli-like’ projections on the hindwing margin (Ni et al., 2002), and in

Hemiptera: Auchenorrhyncha the coupling system is composed of grooves on the fore- and hindwing margins (D'Urso and Ippolito, 1994). The wing-coupling system of some Heteroptera is morphologically and functionally similar to that of Psocoptera (Bohne and Schneider, 1979; Stocks, 2008), although the forewing components are on the A vein.

Phylogenetic significance

The retinacular spines are clearly separated in Trogiomorpha and Troctomorpha, although they are more closely set in the latter (cf. Fig. C3-5E) and are fused in Psocomorpha (cf. Fig. C3-8P). Because outgroup taxa lack this structure, there is no basis on which to assess the polarity of the transformation series. However, homology of the retinacular spines and the ribs on the CuP vein permit estimation of character polarity; I might for example consider distinctly separated retinacular spines as a plesiomorphy. The surface structure of the CuP-tip is identical to the that of the ribs on the basal CuP vein in the Trogiomorpha (cf. Fig. C3-3A) and is also considered a plesiomorphy with gradual modification throughout Troctomorpha and Psocomorpha taxa. The troctomorphan CuP-tip has multiple rows of diagonally to vertically arranged ribs (cf. Fig. C3-7A), but the ribs are clearly separated from the retinaculum, whereas the psocomorphan CuP-tip is arranged diagonally (cf. Fig. C3-8P) with anterior ribs integrated into the retinaculum (cf. Fig. C3-1B).

The wing coupling apparatus in Trogiomorpha and Troctomorpha is less modified. However, the multiple rows of ribs on the CuP-tip (Character 9:1), which have not been reported previously, is an autapomorphy supporting the monophyly of Troctomorpha. Troctopsocidae Gen. (Fig. C3-5B) has a single row of diagonal ribs, as observed in Trogiomorpha and Psocomorpha (Character 9:0), but this trait is a reversal

in the most parsimonious reconstruction (Fig. C3-11). However, this condition may be plesiomorphic, because the higher-level relationships among troctomorphan families are poorly understood (Yoshizawa and Johnson, 2014).

A close relationship between Troctomorpha and Psocomorpha is supported by molecular data (Yoshizawa and Johnson, 2014), but there is little support based on morphology. The ribs arranged diagonally to vertically (Character 8:1) was identified here as a synapomorphy supporting a close relationship. Based on light microscopy, Mockford (1967) suggested that a retinaculum composed of truncated spines fused at their bases is a potential synapomorphy of Troctomorpha and Psocomorpha. However, the SEM-images reveal more detail, and I could not discern such fusion in Troctomorpha. The following character states were recovered as autapomorphies of Psocomorpha: retinacular spines fused (Character 4:1) (Yoshizawa, 2002, 2005) and anterior ribs on the CuP-tip merged into the retinaculum (Character 7:1; Fig. C3-11).

The retainer is less variable throughout Psocoptera. The posterior forewing margin covered by scale-like studs and curved inward (Character 10:1) is considered an autapomorphy of Psocoptera, and its absence in Nanopsocetae (Pachytroctidae and Liposcelididae: Fig. C3-6A, B) is a secondary loss. A bent retainer (Character 11:1) may be an autapomorphy of the order, but several taxa of Troctomorpha and Psocomorpha (Figs. C3-3A; C3-6A, B; C3-8N, O) indicate secondary reversal. The shapes of the surface studs on the posterior wing margin (Characters 14:0; 15:0) are highly variable. Trichome-like studs were observed only in Caeciliusetae and Philotarsetae (Characters 14:1; 15:1; Fig. C3-8B, D, E, I–L), but the distant phylogenetic relationship of the families suggests independent origins (Fig. C3-11). Trichome-like studs occur in *Matsumuraiella* and *Amphipsocus* (Caeciliusetae) but are restricted to the distal region with scale-like studs proximally (Character 14:0). The

retainer of *Archipsocus* is almost completely devoid of surface decorations (Fig. C3-8A), and those of *Echmepteryx* (Lepidopsocidae) and *Goja* (Epipsocidae) diminish gradually distally from the junction of the CuP (Character 14:2; 15:2; Fig. C3-11). Modifications of the bent retainer, retinaculum and CuP-tip occurred independently in different taxa and overall appear to be highly homoplasious. If variation in these character systems contains a phylogenetic signal, a much denser taxon sampling is needed.

The modifications observed in the retainer displayed by some taxa may be phylogenetically informative at a lower level of phylogeny. For example, the strongly developed, thumb-like protrusion in *Calopsocus* (Calopsocidae; Figs. C3-8K; C3-9K, L) and *Heterocaecilius* (Pseudocaeciliidae) (Figs. C3-8L; C3-9L; Character 12:2) may be a synapomorphy that supports a close relationship; this is supported by other morphological (Yoshizawa, 2002) and molecular (Yoshizawa and Johnson, 2014) data.

Among Psocoptera, there are two different cases of reduction of the wing coupling apparatus. *Neotrogla* (Prionoglarididae) has a simplified wing coupling structure in which the retinaculum consists of a few recumbent spines (cf. Fig. C3-4B), and the hindwings are largely diminished. In most analyses, taxa of Prionoglarididae are considered to be the most plesiotypic overall, and the simplified retinaculum may also represent a plesiomorphy. However, the most parsimonious reconstruction (MPR) of the retinacular character (Character 6) implies that the simplified retinaculum of *Neotrogla* is an autapomorphy (Fig. C3-11). *Neotrogla* species inhabit caves and exhibit many specialized behaviors (Yoshizawa et al., 2014), and the simplified retinaculum is probably associated with diminution of the hindwing (Lienhard and Ferreira, 2013).

Liposcelididae and Pachytroctidae also have simplified wings, and they completely lack a wing coupling apparatus. However, their hindwings keep their size, unlike *Neotrogla*. Based on phylogenetic analysis and MPR, loss of wing coupling

structures and the nodulus in Liposcelididae (Fig. C3-6A) and Pachytroctidae (Fig. C3-6B; Troctomorpha: Nanopsocetae) is considered as a secondary loss (Characters 10:0: 11:0; Fig. C3-11). The insects can flap the fore- and hindwings independently during flight, and the reduction of their wing coupling apparatus may be involved with the different ecology of *Neotrogla* in its functional aspect.

In summary, the common ancestor of Psocodea gained a unique wing coupling apparatus composed of the retainer, CuP-tip and a retinaculum at the end of the CuP vein. These structures are consistently retained throughout the Psocodea, and some modifications reflect their deep phylogenetic relationships, including the first potential autapomorphy of Troctomorpha (Character 9:1) and synapomorphy of Troctomorpha and Psocomorpha (Character 8:1). Independent origins of the wing coupling apparatus among the paraneopteran orders were suggested by the morphological analysis. The factors driving the independent evolution of the wing coupling apparatus are still unknown. To answer this question, comprehensive observations of the morphology and flight behavior must be conducted throughout the paraneopteran orders.

Table C3-1. Taxa examined for this study. The specimens examined are stored at Systematic Entomology, Hokkaido University (SEHU).

ORDER HEMIPTERA

Cinara sp. (Aphididae). 1ex. VI. 2015. Hokkaido, Japan. Naoki Ogawa leg. [Collection No: NOJM-STN01]

ORDER THYSANOPTERA

Aeolothrips kurosawai Bhatti, 1971 (Aeolothripidae). 1ex. VI. 2015. Fukushima, Japan. Tadaaki Tsutsumi col. [Collection No: NOJM-THY01]

ORDER PSOCODEA

SUBORDER TROGIOMORPHA

Prionoglaris stygia Enderlein, 1909 (Prionoglarididae). 1ex. 25. XI. 1986. Camou, France. B. Houses leg. [Collection No: NOJM-PS17]

Neotrogla curvata Lienhard & Ferreira, 2013 (Prionoglarididae). 1 ex. 19. X. 2012.Bahia, Brasil. Ferreira R.L. leg. [Collection No: NOJM-PS13]

Psyllipsocus yucatan Gurney, 1943 (Psyllipsocidae). 1 ex. 2011. Bahia, Brasil [Collection No: NOJM-PS18]

Psoquilla sp. (Psoquillidae). 1 ♂. 2011. Ghana [Collection No: NOJM-PS22]

Echmepteryx hageni (Packard, 1870) (Lepidopsocidae). 1 ex. 12. VII. 2010. Illinois, USA. Kazunori Yoshizawa leg. [Collection No: NOJM-PS07]

Table C3-1. *Continued.*

SUBORDER TROCTOMORPHA

Stimulopalpus japonicus Enderlein, 1906 (Amphientomidae). 1ex. 07. X. 2005. Nepal. Kazunori Yoshizawa leg. [Collection No: NOJM-PS12]

Gen. sp. (Troctopsocidae) . 3-10. II. 2008. Mae Hong Son, Thailand. A. Kamkoon leg. T3497 [Collection No: NOJM-PS24]

Selenopsocus sp. (Troctopsocidae). 1ex. 9-16. IV. 2009. Kanchanaburi, Thailand. Boonnam & Phumarin leg. T4793 [Collection No: NOJM-PS25]

Musapsocus sp. (Musapsocidae). 1ex. 20-31. VII. 1987. Monagas, Venezuela. S. & J. Peck. [Collection No: NOJM-PS23]

Manicapsocus alettae Smithers, 1966 (Electrentomidae). 1♂. 30. I.—13. II. 2001. Kenya. Minakawa et al. leg. [Collection No: NOJM-PS27]

Embidopsocus sp. (Liposcelididae). 1ex. 06-18. X. 2015. Roura, French Guiana. Naoki Ogawa leg. [Collection No: NOJM-PS21]

Tapinella sp. (Pachytroctidae). 1ex. 22. XI. 1997. Kaohsiung, Taiwan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS19]

Table C3-1. *Continued.*

SUBORDER PSOCOMORPHA

Archipsocus sp. (Archipsocidae). 1ex. 18. XI. 1997. Taichung, Taiwan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS14]

Matsumuraiella radiopicta Enderlein, 1906 (Dasydemellidae). 1ex. 8-17. 07. 1998 Aichi, Japan. Kenzo Yamagishi leg. [Collection No: NOJM-PS09]

Stenopsocus nigricellus Okamoto, 1907 (Stenopsocidae). 1ex. 20. VII. 2014. Hokkaido, Japan. Naoki Ogawa leg. [Collection No: NOJM-PS05]

Amphipsocus japonicus (Enderlein, 1906) (Amphipsocidae). 1ex. 19-26. VII. 2007. Hokkaido, Japan. K. Konishi leg. [Collection No: NOJM-PS01]

Valenzuela flavidus (Stephens, 1836) (Caeciliusidae). 1ex. 20-27. VIII. 2003. Hokkaido, Japan. K. Konishi leg. [Collection No: NOJM-PS11]

