BIOGEOGRAPHY AND PHYLOGENETICS OF HAWAIIAN BARK LICE

BY

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DISSERTATION

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ABSTRACT

In a redefinition of the genus *Ptycta* (Psocidae), the history of the genera *Ptycta* Enderlein, 1925, and *Copostigma* Enderlein, 1903, is assessed. Previously defined as a monophyletic complex based on male genital morphology, *Ptycta* is redefined as those species of the *Ptycta-Copostigma* complex with forewing veins Rs+M fused for a length. Two new species of *Ptycta* from Japan are described, *P. recava* sp. nov. and *P. johnsoni* sp. nov., increasing the number of Japanese species to four, along with *P. parvidentata* Tsutsumi, 1964, and *P. micromaculata* Thornton, Lee, and Chui, 1972. Distributional information and illustrations of each species, and a key to Japanese species of *Ptycta* are included.

*Ptycta* has diversified in the mid- and high-elevation forests of the six main Hawaiian Islands. To investigate the diversity, distribution, and evolutionary history of the group, I used morphological characters of the male genitalia, wings, and head, and DNA sequences of the nuclear gene *wingless* and mitochondrial genes 12S, 16S, and COI. Molecular and morphological data indicate that the ~50 species of Hawaiian *Ptycta* are a monophyletic group. The lineage includes two well-supported clades that are united by two synapomorphic characters of the male genitalia. The two clades are likely the products of a single colonization event and include many independent lineages with radiations within and between islands. The Hawaiian *Ptycta* is likely slightly less diverse than previously described, with an estimated diversity of 40 species, rather than the 61 taxa previously. These data also suggest that the lineage originated in the west or south Pacific region.

Further, I revise the systematic status of subfamily Kaindipsocinae (formerly Kaindipsocini) based on morphology of the male terminalia and on molecular data. *Clematostigma, Lasiopsocus*, and *Tanystigma* are newly assigned to this subfamily, and a new
tribe, Clematostigmini, is established for these genera. The *Blaste lunulata* species group is also placed within Kaindipsocinae and is probably closest to *Kaindipsocus*. Both morphological and molecular data provide strong support for monophyly of Kaindipsocinae and molecular data support a sister relationship between this subfamily and the rest of Psocidae.
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Chapter 1. Redefinition of Ptycta Enderlein (Psocodea: ‘Psocoptera’: Psocidae) and a Taxonomic Revision of the Japanese Species

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**Abstract**

The genera *Ptycta* Enderlein, 1925, and *Copostigma* Enderlein, 1903, are defined as a monophyletic complex based on male genital morphology. *Ptycta* is redefined as those species of the *Ptycta-Copostigma* complex with forewing veins Rs+M fused for a length. Two new species of *Ptycta* from Japan are described, *P. recava* sp. nov. and *P. johnsoni* sp. nov., increasing the number of Japanese species to four, along with *P. parvidentata* Tsutsumi, 1964, and *P. micromaculata* Thornton, Lee, and Chui, 1972. Distributional information and illustrations of each species, and a key to Japanese species of *Ptycta* are included.
INTRODUCTION

*Ptycta* Enderlein, 1925, is a large genus in the family Psocidae ('Psocoptera') that includes more than 170 species from all zoogeographic regions of the world, with the greatest diversity in subtropical and tropical regions of Africa (29 species), Asia (43), and the Pacific Islands (63) (Lienhard & Smithers 2002, Lienhard 2003-2006 in Yoshizawa 2006). Two species of *Ptycta* are currently known from the subtropical region of southern Japan: *P. parvidentata* Tsutsumi, 1964, described from Ishigakajima Island, Ryukyus and *P. micromaculata* Thornton, Lee & Chui, 1972, from Chichijima Island, Ogasawara Islands.

The genus *Ptycta* was erected by Enderlein (1925) to include the Indonesian species *Clematostigma schillei* Enderlein, 1903, and the Hawaiian species *Psocus distinguenda* and *Psocus haleakalae* Perkins, 1899, with *P. haleakalae* designated as type species. *Ptycta* was characterized as having the first section of forewing vein CuA1 shorter than the second section.

Badonnel (1967) argued that the characters used to define the genera *Ptycta*, *Copostigma* Enderlein, 1903, *Clematostigma* Enderlein, 1906, and *Maheella* Enderlein, 1931, were insufficient for distinguishing the genera and suggested that this complex of genera should be dealt with as a single unit until the relationships among them are clarified. He also suggested the synonomy of *Maheella* with *Ptycta*, which was accepted by Lienhard and Smithers (2002).

Wing vein characters have been the focus of taxonomy within the species complex. Smithers (1972) added a forewing character to Enderlein’s description of *Ptycta*: a spur vein at the apex of the pterostigma. Thornton (1981) studied Fijian species of *Ptycta* and concluded that the 8 species with a crossvein between Rs and M represent an endemic complex, while the single species with a Rs+M fused for a length represented a separate lineage. Smithers (1983) addressed the *Copostigma-Clematostigma-Ptycta-Maheella* complex and concluded that the
character of forewing vein CuA1 with the first section of shorter than the second as defined by Enderlein (1925) is variable within Ptycta and is shared by both Copostigma and Indiopsocus. Smithers (1983) also redefined Clematostigma, distinguishing the genus as a separate lineage from Copostigma-Ptycta. Later, Smithers (1985) defined Ptycta as having forewing veins Rs+M fused for a short distance, whereas Copostigma has a crossvein connecting veins Rs-M in the forewing.

Smithers (1985) also described a novel character of the male terminalia, the rugose basal lobe of the paraproct, as a diagnostic character of Copostigma. Using the new definition, Smithers (1985) moved several species to Copostigma: four New Guinea species of Mecampsis Enderlein, 1925, with a basal paraproct lobe, and eight Fijian Ptycta species with an Rs-M crossvein.

Concurrently, the basal paraproct lobe was also observed in Hawaiian Ptycta by Thornton (1984). In redefining Ptycta, Thornton (1984) considered all 51 Hawaiian endemic species to be from a single lineage, a diagnosis that required “wider parameters than is usual in genera of Psocidae,” (p. 109). He referred to Enderlein’s original description of vein CuA1 and added three male genital characters: the presence of a distinct basal paraproct lobe, a rugose epiproct lobe, and denticles on the lateral margins of the hypandrial strap.

Several taxa have been added to Ptycta based on the Rs+M fusion, including species from the Melanesian islands (Smithers & Thornton 1990) and Indonesia (Endang et al. 2002). However, this character is plesiomorphic within Psocidae (Yoshizawa 2005) and occurs in most other genera of the tribe Ptyctini. As a result, Ptycta appears to be an assemblage of many heterogeneous species (Lienhard & Smithers 2002). Substantial variation of the Rs+M character.
has been observed within species (i.e. Thornton 1981 discussion of *P. vitiensis*), and occasionally between the two wings of a single specimen (Yoshizawa, pers. obs.).

On the other hand, the basal paraproct lobe observed in *Copostigma* from New Guinea and Fiji (Smithers 1985; Yoshizawa, pers. obs.) and in *Ptycta* from Hawaii (Thornton 1984; Yoshizawa & Bess, pers. obs.), Indonesia (Endang et al. 2002), Australia (Yoshizawa & Smithers 2006), Malaysia (Yoshizawa, pers. obs.), and North and South America (Bess, pers. obs.) is likely to be a synapomorphy uniting *Ptycta + Copostigma*. The character is not observed in other genera of the tribe Ptyctini. Although the boundary between *Ptycta* and *Copostigma* remains unclear, their close relationship can be justified by the presence of the basal paraproct lobe. It should be noted that although the subgenital plate of the female is used for species delimitation in other Psocidae, this structure is poorly scleritized and is not useful for identification in *Ptycta-Copostigma* species.

Here, we redefine the genus *Ptycta* as those members of the *Ptycta-Copostigma* complex with Rs+M fusion and we describe two male genital characters that are synapomorphies of *Copostigma* and *Ptycta*. We also describe two new species of *Ptycta* from Japan, including a key to Japanese *Ptycta* and illustrations and distributional data for all species.

**MATERIALS AND METHODS**

The specimens used in this study were fixed in 80% or 99% ethanol. Some specimens were preserved in 65% glycerol after fixation in ethanol. Description of the color of specimens is based on alcohol material, with the exception of *Ptycta micromaculata*, preserved in glycerol. A Leica MZ12 binocular stereoscopic microscope and Zeiss Axiphoto compound light microscope
were used for observation and illustration. Wing photographs were taken with a digital camera on a Zeiss Axiphoto compound microscope. All measurements are in mm.

The ratio between interocular space and eye-diameter (IO/D) is calculated from measurements on the front of the head (Pearman’s method: Pearman 1934). On the phallosome, width and length (W/L) were measured from the internal margins of the phallosome ring; length of distal process (DP) was measured from internal margin of the distal process to the apex of the distal process of the phallosome, and divided by the length of the phallosome ring (DP/L). Body measurements are recorded as B (body length), F (forewing length), and H (hindwing length).

In illustrations, membranous areas are indicated by stippling, whereas sclerites are illustrated as plain areas. The internal surface of body walls is indicated with solid diagonal, parallel lines. Broad cross-hatches mean that a structure has been left out. In figures of the female subgenital plate, structure (left half) and pigmentation (right half) are shown. Illustrations of genital structures share a common scale and wing photos share a different common scale.

Methods and terminology for body morphology followed Yoshizawa (2005). Terminology for wing morphology followed Günther (1974). The following abbreviations were used in the text: ELKU (Entomological Laboratory, Kyushu University, Fukuoka, Japan), INHS (Illinois Natural History Survey, USA), KY (K. Yoshizawa). Unless specified, specimens are stored in the Hokkaido University Insect Collection.
SYSTEMATICS

Tribe Ptyctini Mockford, 1993

Genus *Ptycta* Enderlein


Synonymy with *Ptycta* suggested by Badonnel, 1967: 193; synonymy accepted by Lienhard and Smithers, 2002: 450.

*Diagnosis.* Ptyctini with body white to pale yellow in ground color, with brown to blackish-brown markings on head, thorax, and abdomen. Antennal flagella with short cilia in both male and female. Forewing (Fig. 1.1) hyaline with brown to blackish-brown markings, usually with dark spot in pterostigma; spur vein of pterostigma variable; veins Rs and M fused for a short distance. Posterolateral region of male clunium strongly concave, widely membranous (e.g., Fig. 1.2A). Male epiproct chair-shaped, epiproct lobe overlaps clunium (e.g., Fig. 1.2A, C). Male paraproct with basal lobe projecting laterally (e.g., Fig. 1.2A).
**Key to Japanese species of the genus Ptycta**

Forewings

1. Subcostal vein ends in costal cell, spur vein absent (Fig. 1.1A-D) ..... 2
   - Subcostal vein continues to vein R, spur vein present (Fig. 1.1E) ..... *P. parvidentata* Tsutsumi

2. Dark basal band present, discoidal band absent (Fig. 1.1A-C) ..... 3
   - Basal band faint or absent, discoidal band of 4 spots from distal margin of pterostigma to basal edge of areola postica (Fig. 1.1D) ..... *P. micromaculata* Thornton, Lee, and Chui

3. Dark band on anterior margin of pterostigma, nodal band fairly dark with large spots in cells cua and cup (Fig. 1.1B, C) ..... *P. johnsoni* sp. nov.
   - Pterostigma with pigmentation only in distal 1/3, nodal band faint (Fig. 1.1A) ..... *P. recava* sp. nov.

**Ptycta recava, sp. nov.**

*Diagnosis.* Forewing with dark basal band, faint nodal band, and subcostal vein ending in costal cell (Fig. 1.1A). *Male terminalia* (Fig. 1.2). Posterodorsal margin of clunium deeply concave, posteroventral process of clunium articulating with paraproct, median strap of the hypandrium wide, parallel-sided, symmetrical. Distributed from central to northern Honshu and on Sadogashima Island.
Description (after 9 years in 80% ethanol). Male. Head. Light brown in ground color; vertical markings broad dark brown bands on both sides of coronal suture; coronal suture black; orbital markings broad brown dorsal bands; epiclunial suture brown; frons with brown triangular marking between ocellar field and clypeus; gena dark brown; eye black, IO/D = 0.7; ocelli black, ocellar field dark brown; antennal socket dark brown; postclypeus with ca. 8 vertical dark brown stripes, ventrolateral corners without marking; anteclypeus with dark brown dorsal band. Antenna brown. Mouth parts pale brown; maxillary palps darker.

Thorax. Prothorax brown. Mesonotum pale brown; anterior surface of scutum dark brown, margins of anterior and lateral lobe sutures white bands; scutellum and postscutellum dark brown with black margins. Metanotum pale brown; scutum dark brown with paler anterior lobe; scutellum dark brown with triangular white markings on anterior margin. Meso- and metapleuron dark brown.

Legs. Brown; coxae dark brown; trochanters pale brown; distal 1/4 of tibiae dark brown; tarsi dark brown.

Forewing. (Fig. 1.1A). Basal band present. Faint nodal band with markings at junction of Rs+M veins and distal end of cell cup. Subcostal vein ends in costal cell. Pterostigma cloudy with basal 2/3 light brown and distal 1/3 dark brown, spur vein absent. Veins of areola postica pigmented except CuA2. Hindwing. Hyaline, veins without pigment.

Abdomen. White, each segment with transverse black band on posterior margin. Terminalia (Fig. 1.2). Clunium with posterovertral projection articulating with paraproct (Fig. 1.2A); posterordorsal margin deeply concave with shallow median process at articulation with epiproct (Fig. 1.2B); posterolateral part deeply concave, widely membranous (Fig. 1.2A). Epiproct lobe (Fig. 1.2A,C) high with nearly straight lateral margins, surface smooth, dorsal
margin recessed slightly, with microtrichia. Paraproctal basal lobe rugose, short, and in anterolateral orientation (Fig. 1.2A). Hyandrium (Fig. 1.2D) symmetrical, median strap wide, parallel sided, slightly broadening at apex, with denticles on basal 2/3 and at apex, apex with shallow notch; lateral corners with broad posterior extension of varying shape. Phalosome (Fig. 1.2E) ovate, twice as long as wide; distal process rugose, wide without expanded lobe at apex; W/L=0.50, DP/L=0.18.

Length. B 3.0-3.1; F 3.1-3.2; H 2.4.

*Female*. Similar to male except frons with white spot in center of brown triangular marking; IO/D=1.4.

*Genitalia*. (Fig. 1.3). Egg guide of subgenital plate (Fig. 1.3A) tapers slightly to rounded apex; sclerite on dorsal surface of egg guide with slightly concave lateral margins, distal margin of sclerite broad and rounded. External valve of gonapophyses (Fig. 1.3B) with rounded anterior and posterior margins; posterior lobe narrow, triangular, with rounded process ventrally.

*Etymology*. The specific epithet refers to the deeply concave posterodorsal margin of the male clunium.

*Distribution*. This species occurs from central to northern Honshu, and on Sadogashima Island.

*Remarks*. This species is quite distinct from the other Japanese *Ptycta* in the reduced pigmentation of the forewing and in the form of the male clunium: the posterodorsal margin is deeply concave with shallow median process at articulation with epiproct, and the posterolateral region is deeply concave and widely membranous.


Ptycta johnsoni, sp. nov.


Ptycta sp. KY2002 GenBank (online database for gene sequences): accession numbers of gene sequences obtained from paratype male (voucher number KY235 collected at Cape Satamisaki) are AY139907 (12S rDNA), AY139954 (16S rDNA) and AY630553 (18S rDNA).

Diagnosis. Forewing (Fig. 1.1BC) with wide basal band and nodal band, pigmentation on anterior margin and apical 1/3 of pterostigma. Male terminalia (Fig. 1.4): Basal paraproct lobe short in anterodorsal orientation, posterior lobe of paraproct rounded; phallosome long with narrow basal margin. Female genitalia (Fig. 1.5): Sclerite of subgenital plate with broad triangular distal margin. Distributed from Tsushima Island to Okinawajima Island.

Description (coloration mainly based on samples freshly collected into 99% ethanol but, for description of wing markings, old 80% ethanol specimens were also used). Male. Head. White in ground color; vertical markings elongate black spots in band around coronal suture; pair of black markings between apex of vertical marking and ocelli; coronal suture black; orbital markings black; epiclunial suture black; frons with four broad bands between epiclunial suture and postclypeus; gena white; eye black, IO/D = 1.3; ocelli black, ocellar field white; antennal
socket dark brown; postclypeus with ca. 14 vertical dark brown stripes; anteclypeus white with dark brown horizontal band in center. Antenna brown, pedicel and scape white. Mouth parts pale brown; maxillary palps darker.

**Thorax.** Prothorax dark brown. Mesonotum dark brown; scutum with yellow band at center and between anterior and lateral lobes; scutellum yellow with triangular brown marking at posterior. Metanotum brown; scutum brown with yellow bands along sutures of lateral lobes, posterior margin yellow; scutellum brown. Meso- and metapleuron brown.

**Legs.** White; middle and hind coxae brown; femora with dark band on distal surface; tibiae with dark brown spines, 1/4 of tibiae dark brown; tarsi dark brown to black.

**Forewing** (Fig. 1.1B, C). Forewing markings with some geographical variation. Basal band present. Nodal band with dark spots at base of pterostigma, along veins Rs+M, in center of cell cua, and at apex of cell cup, and faint spots in cell r (nodal band fainter in specimens from Okinawajima and Tsushima). Subcostal vein ends in costal cell. Pterostigma with anterior margin pigmented (faint in specimens from Okinawajima), distal 1/3 pigmented with marking extending past posterior margin, spur vein absent. Veins of areola postica pigmented except CuA2. **Hindwing.** Hyaline, veins pigmented on apical 2/3.

**Abdomen.** Yellow to white, each segment with narrow transverse brown band at center. **Terminalia** (Fig. 1.4). Clunium posterodorsal margin nearly straight at articulation with epiproct (Fig. 1.4B); posterolaterally with moderate-sized membranous region (Fig. 1.4A). Epiproct lobe low (Fig. 1.4A), with nearly straight, wide dorsal margin, not rugose in texture (Fig. 1.4C). Paraproct basal lobe rugose, short, and in anterolateral orientation; posterior lobe rounded (Fig. 1.4A). Hypandrium (Fig. 1.4D) nearly symmetrical, median strap broad basally, constricted medially and broadening at apex, with denticles in comb-like arrangement on entire margins,
apex with deep asymmetric notch; lateral corners narrow and tapering to point or small rounded process; hypandrial membrane with sclerites of varying size, sometimes absent. Phallosome (Fig. 1.4E) ring elongate, twice as long as wide: apical distal process rugose, wide without expanded lobe at apex; W/L=0.45, DP/L=0.18.

Length. B 2.8-3.0; F 3.0-3.1; H 2.1.

Female. Similar to male, except; IO/D=1.5; abdominal segments with wider transverse brown bands.

Genitalia. (Fig. 1.5). Egg guide of subgenital plate (Fig. 1.5A) tapers slightly to truncate apex; sclerite on dorsal surface of egg guide triangular. External valve of gonapophyses (Fig. 1.5B) rectangular with rounded posterior margin; posterior lobe small and rounded.

Etymology. Specific epithet is dedicated to Kevin P. Johnson of Illinois Natural History Survey. The species was first mentioned as Ptycta sp. in co-authored paper by Yoshizawa & Johnson (2003).

Distribution. This species occurs in southern Japan, from Tsushima Is. to Okinawajima Is.

Remarks. This species is very similar to Ptycta furcata Li, 1993, from Guangdong Province, China. It can be distinguished by the pigmentation on the anterior margin of the pterostigma, the rounded posterior lobe of the paraproct, and the narrow, tapering lateral corners of the hypandrium.


Paratypes. [Tsushima] 1 female, Kamisaka, 17. vi. 1995; 4 males 1 female, Nita, 17. vi. 1995. [Kyushu ] 2 females, same locality as holotype, 21. vi. 1993; 12 males 24 females, same data as for holotype (some specimens were collected as nymphs, reared to adults and fixed on 21.
Ptycta micromaculata Thornton, Lee & Chui

* Ptycta micromaculata * Thornton, Lee & Chui, 1972: 139.

**Diagnosis.** Forewing (Fig. 1.1D) with basal band faint or absent, discoidal band of 4 spots from distal margin of pterostigma to basal edge of areola postica. *Male terminalia* (Fig. 1.6) with posterolateral region of clunium broadly membranous, paraproct with long basal lobe in anterolateral orientation, posterior lobe of paraproct rectangular; hypandrium asymmetrical. Distributed on Chichijima and Hahajima of Ogasawara Islands.

**Redescription of male terminalia** (after 13 years in glycerol; Fig. 1.6). Clunium posterior margin only slightly concave at articulation with epiproct (Fig. 1.6B); posterolateral region broadly membranous (Fig. 1.6A). Epiproct lobe low (Fig. 1.6A), with narrow, lobed dorsal margin, surface spinous (Fig. 1.6C). Paraproct basal lobe rugose, narrow and long, and in anterolateral orientation; posterior lobe rectangular (Fig. 1.6A). Hypandrium asymmetrical (Fig. 1.6D), median strap narrow, curved, fine denticles along lateral margins visible with compound microscope, apex with deep notch; lateral corners shallow; hypandrial membrane with large
asymmetric sclerites of varying size. Phallosome (Fig. 1.6E) with long, slender rugose distal process with expanded lobe at apex; W/L=0.68, DP/L=0.33.

Redescription of female genitalia (after 13 years in glycerol; Fig. 1.7). Egg guide of subgenital plate tapers slightly and widens to slightly rounded apex; sclerite on dorsal surface of egg guide rounded, distal margin of sclerite 1/3 width of sclerite and straight. External valve of gonapophyses large with rounded anterior and posterior margins; posterior lobe broad and rounded.

Distribution. This species occurs on Hahajima Island and Chichijima Island, Ogasawara Islands, in southern Japan.

Remarks. This species can be distinguished from other Japanese Ptycta by the dark discoidal band on the forewing, the shallow concavity of the posteroventral corner of the male clunium, and the long paraproct basal lobe with posterolateral orientation.


**Ptycta parvidentata** Tsutsumi

*Ptycta parvidentata* Tsutsumi, 1964: 267

Diagnosis. Forewing (Fig. 1.1E) with subcostal vein continuing to vein r, spur vein present. Male terminalia (Fig. 1.8) with basal paraproct lobe mid-length in anterolateral
orientation, clunium with moderately-size concave membranous region posterolaterally. Hypandrium symmetrical with median strap gradually narrowing and deep slightly asymmetrical notch at apex. Distributed on the Yaeyama Islands.

**Redescription of male terminalia** (after ten years in 80% ethanol; Fig. 1.8). Clunium posterior margin slightly concave at articulation with epiproct (Fig. 1.8B); posterolateral membranous region moderate in size (Fig. 1.8A). Epiproct lobe low (Fig. 1.8A) with rounded dorsal margin, finely rugose surface (Fig. 1.8C). Paraproct basal lobe of moderate length, rugose, and in anterolateral orientation; posterior margin rectangular (Fig. 1.8A). Hypandrium symmetrical (Fig. 1.8D), median strap gradually narrowing, with denticles in comb-like arrangement on almost entire margins, apex with deep slightly asymmetric notch; lateral corners narrow and tapering to point or small rounded process; hypandrial membrane with large sclerites of varying shape. Phallosome (Fig. 1.8E) with long, slender, rugose apical distal process without expanded lobe at apex; \( W/L = 0.51, \) \( DP/L = 0.26. \)

**Redescription of female genitalia** (after ten years in 80% ethanol; Fig. 1.9). Egg guide of subgenital plate tapers slightly to rounded apex; sclerite on dorsal surface of egg guide oval. External valve of gonapophyses with rounded anterior and posterior margins; posterior lobe broad triangular and rounded.

**Distribution.** This species occurs on the Yaeyama Islands (Ishigakijima and Iriomotejima) of southern Japan.

**Remarks.** This species can be distinguished from other Japanese *Ptycta* by the forewing with subcostal vein continuing to vein *r*, presence of a pterostigma spur vein, and the wide, gradually narrowing median strap of the hypandrium.