Peripsocus quercicola Enderlein, 1906 (Peripsocidae). 1 ex. 21. VI. 1993. Fukuoka, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS04]

Ectopsocus briggsi McLachlan, 1899 (Ectopsocidae). 1 ex. 21. VI. 1993. Fukuoka, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS06]

Idatenopsocus orientalis (Vishnyakova, 1986) (Mesopsocidae). 1 ex. 22. VI. 1994. Fukuoka Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS08]

Aaroniella badonneli (Danks, 1950) (Philotarsidae). 1 ex. 29. VII. 1999. Ōita, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS02]

Trichopsocus clarus (Banks, 1908) (Trichopsocidae). 1 ex. 20. VIII. 2004. California, USA. Kazunori Yoshizawa leg. [Collection No: NOJM-PS26]

Table C3-1. *Continued.*

Calopsocus furcatus (New, 1978) (Calopsocidae syn: Pseudocaeciliidae). 1ex. 14. III. 2003. Malaysia. Kazunori Yoshizawa leg. [Collection No: NOJM-PS20]

Heterocaecilius solocipennis (Enderlein, 1907) (Pseudocaeciliidae). 1ex. 17. VII. 1993. Hokkaido, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS03]

Goja sp. (Epipsocidae). 1ex. 23. VI. 2014. Mexico DF, Mexico. Kazunori Yoshizawa leg. [Collection No: NOJM-PS16]

Hemipsocus chloroticus (Hagen, 1958) (Hemipsocidae). 1ex. 01. XII. 1991. Fukuoka, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS10]

Psilopsocus malayensis New & Lee, 1991 (Psilopsocidae). 1ex. 07. III. 2003. Malaysia. Kazunori Yoshizawa leg. [Collection No: NOJM-PS28]

Metylophorus sp. (Psocidae). 1ex. 02. X. 2002. Hokkaido, Japan. Kazunori Yoshizawa leg. [Collection No: NOJM-PS15]

Table C3-2. Data matrix used for the parsimonious reconstruction. See text for characters and their states.

					5					10					15
Cinara sp. (Aphididae)	1	0	0	?	?	?	?	0	?	0	0	0	?	0	0
Aeolothrips kurosawai (Aeolothripidae)	0	0	0	?	0	0	0	0	0	0	0	0	?	1	1
Prionoglaris stygia (Prionoglarididae)	1	0	1	0	1	0	0	0	0	1	1	0	?	0	0
Neotrogla curvata (Prionoglarididae)	1	0	1	0	0	1	?	0	0	1	1	0	?	0	0
Psyllipsocus yucatan (Psyllipsocidae)	1	0	1	0	0	0	0	0	0	1	1	0	?	0	0
Psoquilla sp. (Psoquillidae)	1	0	1	0	1	0	?	0	0	1	1	0	?	0	0
Echmepteryx hageni (Lepidopsocidae)	1	0	1	0	1	0	0	0	0	1	1	0	?	0	2
Stimulopalpus japonicus (Amphientomidae)	1	0	1	0	2	0	0	1	1	1	0	1	1	0	0
Gen. sp. (Troctopsocidae)	1	0	1	0	2	0	0	1	0	1	1	0	?	0	0
Selenopsocus sp. (Troctopsocidae)	1	0	1	0	2	0	0	1	1	1	1	1	0	0	0
Musapsocus sp. (Musapsocidae)	1	0	1	0	0	0	0	1	1	1	1	1	?	0	0
Manicapsocus alettae (Electrentomidae)	1	0	1	0	2	0	0	1	1	1	1	1	?	0	0

Table C3-2. *Continued.*

Embidopsocus sp. (Liposcelididae)	1	1	0	?	?	0	?	?	?	0	0	?	?	?	?
Tapinella sp. (Pachytroctidae)	1	0	0	?	?	0	?	?	?	0	0	?	?	?	?
Archipsocus sp. (Archipsocidae)	1	0	1	1	0	0	1	1	0	1	1	0	0	2	2
Matsumuraiella radiopicta (Dasydemellidae)	1	0	1	1	2	0	1	1	0	1	1	0	?	0	1
Stenopsocus nigricellus (Stenopsocidae)	1	0	1	1	0	0	1	1	0	1	1	0	?	0	0
Amphipsocus japonicus (Amphipsocidae)	1	0	1	1	2	0	1	1	0	1	1	0	?	0	1
Valenzuela flavidus (Caeciliusidae)	1	0	1	1	1	0	1	1	0	1	1	1	0	1	1
Peripsocus quercicola (Peripsocidae)	1	0	1	1	0	0	1	1	0	1	1	1	0	0	0
Ectopsocus briggsi (Ectopsocidae)	1	0	1	1	0	0	1	1	0	1	1	1	?	0	0
Idatenopsocus orientalis (Mesopsocidae)	1	0	1	1	0	0	1	1	0	1	1	0	?	0	0
Aaroniella badonneli (Philotarsidae)	1	0	1	1	2	0	1	1	0	1	1	1	0	1	1
Trichopsocus clarus (Trichopsocidae)	1	0	1	1	0	0	1	1	0	1	1	1	0	1	1

Table C3-2. *Continued.*

Calopsocus furcatus (Calopsocidae)	1	0	1	1	0	0	1	1	2	1	1	2	0	1	1
Heterocaecilius solocipennis (Pseudocaeciliidae)	1	0	1	1	0	0	1	1	0	1	0	2	0	1	1
Goja sp. (Epipsocidae)	1	0	1	1	2	0	1	1	0	1	1	0	?	0	2
Hemipsocus chloroticus (Hemipsocidae)	1	0	1	1	0	0	1	1	0	1	0	1	0	0	0
Psilopsocus malayensis (Psilopsocidae)	1	0	1	1	1	?	1	1	0	1	0	1	?	0	0
Metylophorus sp. (Psocidae)	1	0	1	1	0	0	1	1	0	1	1	0	?	0	0

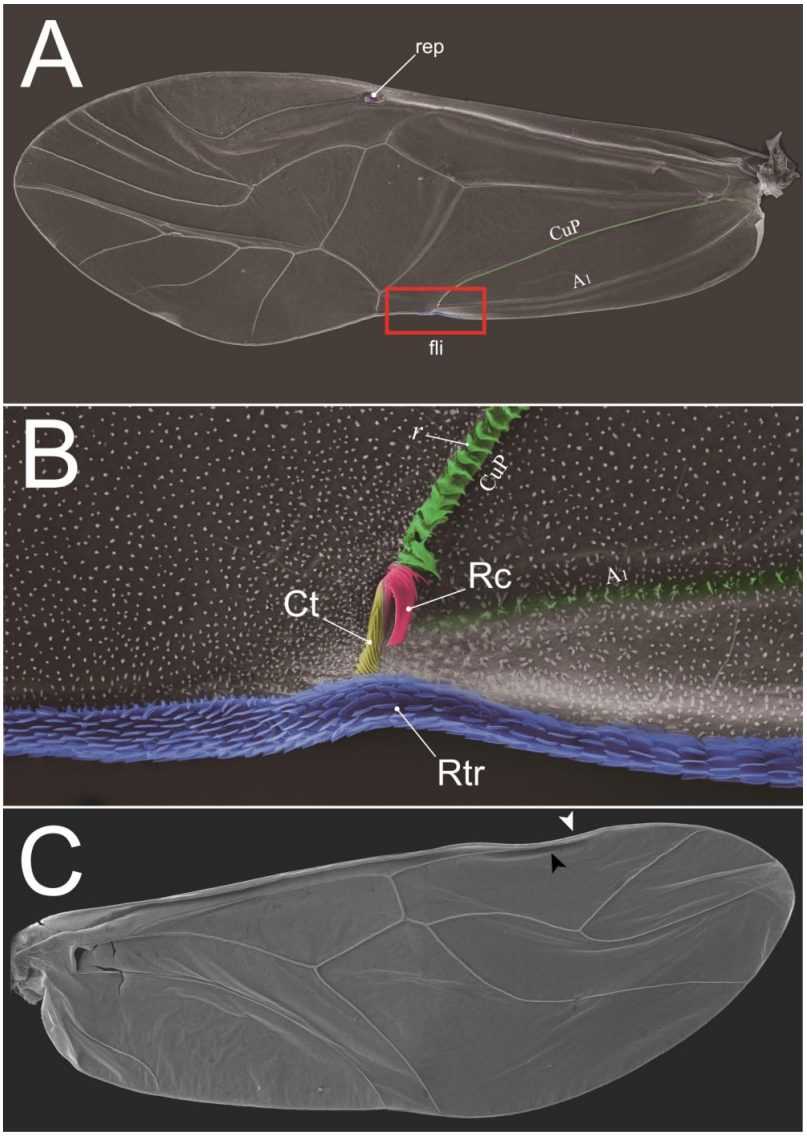


Fig. C3-1. Flipped left fore- and hindwing of *Metylophorus* sp. (Psocidae). A. Ventral view of the forewing, with names of relevant veins. Red rectangle indicates the in-flight wing coupling region (fli). Abbreviations: fli, in-flight coupling apparatus; rep, repose-coupling apparatus. B. Enlarged in-flight wing coupling structure. Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; *r*, rib-like structure; Rc, retinaculum; Rtr, retainer. C. Dorsal view of the hindwing. White and black arrows indicate engagement point with repose-coupling appatarus and in-flight coupling apparatus, respectively. Scale (A, C) 1 mm, (B) 0.05 mm.

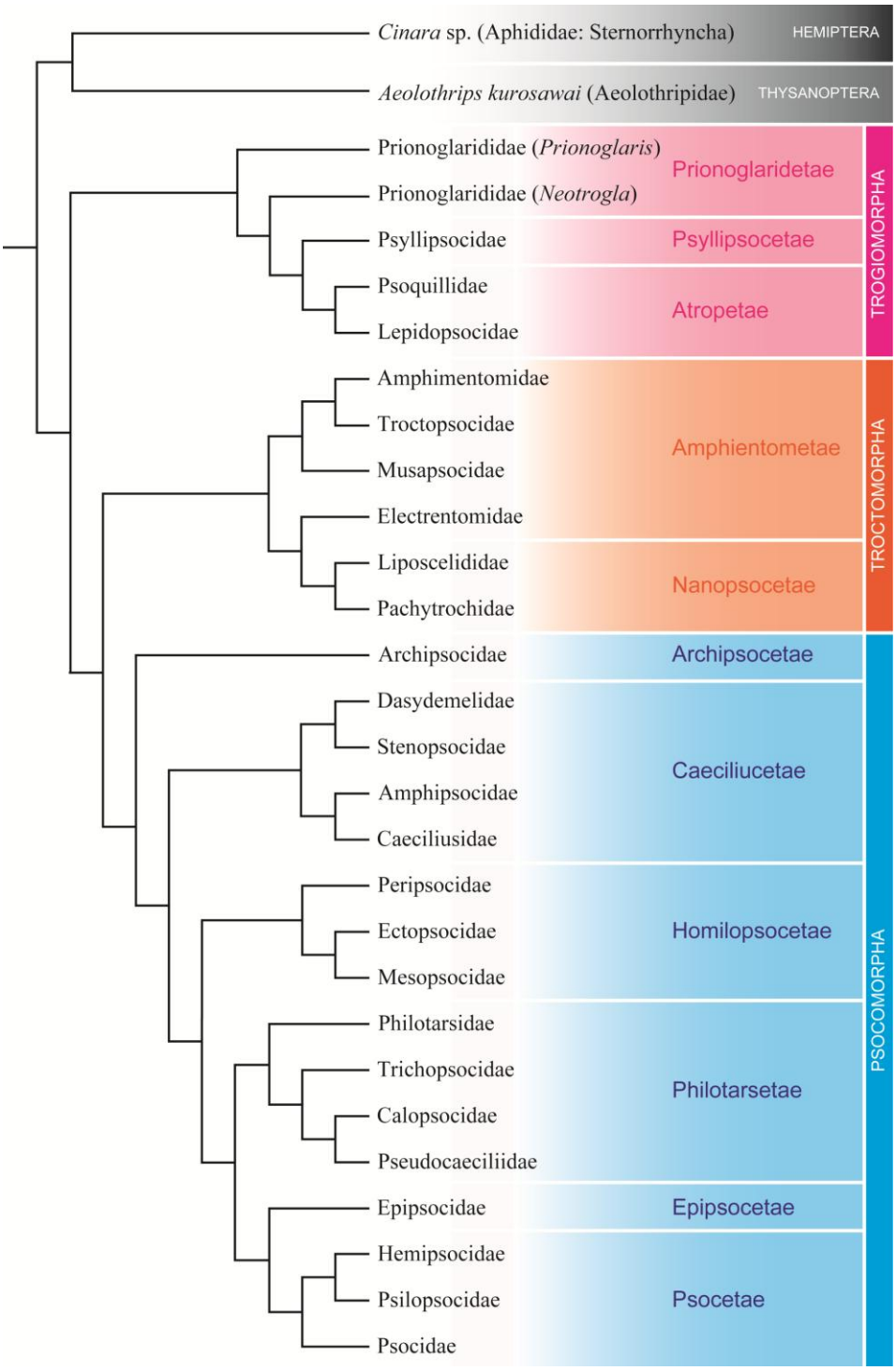


Fig. C3-2. Phylogeny of ‘Psocoptera’ and relatives adopted in this study. This tree was constructed based on Yoshizawa & Johnson (2010, 2014) (for ‘Psocoptera’) and Friedmann et al. (Friedemann et al., 2014) and Yoshizawa & Lienhard (2016) (for the relationship with the outgroups).

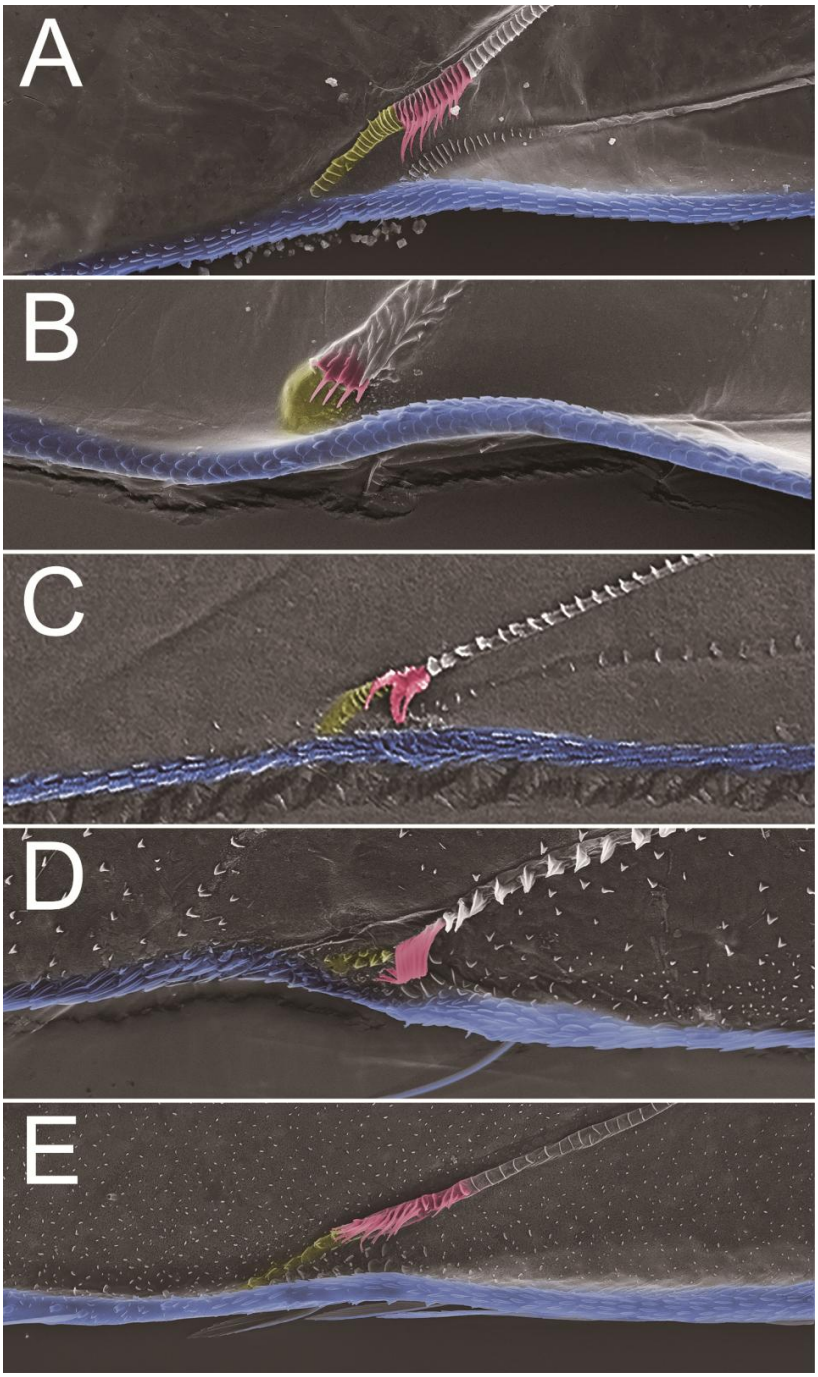


Fig. C3-3. In-flight wing coupling structures in Trogiomorpha, ventral view. A. *Prionoglaris stygia* (Prionoglarididae). B. *Neotrogla curvata* (Prionoglarididae). C. *Psyllipsocus yucatan* (Psyllipsocidae). D. *Psoquilla* sp. (Psoquillidae). E. *Echmepteryx hageni* (Lepidopsocidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.05 mm.

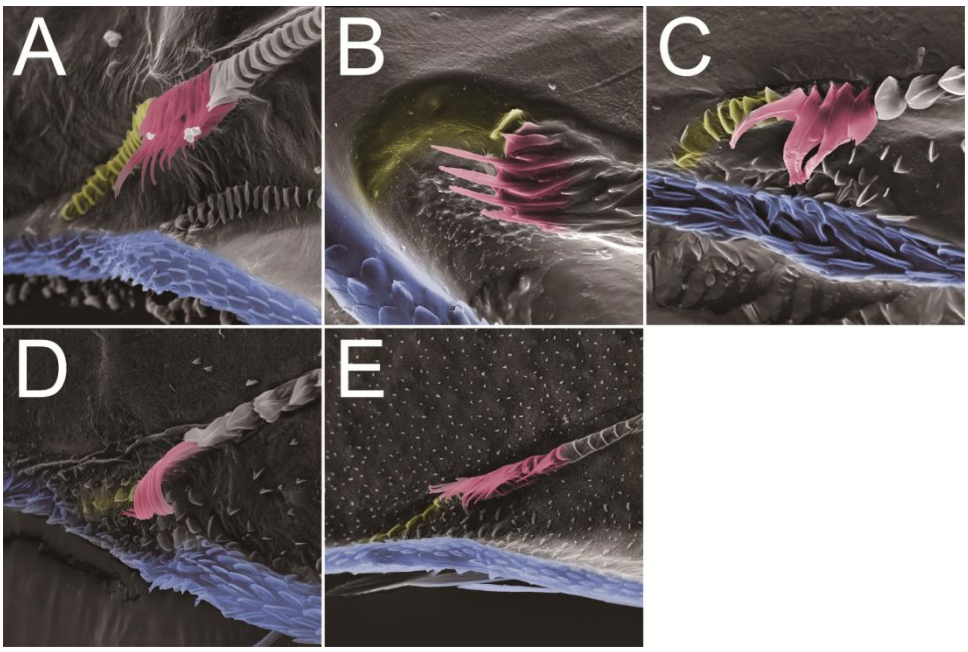


Fig. C3-4. In-flight wing coupling structures in Trogiomorpha, ventrolateral view. A. *Prionoglaris stygia* (Prionoglarididae). B. *Neotrogla curvata* (Prionoglarididae). C. *Psyllipsocus yucatan* (Psyllipsocidae). D. *Psoquilla* sp. (Psoquillidae). E. *Echmepteryx hageni* (Lepidopsocidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.01 mm.