**DISCUSSION**

*Definition of Ptycta and monophyly of the Copostigma-Ptycta complex*

As discussed above, the original description and subsequent redefinitions of *Ptycta* have relied on plesiomorphic and/or highly variable characters of forewing venation (Fig. 1.1). Enderlein (1925) erected the genus based on the vein CuA1 having the first section shorter than the second. However, this character varies among species of *Ptycta*, as does the pterostigma spur vein, suggested by Smithers (1972) to be diagnostic of *Ptycta*. Most recently, *Ptycta* was redefined by Smithers (1985) as having veins Rs+M fused for a short distance, whereas *Copostigma* has a cross vein connecting Rs-M. Although the Rs+M fusion is plesiomorphic within Psocidae and varies within species of *Ptycta*, this definition has been accepted by subsequent authors (e.g., Endang et al., 2002).

The use of plesiomorphic and variable venation characters in defining *Ptycta* has led to a heterogeneous “holding genus” (Endang et al. 2002; Lienhard & Smithers 2002). The primary problem with the current taxonomy of *Ptycta* is in defining the basal limit of the genus because
there has been no synapomorphic character to define the genus and exclude heterogeneous species.

The secondary problem is in differentiating Ptyctia from Copostigma. The Rs+M fusion (Fig. 1.1) is the only character that we are aware of that establishes a boundary between Ptyctia and Copostigma. Until further study, we will retain this distinction between Ptyctia and Copostigma, although we are aware that the relationship between veins Rs and M is variable and unreliable, and that defining Ptyctia based on the Rs+M fusion will maintain its status as paraphyletic.

Although the boundary between Copostigma and Ptyctia remains unclear, the two genera are strongly united by male genital morphology. The basal paraproct lobe described by Thornton (1984), Smithers (1985) and Yoshizawa & Smithers (2006) is a prominent synapomorphy of the two genera (e.g., Fig. 1.2A). Here, we also describe a second synapomorphy, the clunium with a strongly concave, membranous posterolateral region (e.g., Fig. 1.2A). The combination of these two synapomorphies will be helpful in excluding heterogeneous species currently held in Ptyctia. These characters also distinguish Ptyctia-Copostigma taxa from the potentially related genera, such as Indiopsocus Mockford, 1974, and Atlantopsocus Badonnel, 1944, which otherwise resemble Ptyctia-Copostigma in genital morphology.

All of the Ptyctia specimens we have examined have both of these apomorphic characters, including specimens from Hawaii (the type locality of the genus), Indonesia, Fiji, Malaysia, Australia, and North America. These observations indicate that many species from these regions are correctly classified in the Ptyctia-Copostigma complex.

Information on these characters, particularly the clunium character, is rarely available in published illustrations, however. This makes it difficult to draw conclusions about the validity of
*Ptycta* from other regions. We found one species with the paraproct lobe clearly illustrated from Madagascar (Badonnel 1967) and another with both characters from the Galapagos Islands (Thornton & Woo 1973). *Ptycta* species that clearly lack these characters also fail to resemble other *Ptycta* in the morphology of the hypandrium, phallosome, and forewing, including species from Madagascar (Badonnel 1967), Angola (Badonnel 1969), and Mediterranean Europe (Lienhard 1998). Based on our redefinition of the genus, these species can be excluded from *Ptycta*. A thorough revision of the genus would be necessary to apply this new definition to all species currently included in *Ptycta* and to establish species groups within the *Ptycta-Copostigma* complex.

Non-homologous structures of the male paraproct similar to those of *Ptycta* and *Copostigma* are seen in other Psocidae taxa. The basal paraproct lobes of *Trichadenotecnum* (Yoshizawa 2001) superficially resemble those of *Ptycta-Copostigma*, but those of *Trichadenotecnum* originated within the genus (Yoshizawa 2001, 2003, 2004). Like the paraproct lobe observed in North American species of *Hyalopsocus* (Psocini) (Yoshizawa, pers. obs.), those of *Trichadenotecnum* extend from the anteroventral margin of the paraproct, whereas the basal paraproct lobe of *Ptycta-Copostigma* is usually apart from the ventral margin of the paraproct (a distinction that is clearer in other *Ptycta* species than in those from Japan, see Yoshizawa & Smithers 2006).

**Distribution of Japanese Ptycta**

The Japanese species of *Ptycta* occur in temperate to subtropical regions from northern Honshu through the Yaeyama Islands on the southern tip of the Japanese archipelago (Fig.10). There is no overlap in the ranges of the species: *Ptycta micromaculata* occurs in the Bonin
Islands in the subtropical southeastern region, *P. parvidentata* occurs only on the Yaeyama Islands of southern Japan, *P. johnsoni* occurs from Okinawa through Kyushu and Tsushima Island, and *P. recava* is found from central through northern Honshu.

**ACKNOWLEDGMENTS**

We thank K. P. Johnson and two anonymous reviewers for critical reading of the ms. ECB thanks S. Akimoto for hosting her stay in Japan and V. S. Smith for helpful comments on the project proposal. KY thanks H. Makihara and T. Yasunaga for assistance in the field. ECB's stay in Japan was supported by the JSPS summer program and the NSF EAPSI program.

**REFERENCES**


Fig. 1.1. Forewings of Japanese Ptycta. A. *P. recava* sp. nov.; B, C. *P. johnsoni* sp. nov., showing geographical variation of wing markings from northern end of Kyushu (Nokonoshima: B) to Okinawajima (C); D. *P. micromaculata*; E. *P. parvidentata*.
Fig. 1.2. Male terminalia of Ptycta recava. A. Terminalia, lateral view; B. Clunium, dorsal view; C. Epiproct, posterior view; D. Hypandrium, posterior view; E. Phallosome, ventral view.
Fig. 1.3. Female genitalia of Ptycta recava, ventral view. A. Subgenital plate; B. Gonapophyses.
Fig. 1.4. Male terminalia of *Ptycta johnsoni*. A. Terminalia, lateral view; B. Clunium, dorsal view; C. Epiproct, posterior view; D. Hypandrium, posterior view; E. Phallosome, ventral view.
Fig. 1.5. Female genitalia of Ptycta johnsoni, ventral view. A. Subgenital plate; B. Gonapophyses.
Fig. 1.6. Male terminalia of *Ptycta micromaculata*. A. Terminalia, lateral view; B. Clunium, dorsal view; C. Epiproct, posterior view; D. Hypandrium, posterior view; E. Phallosome, ventral view.
Fig. 1.7. Female genitalia of *Ptycta micromaculata*, ventral view. A. Subgenital plate; B. Gonapophyses.
Fig. 1.8. Male terminalia of *Ptyeta parvidentata*. A. Terminalia, lateral view; B. Clunium, dorsal view; C. Epiproct, posterior view; D. Hypandrium, posterior view; E. Phallosome, ventral view.
Fig. 1.9. Female genitalia of *Ptycta parvidentata*, ventral view. A. Subgenital plate; B. Gonapophyses.
Fig. 1.10. Map of the distribution of Japanese Ptycta species: j = P. johnsoni, m = P. micromaculata, p = P. parvidentata, r = P. recava.
Chapter 2. A review of the Hawaiian Ptycta with new specimen records and an interactive key to species.

DISCLAIMER: NOMENCLATURAL ACTS OR PROPOSED RECLASSIFICATIONS INCLUDED IN THIS DISSERTATION ARE NOT CONSIDERED VALIDLY PUBLISHED UNDER ARTICLE 8 OF THE INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE.

INTRODUCTION

The bark louse genus Ptycta (Psocidae) represents one of the largest hemimetabolous arthropod radiations yet documented on the Hawaiian Islands. These small, fungus-eating insects live on the surface of trees and shrubs, on bark, twigs, and leaves. Generally black and white in appearance, their colors vary from black to medium brown markings on cream to yellow ground colors. Their appearance is cryptic on uneven surfaces of woody plants. Ptycta are abundant in native forests of Hawaii and in many agricultural areas, such as macadamia and Casaurina plantations (Gange and Haworth, 1981).

Perkins (1899) published the first descriptions of Hawaiian bark lice, including 14 species in the genus Psocus Fabricius (Psocidae), distributed across the six main Hawaiian Islands. Enderlein (1913) transferred these species to the genus Clematostigma Enderlein and reduced two of them to varieties of the species distinguendus (Perkins). Enderlein (1920) transferred the taxa back to the original genus and further reduced Perkins’ species to two full species: Psocus haleakalae included five varieties and six synonymies, and Psocus distinguendus included three varieties. He later (1925) included these two species in the new
genus *Ptycta*, designating *Psocus haleakale* Perkins, 1899, as the type species and also including the Indonesian species *Clematostigma schillei* Enderlein, 1906.

Banks (1931) did not acknowledge Enderlein’s synonymies or his creation of the genus *Ptycta* when he listed two species, *Psocus distinguendus* and *Psocus kauaiensis*, in the collection of the Museum of Comparative Zoology at Harvard. Zimmerman (1948, p. 244) offers an explanation for the names used by Banks; he notes that the type specimens may not have been among those examined by Enderlein and states that Banks, who examined some of Perkins’ original material, disagreed with Enderlein’s synonymy. Likewise, Zimmerman (1949) disregarded Enderlein’s opinions, listed each of Perkins’ 14 species of *Psocus* in *The Insects of Hawaii*, and included illustrations of the wings of each species.

In the early 1960’s, I. W. B. Thornton made extensive collections of bark lice on the six main Hawaiian Islands. Among his collections were more than 2000 specimens of *Ptycta*. Using techniques of numerical taxonomy, Chui and Thornton (1972) designated 47 species of Hawaiian *Ptycta* based on 79 morphological characters. Thornton (1984) published formal descriptions of 51 species and 10 subspecies of Hawaiian *Ptycta* and included further diagnosis of the genus. Thornton retained 12 of Perkins’ 14 original species and included detailed illustrations of the male and female genitalia and of the female forewing of each taxon.

Many of the 61 taxa designated by Thornton (1984) are described from very few specimens: 32 (52%) are known from five or fewer specimens and eight (13%) are known from a single specimen. Most species are known from very few localities: 24 (39%) from a single locality, 15 (25%) from two localities, and only seven (11%) from five or more localities (Thornton 1984). This large proportion of rare and narrow range species indicates that the full diversity of these insects may not be represented in current collections (Novotny & Bassett 2000).
METHODS

To review the current species of Hawaiian Ptycta, I first requested a loan of the material from the Bishop Museum, but discovered that only the types were housed there. In the time preceding his death in 2002, I. W. B. Thornton continued working on the Hawaiian bark lice and had most of the specimens in his collection at La Trobe University in Melbourne, Australia. The specimens were temporarily misplaced and rediscovered in 2007 in an informal storage area at La Trobe. The specimens were then sent to the Australian Museum (AM) in Sydney. I examined the specimens at AM and, using Thornton’s handwritten notebooks, labeled the specimens (which were marked only with code numbers) and catalogued the collection data into the AM database. These collection data are listed here (Table 1) and available as part of the 3i online database at http://ctap.inhs.uiuc.edu/dmitriev/3i_keys.asp (Bess, 2011).

Based on 2000+ specimen records reported in Thornton’s notebooks, I focused collecting effort for the current study on regions of the Hawaiian Islands that have not been collected and on the type localities of Ptycta species. The highest diversity of Ptycta was found in the regions that have received the most collecting effort. Thus, collecting in new regions was expected to yield new species.

In order to study the diversity of Hawaiian Ptycta, I used characters established by Chui and Thornton (1972), Thornton (1984), Günther (1974), Bess and Yoshizawa (2007), and Yoshizawa (2002) to produce preliminary descriptions of each distinct morphospecies in the new collections from Hawaii. I used the cladogram and species descriptions in Thornton (1984) to identify specimens to species or species group. I used these data to compile species descriptions and construct an Internet based interactive key in the program 3i (Dmitriev 2011), including photographs of all type specimens. The Interactive Key to Hawaiian Ptycta is available online at
http://ctap.inhs.uiuc.edu/dmitriev/3i_keys.asp and will be published as a monograph with a dichotomous key to species.

DISCUSSION

In an effort to sample from the widest possible diversity of habitats, I collected an additional 5932 adult *Ptycta* that were examined for this study. Of the 61 established taxa, I identified 26 in the new samples (Table 2.2). Nine undescribed species were also collected. Although I expected that collecting in previously un-collected habitats and quadrupling the number of available specimens would dramatically increase the number of Hawaiian *Ptycta* species, I found that, to the contrary, Thornton’s (1984) treatment of the group is very nearly comprehensive. Further, his use of numerical taxonomic analysis (Chui and Thornton 1972) provided exceptionally detailed analysis of morphological characters. To this list, I have added a single character, the form of the anterior lobe of the paraproct, as described by Bess and Yoshizawa (2007).

With many taxa described from singletons, I predicted that many undiscovered species were present on the islands. To the contrary, I found that many of the species described by Thornton were indistinguishable from one another. In many cases, specimens matched more than one species description and could be placed in a particular species by only a single morphologically plastic character, such as eye size or wing markings. Emphasizing the male genital morphology, which has more characters and varies more than that of the females, I am proposing the following synonymies (Table 2.2).

PROPOSED SYNONYMIES
**sylvestris group (proposed group)**

*Ptycta lanaiensis* (Perkins)

- *P. lanaiensis lanaiensis*
- *P. lanaiensis fusca*
- *P. lanaiensis halawa*
- *P. lanaiensis persclera*

These four subspecies vary in wing markings and head markings, and some slight differences in the shape of the female subgenital plate and posterior lobe of the outer valve of the gonapophyses. These differences indicate that these taxa may represent a species complex, but the characters currently used to distinguish among them are not adequate for consistent identification.

**apicantha group**

*Ptycta palae* (Thornton)

- *P aaroni aaroni* (Thornton)
- *P. aaroni mauiensis* (Thornton)
- *P. peleae* (Thornton)

*Ptycta palae* and the two subspecies of *P. aaroni* vary only in the size of the eyes. Genital morphology is identical among the three taxa.

**kauaiensis group**

*Ptycta kauaiensis* (Perkins)
*P. kauaiensis* (Perkins)

*P. diastema* (Thornton)

*P. persimilis* (Thornton)

*P. zimmermani* (Thornton)

These four species from the *kauaiensis* group differ only in coloration of the abdomen, and minor differences in wing and clypeus markings. There are no consistent differences in genital morphology.

*Ptycta simulator* (Perkins)

*P. simulator simulator* (Perkins)

*P. simulator kilauea* (Thornton)

Two subspecies vary in wing markings, eye size, and the presence of a sclerotized flap on the subgenital plate of the female in *P. simulator kilauea*. The taxa do not vary further in morphology of the male or female genitalia.

**haleakalae group**

*Ptycta haleakalae* (Perkins)

*P. haleakalae haleakalae* (Perkins)

*P. haleakalae hualalai* (Perkins)

*P. haleakalae konae* (Perkins)

These three subspecies vary in wing markings, eye size, and subtle differences in hypandrial morphology in the male. Morphology of the male and female genitalia and body size are consistent among the three taxa.
*Ptycta palikea* (Thornton)

*P. palikea* Thornton

*P. stena* Thornton

The two species do not vary in male genital morphology. *Ptycta stena* is described from a single male specimen and the hypandrium is the only part of the specimen that is available for examination.

*Ptycta unica* (Perkins)

*P. unica* (Perkins)

*P. hawaiiensis* Thornton

*Ptycta unica* was described from only two specimens, one male and one female. These two taxa show subtle variation pigmentation of the forewing and head, as well as in the size of the 3rd valve of the female gonapophyses. An unusual elongate wing shape, large body size, and other genitalia characters are strikingly similar between the two taxa.
LITERATURE CITED


http://ctap.inhs.uiuc.edu/dmitriev/3i_keys.asp


Table 2.1. Localities of Hawaiian *Ptycta* collections. Nomenclature follows Thronton, 1984.

**Ptycta aaroni** (incl. *P. aaroni mauiensis*)


**Ptycta apicantha**

<table>
<thead>
<tr>
<th>Location</th>
<th>Specimen Details</th>
</tr>
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<tbody>
<tr>
<td>1m, Hawaii Co., Volcano, Kipuka Ki, 1200m, on <em>Pipturus</em>, 30 I 1963 (I.W.B. Thornton).</td>
<td></td>
</tr>
<tr>
<td>5m, 1f, 4n, Hawaii Co., Waimea, 2700ft, on <em>Datura</em>, 27 VI 1963 (I.W.B. Thornton).</td>
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<td>1f, Honolulu Co., Oahu, Honolulu, on <em>Plumeria</em>, 14 III 1923 (J.F.I.).</td>
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<tr>
<td>1m, Honolulu Co., Oahu, Honolulu, 28 V 1943 (E.C. Zimmerman).</td>
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<tr>
<td>2f, Maui Co., Lanai, Lanai, 14 XII 1916 (W.M. Giffard).</td>
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<tr>
<td>13m, 8f, 14n, Maui Co., Maui, Haiku Farm, Haiku, mango orchard, on <em>Auracaria</em>, 13 V 1963 (I.W.B. Thornton).</td>
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<tr>
<td>1m, Maui Co., Maui, Thronton's lookout, 18 IX 1963 (I.W.B. Thornton).</td>
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<tr>
<td>1m, 3f, 6n, Maui Co., Maui, Upper Ioa valley, stream from Puu Kukui, 2500ft, on <em>Cheirodendron and guava</em>, 17 IX 1963 (I.W.B. Thornton).</td>
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<tr>
<td>1m, Hawaii Co.: HVNP Bird Park, 16 VII 1998 (D. Percy).</td>
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<td>1m, Maui Co., Maui, Kainalu, 21.0936°N 156.7783°W, 27 II 1927 (E.H. Bryan, Jr.).</td>
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<tr>
<td>1m, Maui Co., Maui, 2400 ft above Kamiloloa, on <em>Diospyros</em>, 19 VII 1963 (I.W.B. Thornton).</td>
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<td>1m, Maui Co., Maui, Kainalu, 27 VII 1927 (W.A. Bryan).</td>
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<td>1m, Maui Co., Maui, middle ridge at head of Ioa valley, 760m, on <em>Metrosideros</em>, 19 IX 1963 (I.W.B. Thornton).</td>
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<td>1m, Maui Co., Maui, Olinda, 4000ft, on <em>Pinus</em>, 31 I 1963 (I.W.B. Thornton).</td>
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<tr>
<td>1m, Maui Co., Maui, Kainalu, 21.0936°N 156.7783°W, 27 II 1927 (E.H. Bryan, Jr.).</td>
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<tr>
<td>1m, Maui Co., Maui, 2400 ft above Kamiloloa, on <em>Diospyros</em>, 19 VII 1963 (I.W.B. Thornton).</td>
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<td>1m, Maui Co., Maui, middle ridge at head of Ioa valley, 760m, on <em>Metrosideros</em>, 19 IX 1963 (I.W.B. Thornton).</td>
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<td>33m, 18 III 2008 (E. Bess).</td>
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<tr>
<td>1m, 15 VII 2008 (E. Bess).</td>
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<tr>
<td>3m, 16 VII 2008 (E. Bess).</td>
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<tr>
<td>6m, 2 VIII 2008 (E. Bess).</td>
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<tr>
<td>4f, Honolulu Co., Oahu, E Koolau Mts, Mt Tantalus, on <em>Acacia koa</em>, 20 VI 1963 (I.W.B. Thornton).</td>
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<td>5m, 3f, Honolulu Co., Oahu, E Koolau Mts, Mt Tantalus, on <em>guava</em>, 20 VI 1963 (I.W.B. Thornton).</td>
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| 2m, 2f, 9n, Honolulu Co., Oahu, E
Table 2.1, cont.


*Ptycta diastema*

**Holotype:** Hawaii: 1m, Kauai Co., Kokee cabin, 22.13802°N 159.65599°W, on *Acacia koa*, 1 VIII 1963 (I.W.B. Thornton), (BPBM). 1m, 1f, 5n, Kauai Co., Kokee area, 1100m, 13 IX 1957 (A.M. Nadler). 3m, 2f, Kauai Co., Kokee cabin, on *Acacia koa*, 1 VIII 1963 (I.W.B. Thornton). 2m, 1f, Kauai Co., Kokee, beginning of Alakai Swamp Trail, ridge b/w Kawaikini and Kawaikoi Streams, on *Acacia koa*, 27 VII 1963 (I.W.B. Thornton). 1m, Kauai Co., Kokee, Kalalau Lookout, 23 VIII 1957 (E.L. Mockford). 3f, Kauai Co., Kokee, Near Camp Slogget, on *Acacia koa*, 1 VIII 1963 (I.W.B. Thornton). 2m, Kauai Co., Koke'e SP, 22.13802°N
Table 2.1, cont.


Ptycta dicrosa


Ptycta disclera


Ptycta distinguenda

Table 2.1, cont.


*Ptycta drepana*


*Ptycta drepana drepanoides*

**Holotype:** Hawaii: 1m, Maui Co., Lanai, Lanai, 20.8306°N 156.9222°W, 600m, 12 XII 1916 (W.M. Giffard), (BPBM). 3m, Maui Co., Lanai, Lanai, 600m, 5 XII 1916 (W.M. Giffard). 4m, Maui Co., Lanai, Lanai, 600m, 7 XII 1916 (W.M. Giffard).

*Ptycta episcia*

Table 2.1, cont.

**Ptycta frogneri**


**Ptycta giffardi**


**Ptycta gynegonia**


**Ptycta haleakalae**

Table 2.1, cont.


*Ptycta hardyi*


*Ptycta hawaiiensis*

**Holotype:** Hawaii: 1m, Hawaii Co., Kilauea area, Kipuka Ki, 19.4467°N 155.3206°W, 1200m, on *Metrosideros and Acacia koa*, 1 I 1963 (I.W.B. Thornton), (BPBM). Hawaii Co., Kiauea, Kipuka Puaulu, on *Acacia koa*.

*Ptycta heterogamias*


*Ptycta iaoensis*

**Holotype:** Hawaii: 1m, Maui Co., Maui, upper Ioa valley, 20.88389°N 156.54889°W, 500m, on *Metrosideros*, 17 IX 1963 (I.W.B. Thornton), (BPBM).
Table 2.1, cont.

**Ptycta kaala**


**Ptycta kauaiensis**


**Ptycta lanaiensis**

(incl. *P. lanaiensis lanaiensis*, *P. lanaiensis fusca*, *P. lanaiensis halawa*, *P. lanaiensis perclera*)

Table 2.1, cont.

**halawa**


**Ptycta leucothorax**


**Ptycta lobophora**

Table 2.1, cont.

**Ptycta maculifrons**


**Ptycta microctena**


**Ptycta microglena**


**Ptycta molokaiensis**

Table 2.1, cont.

*Ptycta monticola*


*Ptycta oahuensis*


*Ptycta oligocantha*


*Ptycta palikea*

**Holotype:** Hawaii: 1m, Honolulu Co., Oahu, eastern Koolau Range, Paika, 156.54889°W, 600m, on *Metrosideros*, 31 X 1963 (I.W.B. Thornton), (BPBM). 2m, 4n, Honolulu Co., Oahu, E Koolau Range, Palikea, rim of Kaau Crater, 580m, on *Metrosideros*, 31 X 1963 (I.W.B. Thornton). 1n, Honolulu Co., Oahu, Pupekea ridge, 1500ft, on *Metrosideros*, 30 XII 1963
Table 2.1, cont.


_Ptycta pardena_

**Holotype:** Hawaii: 1m, Hawaii Co., Kilauea, Kipuka Puaula (Bird Park), 19.406267°N 155.2628333°W, on Osmanthus sandwicensis, 24 VI 1963 (I.W.B. Thornton), (BPBM). 1m, 1n, Hawaii Co., Kilauea, Kipuka Puaula (Bird Park), 1200m, on _Osmanthus sandwicensis_, 25 VI 1963 (I.W.B. Thornton). Hawaii Co., Kipuka Puaula, 1200m, on _Osmanthus sandwicensis and Charpentiera obovata_, 1 VI 1963 (I.W.B. Thornton).


_Ptycta pedina_


_Ptycta peleae_


_Ptycta perkinsi_

Table 2.1, cont.

_Ptycta persimilis_


_Ptycta pikeloi_


_Ptycta placophora_

Table 2.1, cont.


**Ptycta pupukea**


**Ptycta rhina**

**Holotype:** Hawaii: 1m, Honolulu Co., Oahu, Koolau Range, Kahauiki, 21.3794°N 157.84°W, on *Metrosideros*, 29 I 1933 (O.H. Swezey), (BPBM).

**Ptycta rhina symmetrica**

Table 2.1, cont.