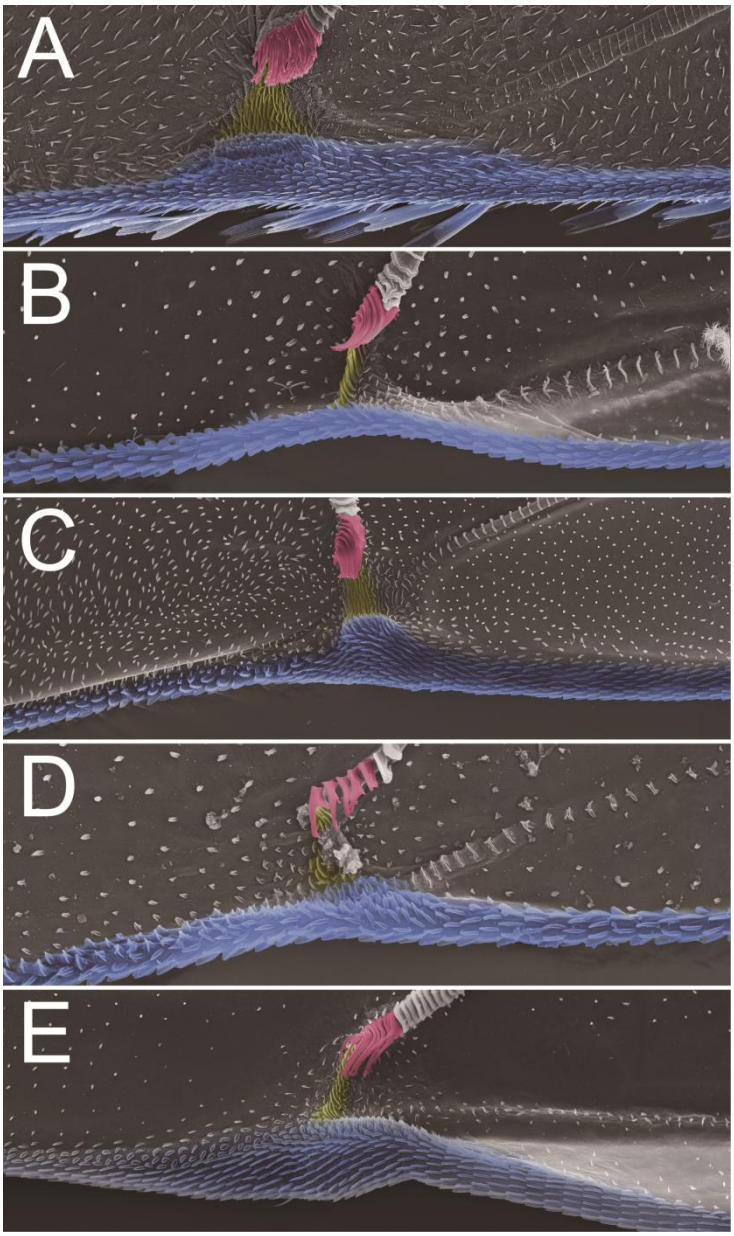


Fig. C3-5. In-flight wing coupling structures in Troctomorpha, ventral view. A. *Stimulopalpus japonicus* (Amphientomidae). B. Gen. sp. (Troctopsocidae). C. *Selenopsocus* sp. (Troctopsocidae). D. *Musapsocus* sp. (Musapsocidae). E. *Manicapsocus alettae* (Electrentomidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.05mm.

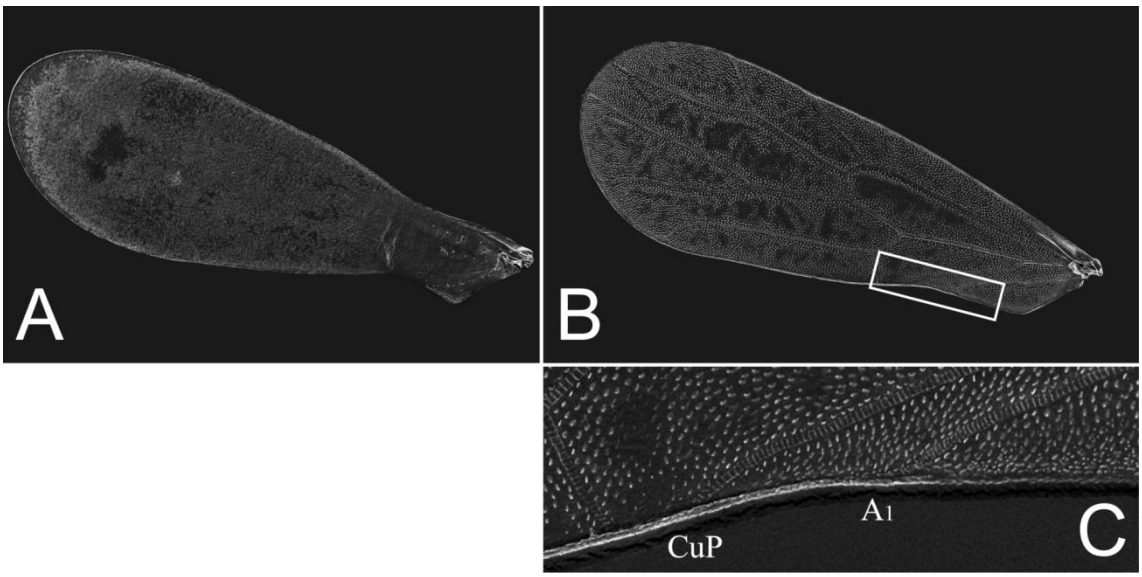


Fig. C3-6. Forewings of Nanopsocetae species (Troctomorpha) lacking the in-flight wing coupling structure, ventral view. A. *Embidopsocus* sp. (Liposcelididae). B. *Tapinella* sp. (Pachytroctidae). C. ditto, enlarged view of the end of the CuP and A1 in B. Abbreviations: A1, First Anal vein; CuP, Posterior Cubital vein; *r*, rib-like structure. Scale (A, B) 0.5mm, (C) 0.01mm.

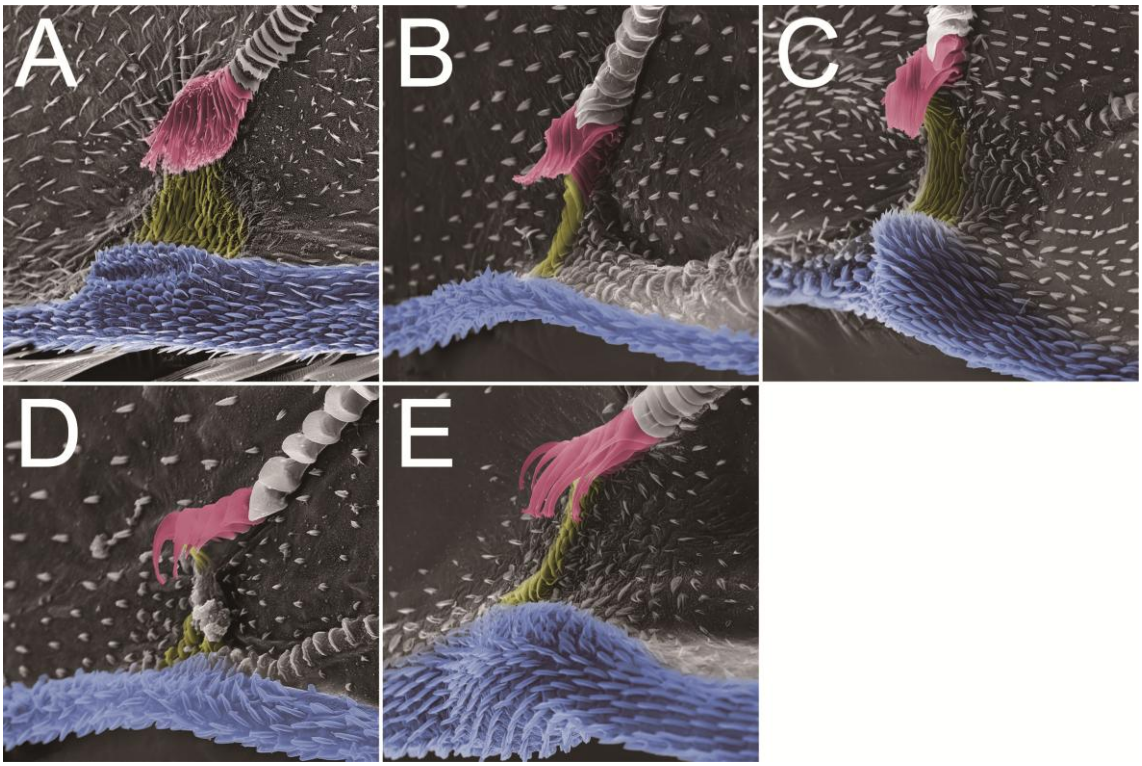


Fig. C3-7. In-flight wing coupling structures in Troctomorpha, ventrolateral view. A. *Stimulopalpus japonicus* (Amphientomidae). B. Gen. sp. (Troctopsocidae). C. *Selenopsocus* sp. (Troctopsocidae). D. *Musapsocus* sp. (Musapsocidae). E. *Manicapsocus alettae* (Electrentomidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.03 mm.

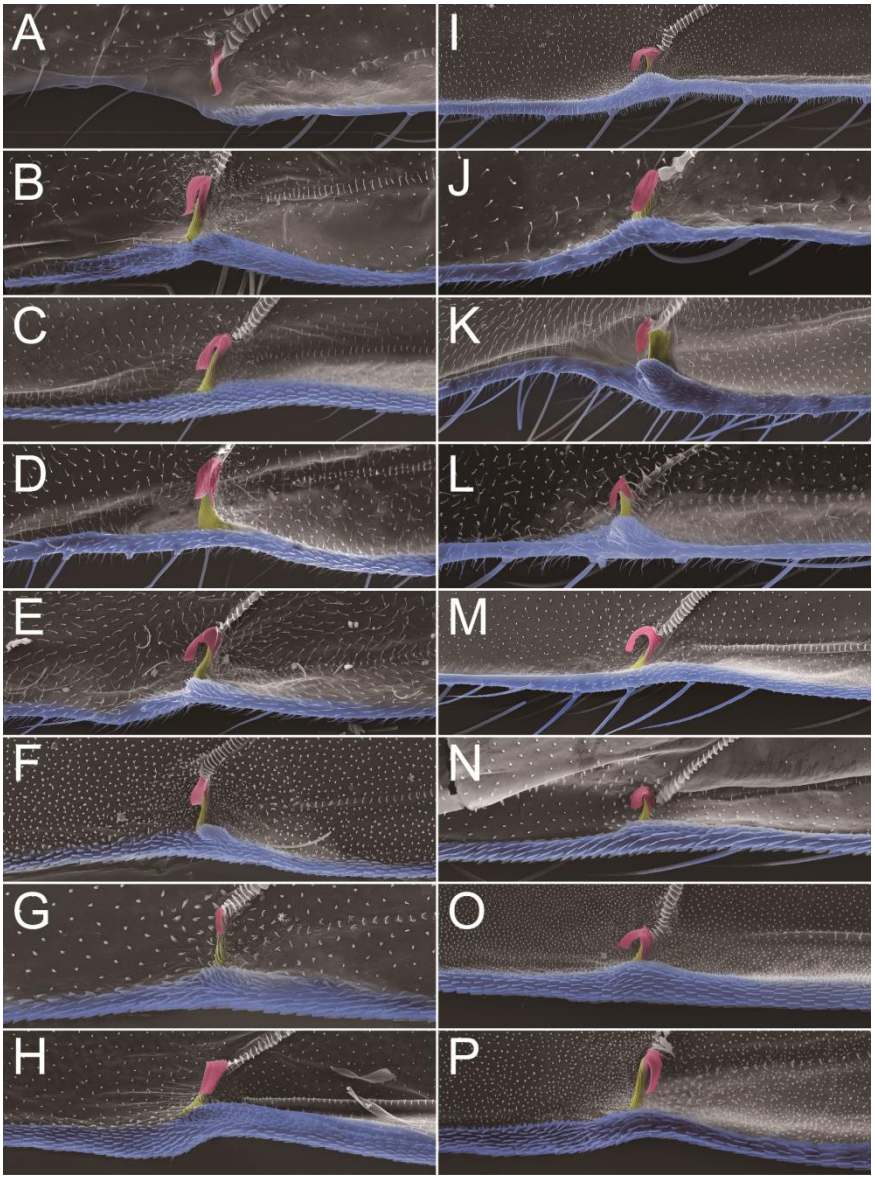


Fig. C3-8. In-flight wing coupling structures in Psocomorpha, ventral view. A. *Archipsocus* sp. (Archipsocidae). B. *Matsumuraiella radiopicta* (Dasydemellidae). C. *Stenopsocus nigricellus* (Stenopsocidae). D. *Amphipsocus japonicus* (Amphipsocidae). E. *Valenzuela flavidus* (Caeciliusidae). F. *Peripsocus quercicola* (Peripsocidae). G. *Ectopsocus briggsi* (Ectopsocidae). H. *Idatenopsocus orientalis* (Mesopsocidae). I. *Aaroniella badonneli* (Philotarsidae). J.*Trichopsocus clarus* (Trichopsocidae). K. *Calopsocus furcatus* (Calopsocidae syn: Pseudocaeciliidae). L. *Heterocaecilius solocipennis* (Pseudocaeciliidae). M. *Goja* sp. (Epipsocidae). N. *Hemipsocus chloroticus* (Hemipsocidae). O. *Psilopsocus malayensis* (Psilopsocidae). P. *Metylophorus* sp. (Psocidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.05 mm.