**Ptycta simulator** (incl. *P. simulator kilauea*)


**Ptycta stena**


**Ptycta stenomedia**


**Ptycta swezeyi**

**Holotype:** Hawaii: 1m, Maui Co., Maui, Halelaau (Kaulalewelewe), 21.5214°N 158.0847°W, on *Metrosideros*, 19 XII 1928 (O. H. Swezey), (BPBM).
Table 2.1, cont.

*Ptycta sylvestris*


*Ptycta telma*

Table 2.1, cont.

*Ptycta unica*


**Ptycta vittipennis**


**Ptycta zimmermani**

Table 2.2. Species groups designated by Thornton (1984) and the current study. Underlined taxa are those collected for this study.

<table>
<thead>
<tr>
<th>Species groups, Thornton (1984)</th>
<th>Species groups proposed in current study</th>
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<tr>
<td>lanaiensis persclera</td>
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Table 2.2, cont.

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- gynegonia
- kauaiensis
- monticola
- persimilis
- placophora
- telma
- zimmermani

**haleakalae group**
- haleakalae
- haleakalae hualalai
- haleakalae konae
- hawaiensis
- microctena
- microglena
- palikea
- simulator simulator
- simulator kilaeua
- stena
- stenomedia
- unica

**kauaiensis group**
- gynegonia
- kauaiensis (incl. diastema, persimilis, zimmermani)
- monticola
- placophora
- telma
- 

**haleakalae group**
- haleakalae (incl. ssp. haleakalae, hualalai, haleakalae, konae)
- 

**oahuensis group**
- distinguenda
- leurothorax
- lobophora
- molokaiensis
- oahuensis
- vittipennis

**oahuensis group**
- distinguenda
- leurothorax
- lobophora
- molokaiensis
- oahuensis
- vittipennis
Chapter 3. The Importance of Molecular Dating Analyses for Inferring Hawaiian Biogeographic History: A Case Study with Bark Lice (Psocidae: Ptycta)

INTRODUCTION

The Hawaiian Islands have fostered some of the most spectacular species radiations on earth and have become a classic model for studies of biogeography and speciation. More than 9000 endemic species have been described from the islands, including more than 6000 insects. Hawaii is unique in being extremely isolated (4100 km from any land mass) and consisting of a series of islands that have arisen from the sea floor in linear chronological order. The ages of these young islands have been established through more than a century of geological research (reviewed in Carson and Clague 1995). Using the geological ages of the islands has allowed biogeographers to estimate the ages of endemic Hawaiian lineages. As early as the 1940s, biologists were using island ages to estimate the age of Hawaiian lineages (reviewed in Wagner and Funk 1995). These age estimates provide important insights on the timescales over which species radiations can occur.

The Hawaiian Islands are composed of a linear series of volcanoes that decrease in age from major islands in the northwest (oldest, Kauai, 5.1my) to southeast (youngest, Hawaii, 0.5my) (Carson and Clague 1995; Fig. 3.1). These islands have arisen as the earth’s crust moves over a volcanic hotspot in the Pacific Ocean. New islands are continually formed and older islands erode into low islands (less that 400 m above sea level), then atolls, and eventually become submerged seamounts. The Hawaii-Emperor Ridge extends far to the northwest of the current high islands, with the oldest of the seamounts dating to approximately 80 million years and lying nearly 7000 km northwest of the youngest island.
The island progression rule provides a biogeographic hypothesis for the temporal pattern of colonization of this linear chronologically arranged archipelago (Wagner and Funk 1995). An island version of Hennig’s (1966) ‘progression rule’, this hypothesis postulates centers of origin as regions in which species display plesiomorphic traits, with the youngest members of a monophyletic lineage on the geographic periphery, in this case the geologically younger islands. In phylogenetic studies of Hawaiian taxa, evidence for this pattern is found in lineages with their origin on the western islands and progressively younger species on eastern islands (i.e. oldest to youngest stepping stone). Many insect lineages appear to follow the progression rule to varying extents, including *Hyposmocoma* moths (350 spp.; Rubinoff 2008), and *Laupala* crickets (25 spp.; Mendelson et al. 2004).

In addition to predicting a pattern of inter-island radiation, the progression rule also allows for the possibility of intra-island speciation (Funk and Wagner 1995). Following an island colonization event, speciation can occur within islands. These speciation events can produce an abundance of single-island endemics, which are more closely related to each other than to species from neighboring islands (Roderick and Gillespie 1998). Intra-island radiations have been documented in several groups, including Succineidae snails (42 species, Holland and Cowie 2009), Platynini beetles (69 species; Cryan et al. 2001), *Tetragnatha* spiders (~60 spp.; Gillespie et al. 1997) and *Drosophila* pomace flies (~700 spp.; Kaneshiro et al. 1995), of which 83%, 97%, 100%, and ~94%, respectively, are single island endemics.

For intra-island radiation to occur, speciation depends on isolation of populations by either ecological specialization (adaptive radiation) or spatial division (non-adaptive radiation) (Roderick and Gillespie 1998). Adaptive radiation results in species with overlapping ranges, differential use of resources, and morphological variation that reflects differences in species
ecology, as seen in _Sarona_ plant bugs (40 spp.; Asquith 1995) and in the ecomorphs of _Tetragnatha_ spiders (Gillespie 2004). Non-adaptive radiation produces a pattern of non-overlapping range, little variation in niche use, and little morphological variation among species (Wagner and Funk 1995), as in generalist feeders such as _Prognathogryllus_ crickets (36 spp.; Shaw 1995).

If a lineage has strong dispersal capabilities, however, a distinct progression rule pattern may not be likely (Holland and Cowie 2009). Dispersal-driven speciation is most common among highly mobile insects that are able to move back and forth between islands (Funk and Wagner 1995). This generally results in few single-island endemics, as seen in _Hylaeus_ bees (60 spp.; Magnacca and Danforth 2006) and _Megalagrion_ damselflies (23 spp.; Jordan et al. 2003).

Many Hawaiian lineages have complex biogeographic histories that involve a combination of speciation by dispersal and intra-island radiation and most lineages follow the progression rule to some extent (reviewed in Holland and Cowie 2009). However most of these studies have examined the overall pattern of relationships among species on different islands, using island ages as the only indicator of lineage age. Colonization can occur much later than island formation and the use of island age to estimate maximum lineage ages can lead to overestimates of lineage age. To evaluate the relative contributions of a forward progression rule versus back colonization, the biogeographic pattern and timing of radiation must be considered, because different evolutionary processes can produce the same biogeographic patterns in some cases, once extinction is accounted for. The use of island chronology alone to estimate the timing of species radiations can potentially be misleading because the effects of extinction can be overlooked.
Large insect radiations make good systems for incorporating both biogeographic patterns and timing of Hawaiian radiations using tools of molecular systematics. Among the most diverse of Hawaiian insect lineages is the bark louse genus *Ptycta* (Psocoptera: Psocidae). Bark lice are small, fungus- and algae-feeding insects that are distributed worldwide and have undergone major radiations within Hawaii. The genus *Ptycta* contains more than 50 endemic Hawaiian species as currently described (Lienhard and Smithers 2000), 85% of which are endemic to a single island (Thornton 1984). Thus, bark louse genus *Ptycta* is one of the largest radiations of hemimetabolous insects yet documented on Hawaii. The Hawaiian *Ptycta* are an important component of the biota in the high elevation forests of the six main Hawaiian Islands and are often quite abundant locally. Their cryptic appearance, crevice-seeking behavior, and reluctance to fly, even when pursued (pers. obs.), suggest that these bark lice are not likely to fly great distances as a means of dispersal. *Ptycta* likely rely on passive dispersal on wind currents, as do many bark louse species (Thornton 1964; Thornton and Harrell 1965). Thornton (1984) proposed cladistics relationships among 11 species groups within the lineage based on 14 morphological characters and concluded that the radiation of Hawaiian *Ptycta* follows the progression rule.

In the current study, I use phylogenetic techniques to investigate the biogeographic history of the Hawaiian *Ptycta* lineage. Using molecular and morphological data, I compare the hypotheses presented by the progression model (west-to-east island colonization) and the “reverse” progression model (east-to-west colonization), using both the pattern of species relationships and the inferred timing of speciation events from a molecular dating analysis.
MATERIALS AND METHODS

Taxon sampling

The genus *Ptycta* includes more than 170 species from all zoogeographic regions of the world, with the greatest diversity in subtropical and tropical regions of Africa (29 species), Asia (43), and the Pacific Islands (63) (Lienhard and Smithers 2002; Bess and Yoshizawa 2007). The genus was erected by Enderlein (1925) to include two Hawaiian species, the type species *Psocus haleakalae* Perkins, 1899, and *Psocus distinguendus* Perkins, 1899, and the Indonesian species *Clematostigma schillei* Enderlein, 1906. Thornton (1984) described many additional species of Hawaiian *Ptycta*, for a total of 51 Hawaiian species and 10 subspecies. A total of 38 of these he reported to be single-island endemics.

I collected bark lice from the Hawaiian Islands of Kauai, Oahu, Lanai, Molokai, Maui, and Hawaii between 2006-2008. Collecting effort focused on (1) type localities of the 61 species and subspecies of Hawaiian *Ptycta* (Thornton 1984) and (2) sampling from the widest possible diversity of habitats and elevation gradients. A total of 316 collections were made at approximately 300 sites, yielding more than 7000 adult specimens of the genus *Ptycta* (Table 3.1). Species of *Ptycta* from non-Hawaiian locations were used as outgroups in phylogentic study. A total of 101 individuals of Hawaiian *Ptycta* and 18 outgroup species (9 *Ptycta*, 9 other genera) were included in this analysis. Hawaiian specimens were chosen to represent each “morphospecies” designated by external morphology; 1 or 2 representatives of each morphospecies was included in the phylogeny. Morphospecies were designated for males from each island independently, by creating abbreviated descriptions of insects with unique characters of the genitalia, wings, head, coloration, and body size. Species of *Ptycta* in the outgroup were sampled from the widest possible diversity of geographic localities of available specimens for
which all four genes used in the study were successfully sequenced, including Fiji, French Polynesia, Japan, Australia, and Peru. Species from the closely related genera *Camelopsocus*, *Indiopsocus*, and *Kaindipsocus* were also included in the outgroup (Table 3.2).

Specimens of Hawaiian *Ptycta* were collected by beating vegetation and removing bark lice from the beating sheet with an aspirator. Samples were preserved in 100% ethanol (original concentration) and transported to the Illinois Natural History Survey for taxonomic and molecular work. Outgroup specimens were collected in the same manner by the author and collaborators, except those from Fiji, which were collected in malaise traps as part of the Fiji Terrestrial Arthropod Survey (Evenhuis and Bickel 2005).

**DNA extraction, amplification, and sequencing**

Recently collected specimens were used for DNA extraction. Total genomic DNA was extracted from a separated abdomen of an adult specimen using a Qiagen DNAeasy Tissue Extraction kit. Extraction was completed following the manufacturer’s instructions, with an extended incubation of 36-48 hours. After extraction, the abdomen was stored in 100% ethanol in a -4°C freezer along with the remainder of the body. Slide mounts of the right forewing, hind wing, hind leg, and antenna were prepared for morphological study. Voucher specimens will be deposited at the Bishop Museum and the Illinois Natural History Survey insect collection.

Four gene regions, three mitochondrial (mt) and one nuclear, were amplified using PCR (Table 3.3). The mt genes consisted of a 513 aligned basepair (bp) fragment of 16S rDNA, a 368 bp region of 12S, and a 384 bp region of cytochrome oxidase I (COI). The nuclear gene wingless (wg) consisted of a 317 bp region. Primers and PCR procedures followed Yoshizawa 2004 (12S, 16S, COI) and Yoshizawa and Johnson 2004 (wg). Sequencing reactions were
performed using the BigDye kit (Applied Biosystems), following the manufacturer’s instructions. The same primers were used for both amplification and sequencing. Sequencing reactions were run on an ABI 3730XL capillary machine at the University of Illinois Keck Center for Comparative and Functional Genomics. Fragments were sequenced in both forward and reverse directions and assembled using Sequencher 4.9 (Gene Codes Corporation 2009). Sequence quality was verified by visual inspection of the chromatograms. The sequences have been deposited in GenBank (Table 3.2).