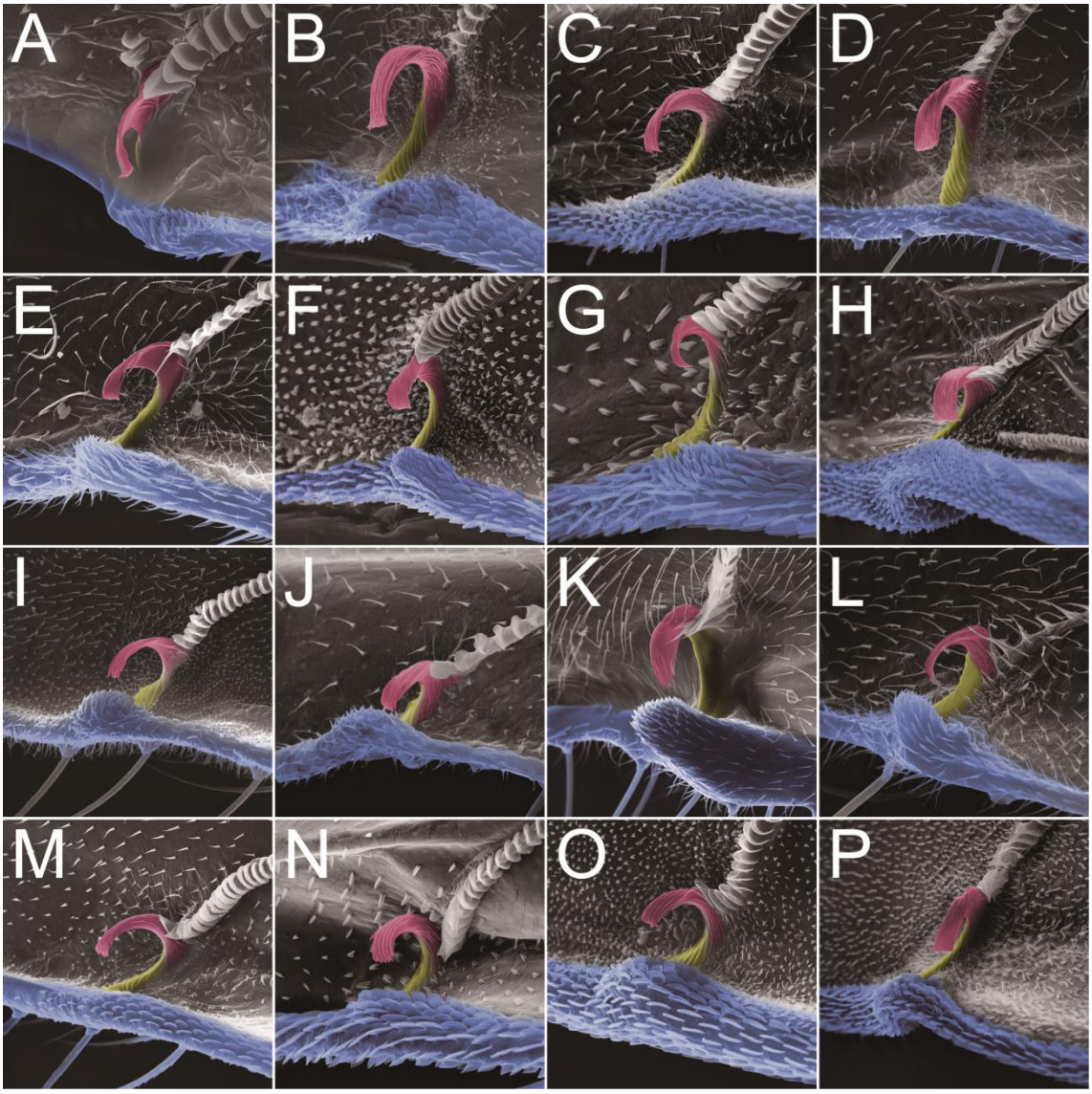


Fig. C3-9. In-flight wing coupling structures in Psocomorpha, ventrolateral view. A. *Archipsocus* sp. (Archipsocidae). B. *Matsumuraiella radiopicta* (Dasydemellidae). C. *Stenopsocus nigricellus* (Stenopsocidae). D. *Amphipsocus japonicus* (Amphipsocidae). E. *Valenzuela flavidus* (Caeciliusidae). F. *Peripsocus quercicola* (Peripsocidae). G. *Ectopsocus briggsi* (Ectopsocidae). H. *Idatenopsocus orientalis* (Mesopsocidae). I. *Aaroniella badonneli* (Philotarsidae). J.*Trichopsocus clarus* (Trichopsocidae). K. *Calopsocus furcatus* (Calopsocidae syn: Pseudocaeciliidae). L. *Heterocaecilius solocipennis* (Pseudocaeciliidae). M. *Goja* sp. (Epipsocidae). N. *Hemipsocus chloroticus* (Hemipsocidae). O. *Psilopsocus* malayensis (Psilopsocidae). P. *Metylophorus* sp. (Psocidae). Abbreviations: A1, First Anal vein; Ct, CuP tip; CuP, Posterior Cubital vein; Rc, retinaculum; Rtr, retainer. Scale 0.03 mm.

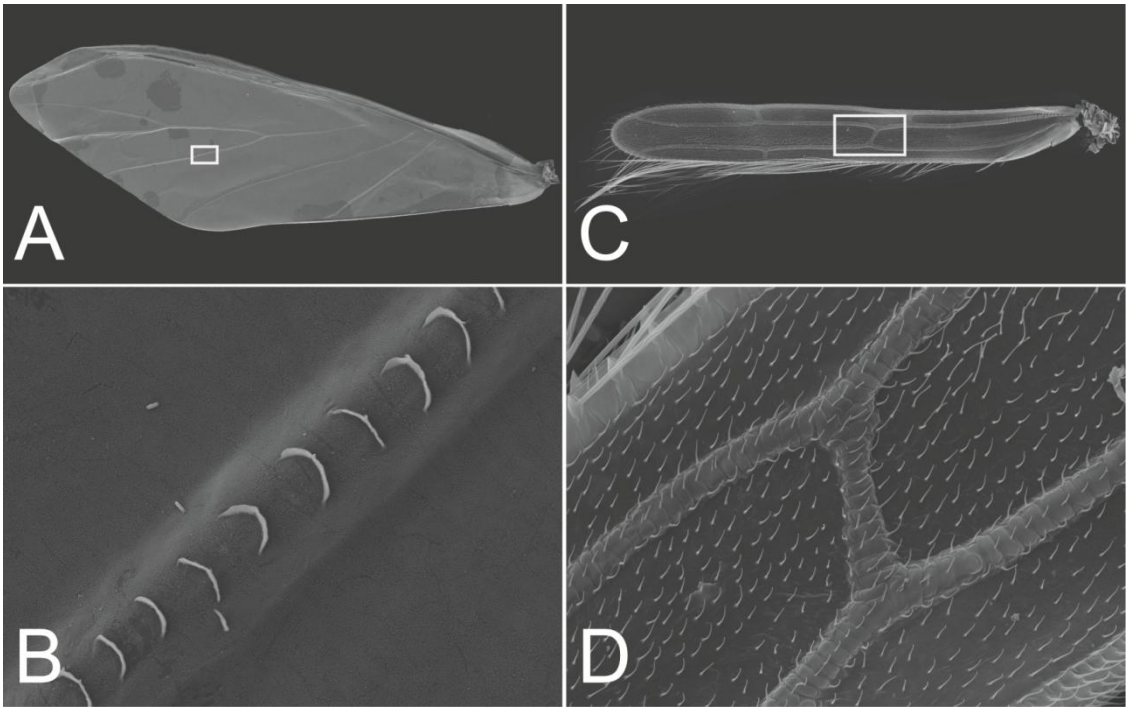


Fig. C3-10. Right forewing and vein decorations of the outgroups. A. *Cinara* sp. (Hemiptera: Sternorrhyncha: Aphididae), ventral view. B. ditto, enlarged view of the vein indicated by white rectangle in A. C. *Aeolothrips kurosawai* (Thysanoptera: Aeolothripidae), ventral view. D. ditto, enlarged view of the vein indicated by white rectangle in C. Abbreviations: *r*, rib-like structure; *t*, tile-like structure. Scale (A) 1 mm, (B) 0.3 mm, (C, D) 0.03 mm.

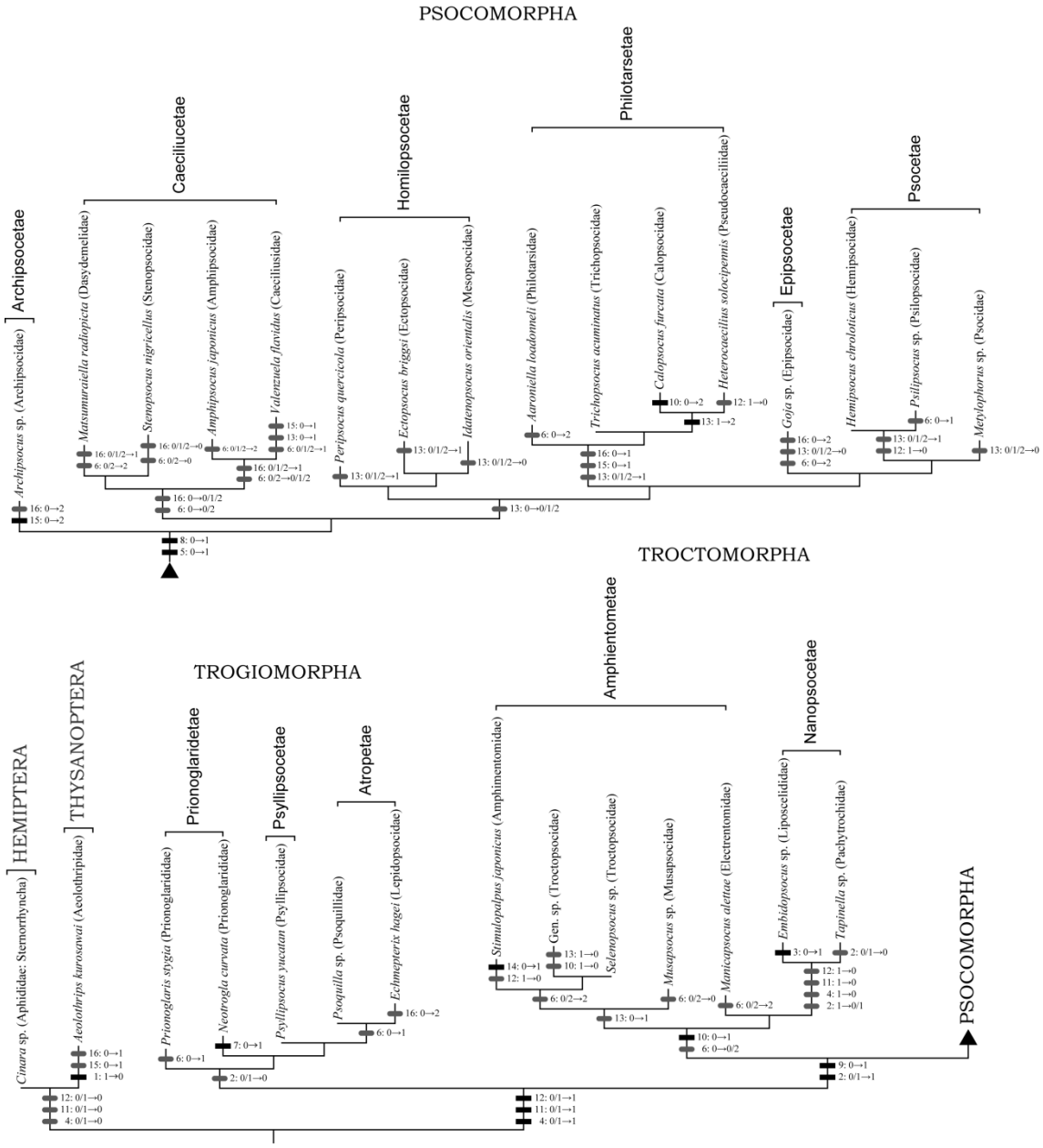


Fig. C3-11. The parsimonious reconstruction of selected 16 characters. Character and character state changes reconstructed on the branches are indicated by black (non-homoplasious) and gray bars (homoplasious).

GENERAL DISCUSSION

本論文では、胸部内跳躍機能の進化史を明らかにするため、半翅目昆虫の胸部内形態の観察と祖先形質復元を行った。また、跳躍機能の進化を実現した要因を検証するため、飛翔筋形態の比較および、準新翅類全体の翅連結構造の形態観察を行い、その進化順序について議論した。

第一章では、頸吻亜目を対象として研究を行い、この亜目に含まれるハゴロモ型下目とセミ型下目がそれぞれ独立して跳躍機能を獲得したことが、筋肉相と内骨格から示唆された。第二章では、半翅目の共通祖先の胸部の祖先形質状態を推測するべく、最も基部に位置する系統である腹吻亜目を対象とした研究を行い、半翅目の祖先が跳躍機能を持たなかったことを示した。この亜目においても、胸部内跳躍機能が 2 回独立に進化したと推定された (Fig. D-1)。以上の結果から、半翅目の祖先は一般的な昆虫と共通した胸部構造や筋肉相を持っていたにもかかわらず、昆虫全体でも稀な胸部内跳躍機能の獲得が繰り返し起こっていたことが示された。また、飛翔筋の改変が跳躍機能の獲得と同時に観察されたことから、半翅目には、飛翔機能を司る筋肉がもたらす胸郭の容積的な制約を打ち消し、後胸に新しい機能を収納する余地を創出する要因が存在すると考えられた。これを実現する具体的な要因として、中胸への飛翔機能の集約が可能性の一つとして想定された。そこで第三章では、この集約を実現した要因として、翅構造、とりわけ前後翅の連結構造に注目して研究を行った。翅連結構造は、飛翔中に 4 枚の翅を結合して 2 枚の翅として振る舞わせる構造である (Chapman, 2013)。この構造は、半翅目を含む準新翅類に幅広く知られているが (Stocks, 2008)、その相同性や進化パターンは十分に検討されたことがなかった。そこで、半翅目の外群にあたる咀顎目（チャタテムシ）についてその形態を検討したところ、チャタテムシは、前翅 Sc 脈上の微細構造の変化によってた

だ一回翅連結構造を獲得していることが分かり、その状態は半翅目のそれとは大きく異なっていた。

胸部内跳躍機能の進化史

跳躍機能は、捕食者からの逃避等に有効であると考えられている運動機能の一つであり (Alexander, 1995; Burrows, 2003)、昆虫においても様々な系統で繰り返し進化してきたと考えられている (Furth and Suzuki, 1992; Picker et al., 2011)。バッタなどの昆虫は、飛翔筋がその容積のほとんどを占める胸郭には跳躍機能を持たず、付属肢である後腿節を長く大きく発達させることによって跳躍を行う (Snodgrass, 1935; Chapman, 2013)。しかし、長すぎる脚は跳躍そのものの効率を低下させ (Burrows and Sutton, 2008)、本来の機能である歩行に対しても悪影響があると考えられる。

一方、胸郭内に跳躍筋を発達させる昆虫も一部知られているが、そのほとんどは飛翔機能を失っているか (Burrows, 2009a, 2011) 、飛翔機能のない前胸に跳躍機能を進化させるなど (Furth and Suzuki, 1992; Ogawa, 2017, unpubl. data; Yoshida, 2018, pers. comm.)、跳躍筋が飛翔筋と干渉しない構造になっている。有翅胸部（中胸＋後胸）の内部において飛翔と跳躍の機能を共存させている昆虫は、半翅目以外では甲虫目、膜翅目のごく一部に知られているに過ぎない (Furth and Suzuki, 1992)。

第一章・第二章で示した形態解析の結果から、半翅目の共通祖先が持つ形質状態と、胸部内跳躍機能の進化プロセスが初めて示された。半翅目の共通祖先は跳躍機能を持っていなかったと推定されたが、これはこれまでに広く受け入れられてきた、跳躍機能が半翅目の共通祖先で起源したとする説 (Shcherbakov and Popov, 1997) を覆す結果である。また、今回の研究から、半翅目は、腹吻亜目および頸吻亜目で 2 回ずつ、少なくとも合計 4 回胸部内に跳躍機能を獲得し

ていることが示唆された (Fig. D-1)。この結果は、全昆虫でも稀な胸部内跳躍機能が近縁な分類群間で繰り返し進化していたことを示し、半翅目昆虫の胸部が昆虫の中でも極めて特異な進化傾向を示すことが明らかとなった。半翅目では、今回対象とした頸吻亜目・腹吻亜目だけでなく、さらに残りの鞘吻亜目および異翅亜目にも跳躍を行う種が知られている (Burrows et al., 2007; Burrows, 2009c)。本研究の結果および半翅目内の系統関係 (Johnson et al., 2018) を考慮すると、これら 2 亜目でも独自に胸部内跳躍機能を獲得している可能性が高く (Fig. D-1)、亜目内における胸部内跳躍機能の進化回数は最低でも 6 回にも上ることが予想される。しかし、鞘吻・異翅両亜目の胸部内部構造の形態学的な精査や、祖先形質の推定は現在まで行われていない。半翅目全体における胸部内跳躍機能の進化史をより明らかにするためには、これらの亜目についても形態の解析が必要である。また、将来的に筋肉量などの連続変量を形質として用いる場合、Kutsukake-Innan 法 (Kutukake and Innan, 2013) など、本研究で用いなかった解析が有用である可能性があり、方法論にも検討の余地があるかもしれない。

半翅目における翅連結構造の進化史

飛翔機能を保ちながら、胸部内に跳躍機能を獲得するためには、飛翔能力を中胸に集約して後胸の飛翔筋を減らし、新しい機能を収納する余地を作り出す必要があると考えられる。実際、第一章・第二章の結果の通り、跳躍を行うグループでは広く後胸の飛翔筋の縮小が観察された。このような、後胸の飛翔機能の縮小を機械的に実現する要因として、第三章では翅連結構造に注目し、半翅目の外群を対象として研究を行った。その結果、咀顎目（チャタテムシ）は前翅 Sc 脈上の微細構造が翅連結構造として進化していた。また、総翅目（アザミウマ）では前後翅縁に列をなして並ぶ毛状突起が翅連結構造として機能することが知られている (Ellington, 1980; Moritz, 1997)。これら外群の形質は、いず

れも半翅目の翅連結構造とは大きく異なっていることから (D'Urso, 2002; Stocks, 2008)、翅連結構造はそれぞれの目で独立に進化したことが推定された。

半翅目の翅連結構造は全ての亜目から知られているが、その構造は亜目ごとに大きく異なることが既に報告されている (Stocks, 2008)。腹吻亜目では、最も原始的なコナジラミ上科および、後翅が偽平均棍に変化しているカイガラムシ上科は翅連結構造を持たないが (Beutel et al., 2014)、アブラムシ上科とキジラミ上科では翅連結構造が知られている (Stocks, 2008)。亜目内での連結機能の分布と系統関係 (Johnson et al., 2018) から判断すると、腹吻亜目においては他の亜目とは独立に翅連結構造が進化したと考えられる (Fig. D-2)。アブラムシ上科では、後翅前縁部に1-数本の細い鉤状突起からなる長くねじれた翅鉤 (hamuli) があり、これが下方に巻いた前翅後縁に掛かることで翅の連結を行う(Ni et al., 2002)。この状態は、アブラムシ科、カサアブラムシ科 (Fig. D-3)、ネアブラムシ科 (Riley, 1874) で知られていることから、アブラムシ上科全体で共有された形質であると考えられる。キジラミ上科も同様に、前翅後縁が下方に巻き、後翅に翅鉤を持つが、後翅の翅鉤はアブラムシのような 1 本の長いねじれた鉤ではなく、前縁の根元付近と中央付近に短い鉤状突起がそれぞれ 1 列に並んだ構造である (Stocks, 2008)。アブラムシ上科とキジラミ上科の翅連結構造はそれぞれ同じ位置にあることから、共通祖先で起源し、その後どちらかの上科で変化した可能性が考えられるが (Fig. D-2; Topology A2, A3)、後翅の翅鉤の構成が大きく異なっていることから、跳躍と同様にそれぞれの上科で独立に獲得された可能性も否定できない (Fig. D-2; Topology A1)。