**Sequence alignment and phylogenetic analysis**

Sequences for the four gene regions were aligned by eye based on amino acid and nucleotide sequences in Sequencher. A few short regions of 12S and COI (15-30 bp) that were difficult to align were realigned using Seaview alignment software (Gout et al. 2010) and the Muscle alignment algorithm (Edgar 2004). Phylogenetic analysis included a total of 1582 aligned base pairs for 101 Hawaiian ingroup specimens and 18 outgroup specimens (Table 3.3).

To compare pairwise homogeneity of each gene region, as well that of mitochondrial versus nuclear regions, the partition homogeneity test (1000 replicates) (Farris et al. 1994, 1995) was performed using PAUP* version 4.0a109 (Swofford 2002). To compare relative rates of substitution, uncorrected pairwise distances were calculated using PAUP* and plotted for each pair of genes. The phylogenetic signal in each gene partition was compared using a set of trees estimated separately for each gene, including 50% Maximum Parsimony (MP) bootstrap consensus trees in PAUP*and Bayesian MCMC analyses using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).
Partition homogeneity tests (Farris et al. 1994, 1995) did not detect significant heterogeneity between gene regions, and thus phylogenetic estimates were based on combined gene regions (see results). Phylogenies were constructed for three data sets: wg alone (317 bp), COI + 12S + 16S concatenated (1265 bp) and all 4 genes combined (1582 bp). For each data set, MP analyses were performed in PAUP*, ML analyses in Garli 0.916b (Zwickl 2006), and Bayesian MCMC using MrBayes. For MP analysis, all data were weighted equally, and TBR branch swapping was performed with 100 random-addition replicates. Parameters for ML and Bayesian analysis were selected using Likelihood Ratio Tests in PAUP* by comparing log likelihood ($lnL$) scores under the most complex parameters (the GTR + I + G model) to increasingly simpler models. The simplest model that returned an $lnL$ score that was not significantly different from the GTR + I + G model was selected; in this case, the GTR + G model was used for all analyses. For ML analyses, 10 independent runs were performed in GARLI, using default settings and the automated stopping criterion, terminating the search when the $lnL$ score remained constant for 50,000 consecutive generations. The highest likelihood of those runs was retained and is presented here. ML bootstrap analyses used GARLI default settings with 400 bootstrap replicates. Bayesian analyses for each data set included two parallel analyses with four chains for 2,000,000 generations, and a tree was sampled every 1000 generations. The first 400 trees were excluded as burnin, and a 50% majority consensus tree of the remaining trees was calculated to determine the posterior probabilities of branches in the tree.

**Molecular Dating**

BEAST 1.5.1 (Drummond and Rambaut 2007) was used to run a relaxed clock uncorrelated lognormal model (Drummond et al. 2006) with the four-gene data set. Parameters used were a GTR model of evolution and a birth-death speciation process for 50,000,000
generations saving trees every 1000 generations. The phylogenetic tree contained several examples of within-island radiations on the youngest islands (Maui Nui and Hawaii), with a close relationship between radiations on these islands (see Results). Thus, calibration points were placed on nodes at the base of these subclades that had radiated on the youngest islands in the archipelago (Fig. 3.3). For these dates, it is assumed that a lineage cannot radiate on an island until that island has been formed, so these are used as maximum ages for these within island radiations. To reflect the mean age of the islands included in each clade, calibration nodes A1, B1, and B3 were set to a maximum age of 0.4my (the age of Hawaii Island); A2 and B2 were set to 1.749my (mean age of Maui Nui Islands; Fig. 3.3). Each node was set to a lognormal prior with a standard deviation of 0.01. Final output was examined visually in Tracer 1.4.1 (Rambaut and Drummond 2007) to determine if the run had reached stationarity after 5,000 samples were removed as burnin.

RESULTS

Data evaluation

For the four gene regions used in this study, pairwise divergence among the Hawaiian species varied from a maximum of 6.26% in 16S to 19.56% in wingless (Table 3.3). Plots of uncorrected pairwise distances of COI against those for 16S (the least divergent gene) reveal considerable multiple substitution in the COI gene (Fig. 3.2-a). In these plots, COI divergences leveled off at around 15%. In contrast, such multiple substitution was not as prevalent for other gene regions. When plotted against 16S, the divergence of 12S and wg gene regions continued to increase with increasing 16S divergence, although the slopes slightly decrease at the far right of the graph (Fig. 3.2-e). The mitochondrial genes 12S and 16S appeared to be similar in the rate
of accumulation of substitutions, although 16S tended to have a lower divergence than did 12S  
(Fig. 3.2-c). The nuclear protein coding gene wg displayed two clusters of divergence values,  
one ranging from about 0-9% and another from about 13-24%; all taxon pairs that exhibited wg  
divergence between 9-13% are in the outgroup. The partition homogeneity test did not detect  
significant heterogeneity among gene regions.

Those species with multiple representatives in the phylogeny showed variation in COI as  
high as 2.79% in Ptycta telma. Most other distance values for COI were under 2%, including P.  
perkinsi (1.83%), P. diadela (0%), P. diastema (1.04%), and P. frogneri (2.09%).

**Phylogenetic Results**

Phylogenetic analyses identified two major well-supported clades of Hawaiian Ptycta  
(Fig. 3.3). Trees estimated from the 4 gene data set (1582 bp) and the concatenated  
mitochondrial genes (1265 bp) data set showed strong support for both clades A and B.  
Combining the 4 genes improved resolution of terminal relationships and increased support for  
most clades, both deep and shallow (support values presented in Fig. 3.3).

The monophyly of Hawaiian taxa was supported in all analyses of the 4-gene dataset  
(Fig. 3.3) and the 3-gene mitochondrial data (trees not shown). ML bootstrap and Bayesian  
analyses of single-gene data sets varied in support of Clades A and B and no single gene offered  
strong support for the monophyly of Hawaiian Ptycta (trees not shown). In analyses of 12S and  
wg, both Clades A and B had strong support, but the relationships among the Clades A, B, and  
the Asian/Pacific taxa were ambiguous. 16S resolved Clade B only. COI resolved some of the  
larger subclades, including the subclades at node A1, B3, and the larger of the two Kauai  
subclades, but did not resolve any relationships as deep as the major Hawaiian clades.
Within the monophyletic Hawaiian lineage resolved by the 4-gene dataset, species from the youngest islands are widespread in the tree, whereas those from the older islands of Kauai and Oahu are almost exclusively grouped together. Within Clade A, the strongly-supported subclades that contain all of the species from Kauai and Oahu (Fig. 3.3) have somewhat ambiguous relationships, since internal branch support is weak for each of them. Clade B includes only 2 Oahu species nested within a clade of Maui and Big Island taxa.

**Molecular Dating**

The time-calibrated phylogeny produced in BEAST (Fig. 3.4) dated the beginning of the radiation of Hawaiian Ptycta at 5.06my, a date that corresponds closely with the age of Kauai (5.1my). The dates on Clade A (2.69my; 1.39 – 3.98my 95% CI) and Clade B (2.88my; 1.88-4.15my 95% CI) are statistically identical and correspond with an origin slightly after the formation of Oahu (3.7my).

**DISCUSSION**

A combined analysis of one nuclear and three mitochondrial gene regions recovered monophyly of the Hawaiian Ptycta. This radiation is split into two strongly supported clades (Figure U). One clade is present on all four of the main Hawaiian Island groups; the other is present on only the three youngest island groups and particularly diverse on Maui Nui and the Big Island of Hawaii. Phylogenetic analysis of the four-gene data set shows strong support for the monophyly of Hawaiian Ptycta and for both Clades A and B. Morphologically, Clades A and B differ in characters of the male genitalia, primarily in the structure of the phallosome and
Species in Clade B have more complex structures than those in Clade A; Thornton (1984) considered the simpler structures of Clade B to be plesiomorphic.

Molecular dating indicates that the two clades diverged about 5mya, an age that corresponds closely with the age of Kauai (5.1my), the western-most and oldest of the current Hawaiian Islands. The two independent radiations of Clades A and B are dated at similar ages, about 2.75 million years.

Within the two Hawaiian clades, most subclades are limited to a single island or two neighboring islands. This suggests that both inter-island dispersal and intra-island radiation are important in species diversification and that there has been repeated movement between islands. The many transitions between the Maui Nui Islands and the Big Island of Hawaii indicate that island proximity is an important factor in successful movement between islands. Within both Hawaiian clades, species from the Maui Nui islands are closely associated with both Big Island and Oahu species, suggesting that the three Maui Nui islands act as a single land mass. The Maui Nui islands split as the result of rising sea levels about 0.2mya (Carson and Clague 1995), and this young vicariance event would be difficult to resolve in the current phylogeny.

As predicted, the ages of the radiations within the younger islands are younger than those of the older islands (e.g. at nodes B1 and B3 in Fig. 3.3). Although the age of the entire Hawaiian radiation dates around 5mya, the age of the oldest island of Kauai, the lineages found on Kauai are estimated to be much younger, only 1.4my. This suggests that there may be unsampled Kauai lineages that are extinct. It is also possible that there are additional surviving Kauai island radiations that are unsampled. However, given the extensive collections made on Kauai (total of 3747 recent and 841 pre-2006 specimens of Ptycta; Table 3.3.1), if these lineages are still extant they are extremely rare. The large Kauai subclade in Clade A is fairly recent
(1.4 my), suggesting that one lineage on Kauai continued to diversify after others went extinct. This large and geographically widespread lineage may have outcompeted other Kauai lineages, leading to their extinction. Alternate, the date of this Kauai radiation and topology are also consistent with back-colonization of Kauai from Oahu with subsequent radiation on Kauai. A third explanation is that Kauai and Oahu were colonized by Ptycta from an ancient island to the west such as Nihoa (7.2 my old) or Necker Island (10.3 my old), both of which likely had habitat suitable for Ptycta 2.75 mya.

Simple parsimony reconstruction alone is not successful in identifying an island of origin for either Hawaiian clade. Clade B has species from the Big Island of Hawaii in a sister relationship to the rest of the clade, but the estimated date of this split of 2.41 my indicates that the divergence between Ptycta stenomedia and the remainder of Clade B predates the formation of the Big Island. This phylogenetic placement of P. stenomedia on the Big Island may indicate that it belongs to a lineage that is otherwise unsampled or extinct and that its presence on the Big Island is the result of a dispersal event from an older island.

From a simple phylogenetic biogeography perspective it might appear that the model of east-to-west reverse colonization has more support than the progression model in the phylogenetic tree. A model of east-to-west reverse colonization predicts that the ancestors of the Hawaiian lineage arrived on one of the youngest islands and dispersed westward. This model finds some support in the topology resolved by ML analysis (Fig. 3.3). In both Clades A and B, taxa from the youngest islands are at the base, sister to the other taxa (Clade A Maui, Lanai, and Big Island taxa; Clade B Ptycta stenomedia). Taxa from the youngest island are spread throughout the tree, suggesting multiple dispersal events from the Big Island westward. Taxa
from the oldest island of Kauai form a monophyletic clade embedded within Clade A, suggesting that they are the youngest members of the lineage.

However, molecular dating in the current phylogeny of Hawaiian Ptycta provides support for the progression model, which predicts advancement of a lineage from west to east as colonizing species move onto newly formed islands. Although the lineage includes two highly divergent clades with overlapping distributions on the Islands, molecular dating supports the establishment of Ptycta on the Hawaiian Islands at about 5mya, a time consistent with the formation of Kauai and prior to the existence of the younger islands. The two Hawaiian clades are dated at 2.7-2.8my, at which time Oahu was inhabitable. Thus, given that the Big Island of Hawaii and Maui-Nui had not emerged from the ocean at these dates, the ancestors of these lineages must have existed on the current older islands of Kauai and Oahu. The fact that there is a single Kauai lineage well embedded within other Hawaiian lineages suggests this may have been a single surviving lineage from a radiation that is now mostly extinct.

The topology of Clade A is also consistent with the progression model. For example, there are many diverse lineages from the Big Island of Hawaii and in nearly every case these are sister to or embedded within radiations on Maui Nui. These relationships of taxa on the youngest islands are consistent with the progression model (forward stepping stone). Within Clade A, the Kauai subclade is embedded with weak support and dated at 1.4my (Fig. 3.3). If the Kauai subclade were sister to the rest of Clade A, the clade would resemble the pattern predicted by the progression model. To test the support for the position of the Kauai subclade within Clade A, I used the SH test (Shimodaira and Hasegawa 1999) to compare the topologies of the current tree and one on which the Kauai subclade was constrained as sister to the remainder of Clade A. The SH test indicated that the constrained topology is not significantly
different from the topology resolved in phylogenetic analysis ($p=0.15$). Thus, in the absence of molecular dating analysis, the progression (oldest to youngest) model hypothesis cannot be rejected for Clade A.