頸吻亜目では、ハゴロモ型下目とセミ型下目はそれぞれ、前翅の後縁が下方に、後翅の前縁が上方にそれぞれ反り返った形状の翅連結構造を持つ (Fig. D-4) (D'Urso and Ippolito, 1994)。ハゴロモ型下目の後翅前縁の形状は細長くて平滑であるのに対し、セミ型下目では丸く突出していて微細な突起で覆われるという

違いがあり区別が可能だが、その位置や基本構造は同一であり、相同な構造であると考えられる (D'Urso & Ippolito, 1994)。異翅亜目では、限られた種の散発的な形態観察が行われているに過ぎないが (Bohne and Schneider, 1979; Czaja, 2012)、いずれも前翅後縁 A 脈附近の裏側に前後 1 個ずつの突起からなる把握器を持ち、この構造が後翅前縁を把握することで翅の連結を行うことが報告されている (Fig. D-5)。後翅前縁は上方に向かって S 字型に曲がっており、前翅の 2 個の突起によって支えられる構造になっている (Bohne and Schneider, 1979) 。鞘吻亜目で唯一飛翔機能を残す *Peloridium hammoniorum* の翅連結構造も異翅亜目とほぼ同様の構造で、やはり S 字状の後翅前縁部を前翅裏側にある 2 個の突起で把握する構造となっている (Fig. D-6) (D'Urso, 1993)。このように、異翅亜目と鞘吻亜目は極めて類似した翅連結構造を持っているが、トランスクリプトーム解析 (Misof et al., 2014b; Johnson et al., 2018) や翅基構造 (Yoshizawa et al., 2017) の結果から、従来主流だった鞘吻亜目と異翅亜目の姉妹群関係 (Kristensen, 1975; Forero, 2008; Cryan and Urban, 2012) は否定され、鞘吻亜目と頸吻亜目との姉妹群関係が強く支持されている。したがって、異翅亜目と鞘吻亜目の翅連結構造は、頸吻亜目＋鞘吻亜目+異翅亜目の共通祖先に由来し、頸吻亜目で二次的に変化したと考えられる (Fig. D-2; Topology B2)。しかし、異翅亜目における翅連結構造の形態情報は前述した通り断片的であり、鞘吻亜目のそれとの相同性に関する議論が不十分であること、また腹吻亜目の例もある通り、亜目への分化後に独立に連結構造が派生した可能性も考えられることから、最節約的ではない各 3 亜目それぞれでの独立起源を想定するべきであろう (Fig. D-2; Topology B2)。このように、半翅目内での翅連結構造の進化史は十分には解明されておらず、今後の研究、特に異翅亜目における網羅的な形態観察が求められる。

翅連結機能と飛翔筋および跳躍機能の進化的関係

胸部内跳躍機能を持つ半翅目昆虫は、多くの場合において後胸内の間接飛翔筋が縮小していたことから、一般的な昆虫において胸部の機能的多様化を妨げているのは飛翔筋の容積的な制約であることが示唆された。このことは、翅を持たないノミやユキシリアゲなど、飛翔機能がもたらす制約が存在しない昆虫で、独立に胸部内跳躍が進化している (Burrows, 2009a, 2011) ことからも支持される。また、半翅目で見られたように、飛翔機能を維持しながら後胸の飛翔筋を縮小するためには、中胸に飛翔機能を集約しつつ翅面積も維持する必要があると考えられる。このことから、翅連結構造の存在は、後胸の飛翔機能を縮小するために必要な条件であることは疑いようがないだろう。本研究の結果から、腹吻亜目で 2 回、頸吻亜目で 2 回の合計 4 回の胸部内跳躍機能が半翅目で進化していたことが推定されたが、その多くで翅連結構造の進化も起こっていることから (Fig. D-1,2)、両形質間に強い関連があり、因果関係が存在する可能性が示唆された。この因果関係は、GLMM などの統計処理を通して今後明らかにしていく必要があるため、方法論の検討が急務である。また、翅連結構造を持たず、後胸に十分な飛翔機能を持つと考えられるコナジラミが胸部内跳躍機能を持つことから、翅連結構造以外の要因も跳躍機能の進化に影響を与えていることが推測される。これに関しては、運動などの機能解析や、遠縁ながら同様の機能を持つグループを対象とした形態解析を行い、可能性のある要因を検討する作業が必要となるであろう。

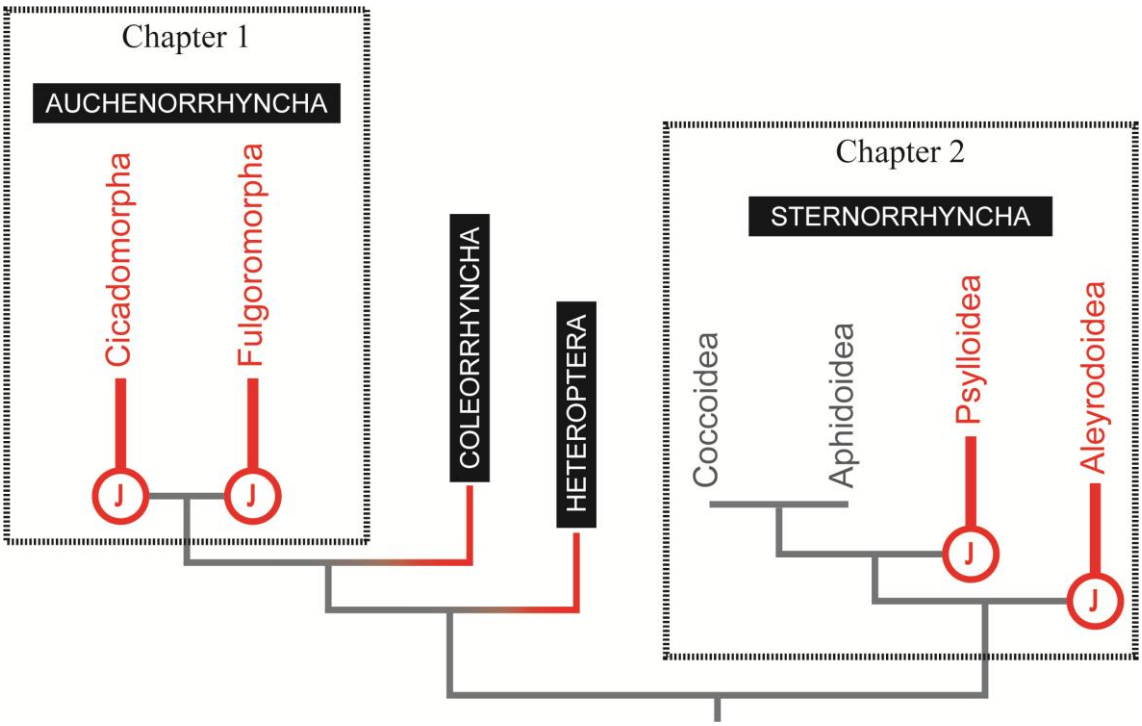


Fig. D-1. Summary of result of hemipteran jumping in this thesis. Outgroups were omitted. Information of jumping behavior in Coleorrhyncha and Heteroptera were referred from Burrows et al. (2007) and Burrows (2009c). "J" indicates implied acquisition events of jumping ability.

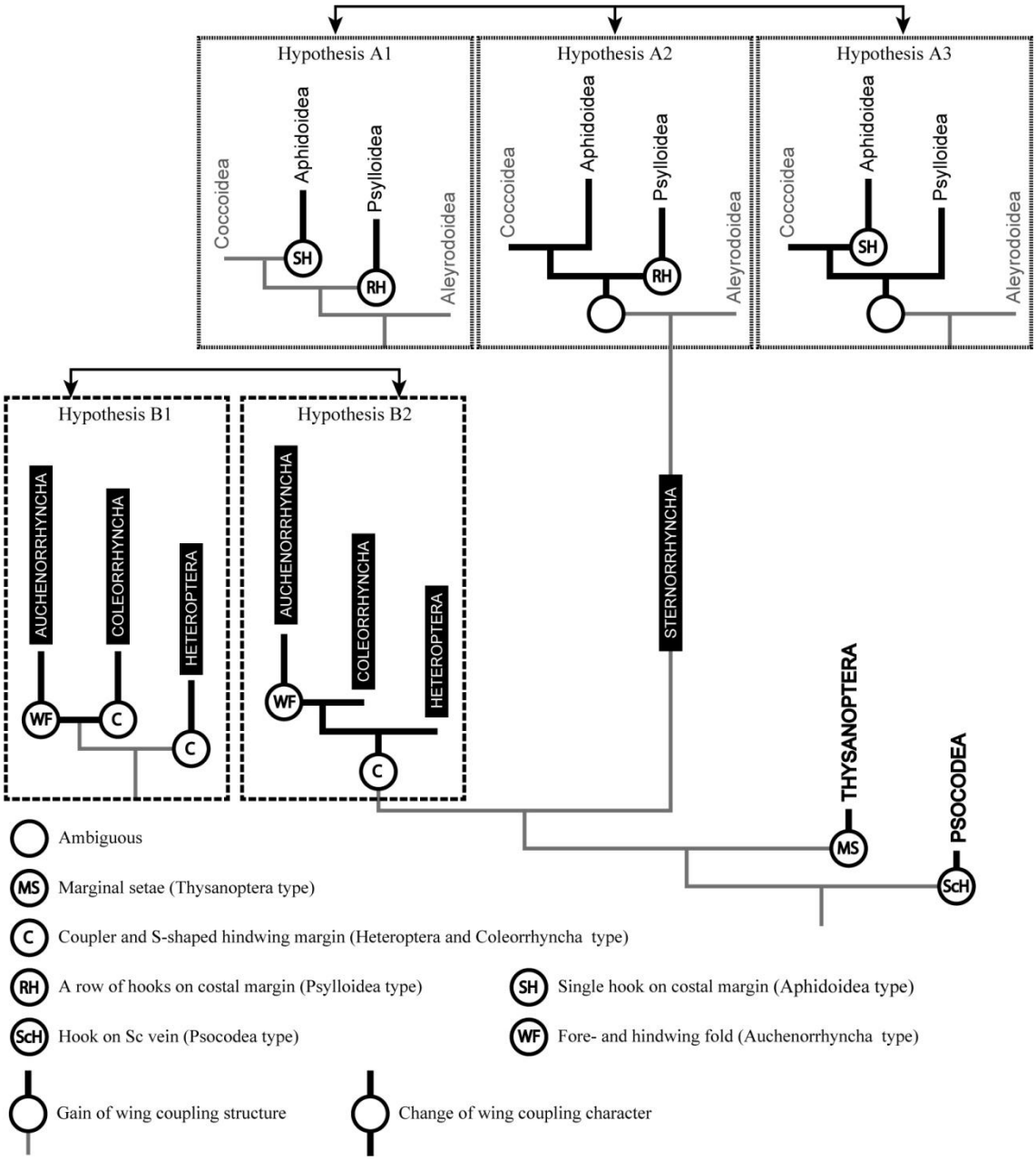


Fig. D-2. Possible evolutional hypothesis of wing coupling structure in hemipteroid orders. The phylogenetic relathionship was cited from Johnson et al. (2018). Topologies A1-A3 and B1-B2 are alternative, respectively. Outgroups are omitted.