The current phylogenies indicate that the closest relatives of the Hawaiian *Ptycta* are in the East Asia and the Pacific Islands, suggesting that this is a likely origin of the Hawaiian colonists. The Pacific Island *Ptycta* form a well-supported clade that is sister to the Hawaiian species with the Australian *Ptycta* as sister to all other *Ptycta* in most phylogenies (Fig. 3.3). This reciprocally monophyletic relationship between Hawaiian and outgroup *Ptycta*, the divergence date of 7.5my, and the establishment of the Hawaiian group at 5mya suggest that the progression model may apply to this radiation.
LITERATURE CITED


Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.
TABLES AND FIGURES

Table 3.1. Adult *Ptecta* specimens available for Thornton’s 1984 revision and specimens collected for the current study between 2005-2008. Number of species designated by Thornton and by current study.

<table>
<thead>
<tr>
<th>Specimens Thorntan, 1984</th>
<th>Kauai</th>
<th>Oahu</th>
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<th>Molokai</th>
<th>Lanai</th>
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<td>378</td>
<td>1080</td>
<td>41</td>
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Table 3.2. Specimens used for this study. GenBank numbers to be included with published data.

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<td>28-Jul-07</td>
<td>E. Bess &amp; S. Cameron</td>
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<td>K. Johnson</td>
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<td>2-13 Aug</td>
<td>2005</td>
<td>M. Whiting</td>
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Table 3.2, cont.

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Table 3.3. Sequence length and uncorrected pairwise genetic divergence of each gene region.

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Figure 3.1. Map of the Hawaiian Islands with island ages. Color coding is used in phylogenies.
Figure 3.2. Plots of the uncorrected pairwise distances for four genes. Markers indicate if paired taxa are in the same clade (AxA or BxB), in different clades (AxB) or include outgroup taxa (Axl, Bxl, Ixl).
Figure 3.3. Phylogeny of Hawaiian Ptycta. Best Maximum Likelihood tree for the 4-gene data set. Branch support: MLBS/BayesPP/MPBS; x = <50% support; 100 (single) = 100% support in all analyses. Yellow circle denotes node used in molecular dating analysis. Islands coded by color: Kauai (green), Oahu (blue), Maui Nui (red; MA = Maui, MO = Molokai, LN = Lanai), Big Island (orange).
Fig 3.4. Time-calibrated phylogeny of the 4-gene data set constructed in BEAST using a relaxed clock uncorrelated lognormal model under the GTR model of evolution. Divergence times are in millions of years, with blue bars denoting the 95% posterior probability densities around point estimates.
Chapter 4. Kaindipsocinae is the sister taxon of the rest of Psocidae (Psocodea: 'Psocoptera')

Note: this is a version of an article accepted by Invertebrate Systematics: K. Yoshizawa, E.C. Bess, C.N. Smithers and K.P. Johnson. Kaindipsocinae is the sister taxon of the rest of Psocidae (Psocodea: 'Psocoptera'). Invertebrate Systematics, submitted January 2011, accepted April 2011.

This chapter was written by Dr. Kazunori Yoshizawa and Emilie Bess and illustrated by Dr. Yoshizawa, based on data collected by Emilie Bess and Dr. Yoshizawa with content input from Dr. Courtenay Smithers and Dr. Kevin Johnson.

Abstract

The systematic status of Kaindipsocinae (formerly Kaindipsocini) is revised based on morphology of the male terminalia and on molecular data. Clematostigma, Lasiopsocus, and Tanystigma are newly assigned to this subfamily, and a new tribe, Clematostigmini, is established for these genera. The Blaste lunulata species group is also placed within Kaindipsocinae and is probably closest to Kaindipsocus. Both morphological and molecular data provide strong support for monophyly of Kaindipsocinae and molecular data support a sister relationship between this subfamily and the rest of Psocidae.
INTRODUCTION

The family Psocidae is the most diverse family of the free-living members of Psocodea ("Psocoptera") (Lienhard & Smithers, 2002). Many different higher-level classification schemes have been proposed for this diverse family (see Yoshizawa & Johnson, 2008 for review), but the classification proposed by Mockford (1993) is now generally accepted (Lienhard & Smithers, 2002; Yoshizawa & Johnson, 2008). Differing from the earlier classifications, which placed greater importance on homoplastic wing vein characters, Mockford's system emphasized more phylogenetically relevant characters, such as male genitalic structures. However, Mockford's system was established based mainly on the Nearctic species and many genera from other regions were not assigned to subfamily or tribe at that time. Later, all unclassified genera of Psocidae were assigned to the subfamilies and tribes of Mockford's system (Lienhard & Smithers, 2002: Mockford, *in litt.* 2001).

One such genus is the enigmatic *Kaindipsocus* Smithers & Thornton, 1981. This genus was originally assigned to the subfamily Psocinae and its affinity with Amphigerontiinae was explicitly rejected (Smithers & Thornton, 1981). New (in New & Lienhard, 2007) accepted this taxonomic treatment and assigned the genus to the tribe Ptyctini of Psocinae. Previously, however, Lienhard & Smithers (2002) had assigned *Kaindipsocus* to the subfamily Amphigerontiinae without mentioning the basis for this placement. Later, the placement of *Kaindipsocus* in Amphigerontiinae was confirmed morphologically (Lienhard, 2008) and, based on molecular phylogenetic analyses, a unique tribal status within Amphigerontiinae was given to the genus (Yoshizawa & Johnson, 2008).

Problems remain with the systematic placement of Kaindipsocini, however. First, although the placement was not rejected statistically, results from the molecular phylogeny
suggested that the tribe does not form a monophyletic group with the rest of Amphigerontiinae (Yoshizawa & Johnson, 2008). Kaindipsocini may represent the most basal divergence event within Psocidae. Thus, the tribe occupies a very important systematic position in understanding the origin, evolution, and biogeography of the family Psocidae. Second, although the tribe is currently represented by a single genus, additional genera may also belong to this tribe.

*Kaindipsocus* has its center of diversity in the Australian region (Lienhard, 2008), and the higher level classification of psocid genera of this region is poorly established. For example, the genus *Lasiopsocus* Enderlein, 1907 of the subfamily Amphigerontiinae is nearly endemic to Australia, but its placement within the subfamily has not been tested (Li, 2002; Yoshizawa & Johnson, 2008). The genera *Clematostigma* Enderlein, 1906 and *Tanystigma* Smithers, 1983 are nearly endemic to the Australian region, as well. Both genera are now only tentatively assigned to the tribe Ptyctini of the subfamily Psocinae, without a detailed examination of their morphological characters (Lienhard & Smithers, 2002: "Assigned to Ptyctini (for present): Mockford, in litt. 2001"). Given their unique distributional pattern, these two genera may share a close affinity with Kaindipsocini.

In this study, we estimate the systematic placements of these Australian psocids based on a highly informative character system, morphology of the male terminalia. We also evaluate the systematic placement of these Australian psocids with molecular data in a combined analysis of nuclear 18S rDNA, Histone 3 and Wingless and mitochondrial 12S rDNA, 16S rDNA and COI.

**MATERIALS AND METHODS**

Taxa examined are listed in Table 4.1. Specimens stored in either 80% or 99% ethanol were used. For specimens stored in 80% ethanol, the abdomen was removed and soaked in 10%
KOH at room temperature for one night before morphological observation. For those stored in 99% ethanol, the abdomen was placed in Proteinase K solution from a Qiagen DNeasy Tissue Kit for both DNA extraction and to clear the tissues for morphological observation. See Yoshizawa & Johnson (2008) for further procedures for preparation of DNA data and Yoshizawa (2005) for methods of morphological observation, illustration, and terminology.

Using the sequences listed in Table 4.2, we performed maximum parsimony (MP) and maximum likelihood (ML) analyses using the portable version of PAUP* 4b10 (Swofford, 2002) and Bayesian MCMC using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). For MP analysis, all data were weighted equally, and TBR branch swapping was performed with 100 random-addition replicates. For ML analyses, TBR branch swapping was performed using the equally parsimonious trees obtained from the MP analysis as starting trees. Parameters for ML analysis were estimated using Modeltest 3.7 (Posada & Crandall, 1998) on the basis of Akaike information criterion (Akaike, 1974). As a result of Modeltest, the GTR+I+G model was selected (unequal base frequencies: A = 0.3232, C = 0.1529, G = 0.1826, T = 0.3413; six substitution categories: A-C = 1.4006, A-G = 4.9710, A-T = 2.9758, C-G = 1.3901, C-T = 8.3099, G-T = 1; gamma distributions shape parameter = 0.5856 based on four rate categories; proportion of invariant sites = 0.5478). Bootstrap support was calculated using 100 replicates with TBR branch swapping, but TBR rearrangement was limited to 3,000 for ML bootstrapping because full TBR rearrangements were unacceptably time consuming. We also applied a constraint strategy to expand tree search space (Yoshizawa & Johnson, 2008). Modeltest-estimated parameters were also adopted for ML bootstrapping. The confidence in monophyly of Amphigerontiinae was also tested using the approximately unbiased test (AU test: Shimodaira, 2002) using CONSEL 0.1h (Shimodaira & Hasegawa, 2001). For Bayesian analyses, we performed two runs each with four
chains for 2,000,000 generations, and trees were sampled every 1000 generations. The first 200 trees were excluded as burnin, and we compared a 50% majority consensus tree of the remaining trees to estimate posterior probabilities of branches in the tree.

RESULTS

*Morphology of the male terminalia*

"*Blaste* lunulata* species group*

Eighth sternum (Fig. 4.1B) with weak but broad sclerotization fused to hypandrium posteriorly. Posterodorsal margin of clunium (Figs. 4.1B, 4.2B) weakly extended posteriorly, with epiproct articulated at posterior margin; posterolateral margin without extension (Fig. 4.1B). Epiproct (Figs. 4.1B, 4.3A) with well-developed single lobe extended from anterior margin. Hypandrium (Fig. 4.1B) fused to clunium laterally, rounded posteriorly, with pair of lateral, incurved, posteriorly projecting processes. Phallosome (Fig. 4.4B) opened posteriorly; phallobase V-shaped, with elongated anterior apodeme, and sometimes with long sclerotized rods laterally arising from base of anterior apodeme and extended posteriorly (erroneously interpreted as "outer paramere" by New, 1974, Smithers, 1984 and Schmidt & Thornton, 1993); paramere ("inner paramere" of the above authors) articulated with phallobase anteriorly, almost straight, and pointed apically.

*Kaindipsocus*

See Lienhard (2008) for illustrations. Eighth sternum with weak but broad sclerotization fused to hypandrium posteriorly. Posterodorsal margin of clunium weakly extended posteriorly
with epiproct articulated at posterior margin; posterolateral margin without extension. Epiproct with well developed single lobe arising from anterior margin. Hypandrium fused to clunium laterally. Phallosome opened posteriorly. Phallobase V- or U-shaped, with short anterior apodeme, paramere articulated with phallobase, strongly curved outwardly, pointed apically.

*Tanystigma*

Eighth sternum (Fig. 4.1CD) without sclerotization. Posterodorsal margin of clunium (Figs. 4.1CD, 4.2CD) with weak extension, with epiproct articulated at posterior margin; posterolateral margin with (Fig. 4.1D) or without (Fig. 4.1C) posterior extension. Epiproct (Fig. 4.3BC) with pair of well developed lobes anterolaterally, their anterior surfaces membranous, and with less- to well-developed sclerotized lobe medially. Hypandrium (Fig. 4.1CD) articulated with clunium. Phallosome (Fig. 4.4CD) opened posteriorly; phallobase V- or U-shaped, without conspicuous anterior apodeme; paramere articulated with phallobase, almost straight or slightly curved outwardly, and pointed or bifurcated apically.