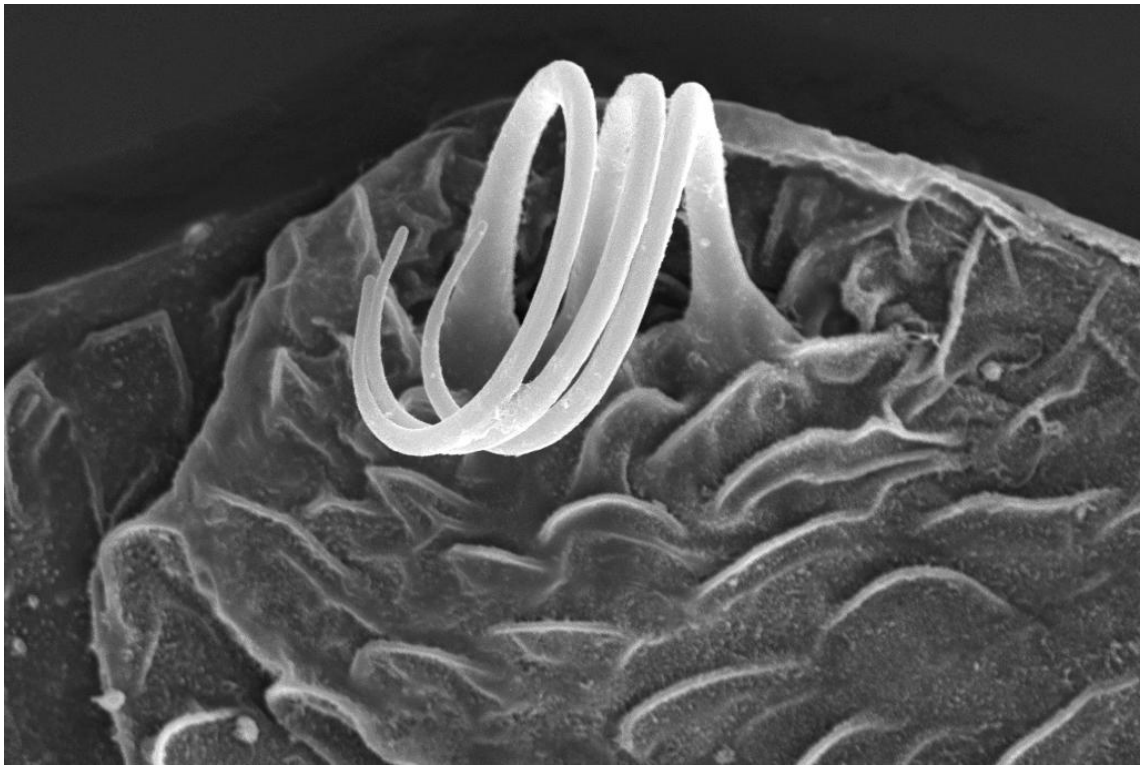


Fig. D-3. Hamulus on hindwing of *Adelges japonicas* (Adelgidae), dorsal view.

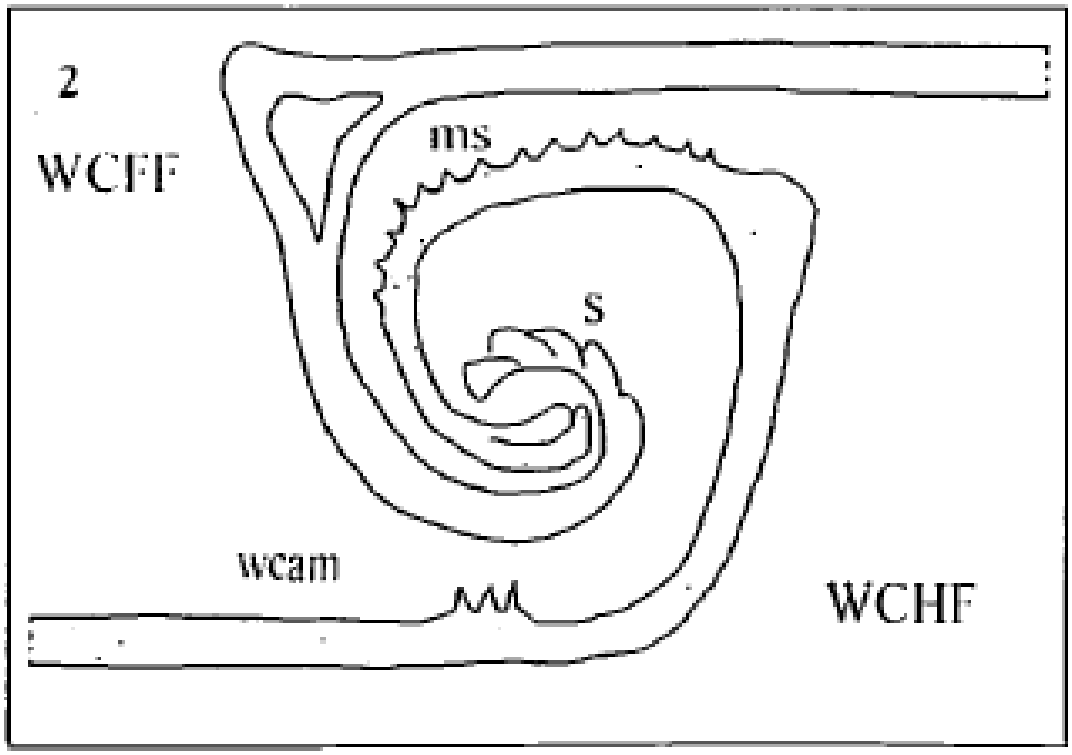


Fig. D-4. Section view of functional wing coupling mechanism in fore- and hindwing of Auchenorrhyncha. ms, microsculptures. s, shovels. wcam, wing-coupling accessory microsculptures, WCFF, wing-coupling fore fold. WCHF, wing-coupling hind fold. The figure and terms were cited from D'Urso (2002).

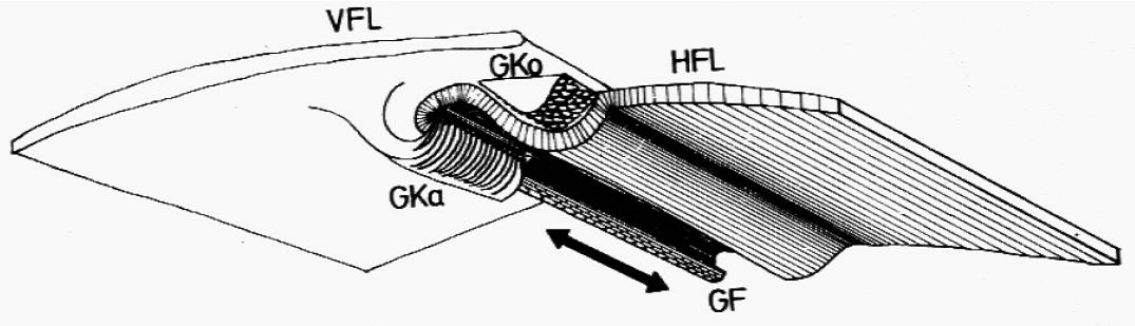


Fig. D-5. Section view of functional wing coupling mechanism in fore- and hindwing of Heteroptera. GF, sliding folding. GKa, sliding comb . GKo, sliding head. HFL, Hindwing. VFL, Forewing. The figure and terms were cited from Bohne and Schneider (1979).

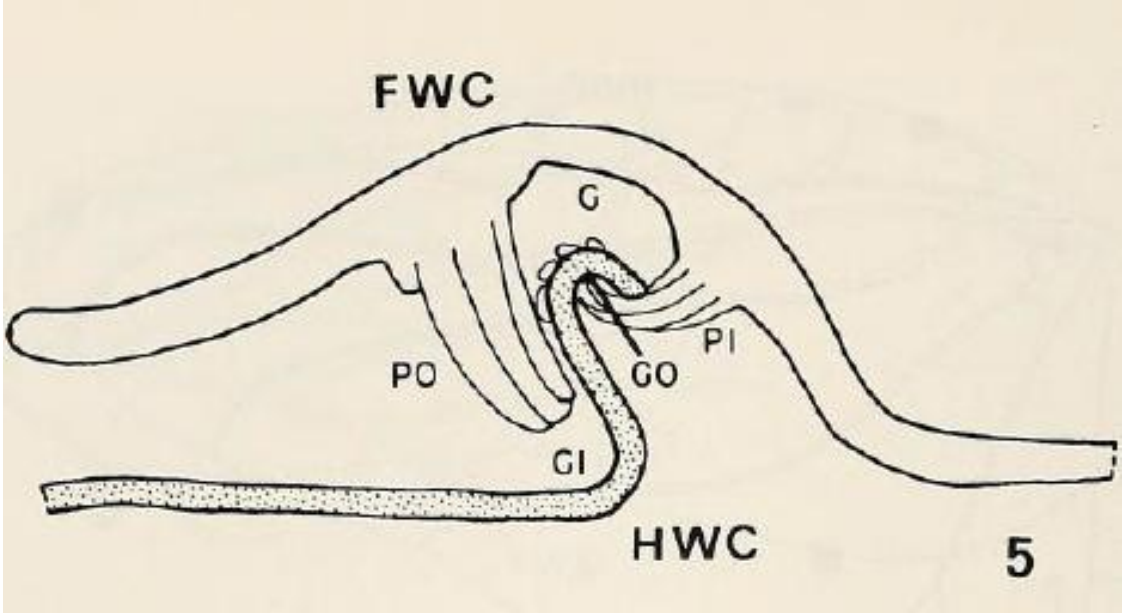


Fig. D-6. Section view of functional wing coupling mechanism in fore- and hindwing of Coleorrhyncha. FWC, wing coupling structure of forewing. G, groove. GI, inner groove. GO, outer groove. HWC, wing coupling structure of hindwing PI, internal position. PO, external position. The figure and terms were cited from D'Urso (1993).

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