*Lasiopsocus*

Eighth sternum (Fig. 4.1E) with weak sclerotization fused to hypandrium posteriorly. Posterodorsal margin of clunium (Figs. 4.1E, 4.2E) with flap-like extension strongly extended posteriorly, with epiproct articulated at posterior margin; posterolateral margin with strong posterior extension (Fig. 4.1E). Epiproct (Fig. 4.3D) with pair of well-developed lobes anterolaterally, their anterior surfaces membranous. Hypandrium (Fig. 4.1E) articulated with clunium. Phallosome (Fig. 4.4E) opened posteriorly; phallobase V-shaped, with short anterior apodeme; paramere articulated with phallobase, short, directed outwardly and bifurcated apically.
**Clematostigma**

Eighth sternum (Fig. 4.1F) with weak but broad sclerotization, fused to hypandrium posteriorly and to clunium laterally. Posterodorsal margin of clunium (Figs. 4.1F, 4.2F) with flap-like extension strongly extended posteriorly, with epiproct articulated at posterior margin; posterolateral margin (Fig. 4.1F) with strong posterior extension. Epiproct (Fig. 4.3E) with pair of well-developed lobes anterolaterally, their anterior surfaces membranous. Hypandrium (Fig. 4.1F) articulated with clunium. Phallosome (Fig. 4.4F) closed posteriorly; phallobase U-shaped, without conspicuous anterior apodeme; paramere articulated with phallobase, very long, curved, and bifurcated apically.

**Molecular phylogeny**

The MP, ML, and Bayesian trees recovered from the present analyses were highly congruent with each other (available online) and with the trees estimated previously (Yoshizawa & Johnson, 2008). Fig. 4.5 shows the ML tree with branch support values obtained from bootstrapping (BP: MP and ML) and Bayesian MCMC (PP). Here, we primarily discuss the position of taxa added to the analyses of the previous study (Yoshizawa & Johnson, 2008).

Representatives of the "Blaste" lunulata group, Kaindipsocus, Tanystigma and Clematostigma composed a clade with weak to strong statistical support (100% PP, 35% ML-BP and 62% MP-BP). The support value for this clade from the ML analysis was extremely low in comparison to those from the MP and Bayesian analyses, but this is likely due to missing data in a sample and the tree searching strategy. The ML analysis is very time consuming, and a NJ tree is employed here as a starting tree for each bootstrap replicate. Also, 100 replicates of full TBR
was too time consuming and thus a rearrangement limit of 3000 was used for each bootstrap replicate. Such limited searching strategies worked well for the relatively complete data set (Yoshizawa & Johnson, 2008). However, a newly added taxon, *Tanystigma* sp. 2, included only 2 of the 6 genes used in phylogenetic analysis (see appendix 2), and there are no data for comparing this sample and *Atlantopsocus personatus, Oreopsocus buholzeri, Kaindipsocus* sp. KY379 and *Clematostigma* sp. KY418 (see Appendix 2 and Yoshizawa & Johnson, 2008). Therefore, placement of this sample within the initial NJ tree could not be calculated correctly, which had the effect of destabilizing the ML bootstrap analysis. By excluding this sample from the ML bootstrapping, monophyly of *lunulata* group + *Kaindipsocus + Tanystigma + Clematostigma* received very strong ML-BP support (91%). Exclusion of *Tanystigma* sp. 2 also improved support values for *Kaindipsocus + lunulata* group (43 -> 51% ML-BP) and *Clematostigma + Tanystigma* (39 -> 99% ML-BP). The *lunulata* group + *Kaindipsocus + Tanystigma + Clematostigma* clade was sister to the remainder of the family Psocidae, and monophyly of the remainder of Psocidae received strong support from the Bayesian analysis (100% PP) but was weakly supported by MP and ML analyses (<50% BP). Monophyly of Amphigerontiinae including Kaindipsocini was never recovered, but results from the AU test did not reject the possibility (P=0.108 by full data set and 0.154 by excluding *Tanystigma* sp. 2).

Within the *lunulata* group + *Kaindipsocus + Tanystigma + Clematostigma* clade, *Tanystigma* and *Clematostigma* composed a clade with strong statistical support (93% MP-BP, 39% full ML-BP, 99% ML-BP ex. *Tanystigma* sp. 2, 100% PP). ML and Bayesian trees both supported monophyly of "*Baste* lunulata group + *Kaindipsocus," but statistical support was weak (at most 54% BP and 78% PP).
DISCUSSION

The molecular phylogeny strongly supports the clade composed of the "Blaste" lunulata group, Kaindipsocus, Tanystigma and Clematostigma (termed Kaindipsocini sensu Yoshizawa & Johnson, 2008 in the following discussion). This clade is further divided into two subclades: lunulata group + Kaindipsocus and Tanystigma + Clematostigma. Although a morphology-based cladistic analysis was not performed, the molecular tree and past morphological analyses (Lienhard, 2008; Yoshizawa, 2002, 2005; Yoshizawa & Johnson, 2008) allow us to evaluate morphological apomorphies supporting this result.

The Kaindipsocini are characterized by the following features: 1) posterodorsal margin of clunium with posterior extension at which epiproct is articulated (Figs. 4.1B-F, 4.2B-F); 2) male epiproct with well developed lobes anteriorly (Fig. 4.3); 3) parameres articulated with the phallobase (Fig. 4.4B-F). The state of Character 1 apparently represents a derived condition, as it is unique to Kaindipsocini among the Psocetae (= infraorder including Psocidae). A similar posterodorsal extension of the clunium is also observed in some Psocidae including all members of Psocini, Atrichadenotecini, Metylophorini, and Thyrsophorini, some species of Indiopsocus and Trichadenotecnum of Ptyctini, and Glossoblaste amamiensis of Amphigerontiiinae (Figs. 4.1A, 4.2A). However, in all these latter cases, the clunial extension always extends over the epiproct (Fig. 4.2A) and thus these structures are not directly articulated with each other, which clearly differs from the clunial extensions of Kaindipsocini. Therefore, character 1 provides unambiguous morphological support for the monophyly of Kaindipsocini. The state of Character 2 is also considered to be apomorphic (e.g., Mockford, 1993), but similar conditions evolved many times independently and several reversals are also evident (Yoshizawa & Lienhard, 2004). In addition, the shape of the epiproct lobe is significantly different between two subclades of
Kaindipsocini (Fig. 4.3A vs. B-E). Therefore, character 2 provides only ancillary support for Kaindipsocini. Character state 3 probably represents a plesiomorphy (see below).

The "Blaste" lunulata group and Kaindipsocus share an apomorphy: the single tongue-shaped epiproct lobe strongly extended dorsally (Figs. 4.1B, 4.2B, 4.3A: Lienhard, 2008). The hypandrium of this group is fused to the clunium (Fig. 4.1B). The presence of clunial-hypandrial articulation is likely the ground plan condition for Psocidae (Fig. 4.1A, C-F: Yoshizawa, 2002), and a similar condition is also widely observed in Myopsocidae (Lienhard, 2004; Yoshizawa, personal observation). Therefore, the clunial-hypandrial fusion of the lunulata group and Kaindipsocus can be regarded as a synapomorphy. In addition to the male terminal characters, these two groups share an apomorphic character of stalked-eyes (Smithers & Thornton, 1981; Smithers, 1984; Schmidt & Thornton, 1993; Lienhard, 2008; Bess & Yoshizawa, present observation)

Monophyly of Tanystigma + Clematostigma is supported strongly by molecular data. Lasiopsocus, which was not included in the molecular analysis, shares a morphological apomorphy with these two genera: the epiproct with a pair of anterolateral lobes with their anterior surfaces membranous (Fig. 4.3B-E). Lasiopsocus also shares the above-mentioned morphological apomorphies of Kaindipsocini. Similar paired epiproct lobes are also observed in some species of Trichadenotecnum but, in all cases, the paired lobes are well sclerotized and are developed as accessory lobes of the main epiproct lobe (e.g., T. auritum Yoshizawa & Lienhard, 2004 and T. barrerai Yoshizawa, Garcia-Aldrete & Mockford, 2008). Trichadenotecnum is also phylogenetically distant from Kaindipsocini (Fig. 4.5), and the lack of homology of these features is obvious. Therefore, the paired and well-developed epiproct lobe is a prominent autapomorphy of the Tanystigma + Lasiopsocus + Clematostigma subclade.
Within this subclade, the following morphological features support the close relationship of *Lasiopsocus* and *Clematostigma*: 1) posterodorsal extension of the clunium well developed (Figs. 4.1EF, 4.2EF); 2) posterolateral margin of the clunium with posterior extension (Fig. 4.1EF); 3) paramere bifurcated apically (Fig. 4.3EF). Among them, Characters 2 and 3 are very prominent and apparently autapomorphic character states. However, these states are also observed in at least one species of *Tanystigma* (e.g., *T. latimentula* examined here: Fig. 4.1D for the clunial extension; Fig. 4.4D for the bifurcated paramere). The genus *Tanystigma* is characterized by the shallow pterostigma in the forewing, but such a wing venational character is also observed in other genera of Psocidae (e.g., *Camelopsocus* of Ptyctini). Therefore, *Tanystigma* is possibly paraphyletic. Character 1 is a quantitative character that requires careful observation, but the difference in this character between *Tanystigma* and *Lasyopsocus + Clematostigma* is obvious (Fig. 4.2CD vs. EF). Therefore, Character 1 provides additional support for the latter clade.

The most important finding of the present analyses concerns the sister relationship of Kaindsayscocini with the remainder of Psocidae. Based on the detailed analysis of a species of *Kaindsayscocus*, Lienhard (2008) concluded that the genus belongs to the subfamily Amphigerontiinae. The broadly sclerotized 8th sternum was considered to be the most important synapomorphy between *Kaindsayscocus* and other genera of Amphigerontiinae. However, *Tanystigma* lacks sclerotization on the 8th sternum (Fig. 4.1CD), and sclerotization on the 8th sternum of the *lunulata* group and other genera of Kaindsayscocini is much less developed compared to other Amphigerontiinae. For example, lateral margins of the 8th sternum always overlap the clunium in other Amphigerontiinae (Yoshizawa, 2010: Fig. 4.1A). This condition was never observed in Kaindsaysonini (Fig. 4.1B-F) including *K. splendidus* Lienhard, 2008, on
which interpretation by Lienhard (2008) was based. The 8th sternum functions as an attachment of the retractor muscles of the phallosome (Badonnel, 1934) and sclerotization of the 8th sternum has evolved many times independently in Psocidae, probably associated with function of the phallosome. For example, a broadly sclerotized 8th sternum fused to the hypandrium posteriorly evolved at least three times independently within a single genus, *Trichadenotecnum* (Yoshizawa et al., 2008). Therefore, this character state only provides weak evidence for Kaindipsocini + other Amphigerontiinae. Lienhard (2008) also pointed out the posteriorly opened phallosome as an additional shared character between *Kaindipsocus* and other genera of Amphigerontiinae. However, the phallosome of *Clematostigma* is closed posteriorly (Fig. 4.4F), which indicates that this character state is inconsistent within Kaindipsocini. Molecular data fail to support monophyly of Kaindipsocini + other Amphigerontiinae. Monophyly of Amphigerontiinae including Kaindipsocini was not rejected by the AU test. However, a sister relationship between Kaindipsocini and the remainder of Psocidae received very strong support in Bayesian analysis (100% PP), that is robust within a variety of taxon sampling schemes (Yoshizawa & Johnson, 2008). Therefore, we conclude that subfamilial status (i.e. Kaindipsocinae) should be given to this group to clarify its significant morphological differences from the other Amphigerontiinae, and also to indicate its distinctiveness from the rest of Psocidae.

Is there any morphological evidence supporting this basal split between Kaindipsocinae and the rest of Psocidae? This is a very difficult question to answer, and more extensive and detailed morphological analysis is needed. However, the phallosomal character (Fig. 4.4: listed as Character 3 of Kaindipsocini above) may provide support for this divergence. In all species of Kaindipsocinae, the parameres are articulated basally with the phallobase (Fig. 4.4B-F). This represents the ground plan condition of Psocodea (Yoshizawa & Johnson, 2006). In the rest of
Psocidae, the parameres are either fused to the phallobase (Fig. 4.1A) or absent (Yoshizawa, 2003, 2005, 2010) which suggests that the articulated condition as observed in Kaindipsocinae represents a plesiomorphy and thus supports their exclusion from the rest of Psocidae. However, interpretation of this character state is not straightforward, because the parameres of many psocomorphan families are fused to the phallobase (Yoshizawa, 2005). The infraorder Epipsocetae often is placed as sister to Psocetae in molecular phylogenies (Johnson et al., 2004; Yoshizawa & Johnson, 2010), and movable parameres are retained in some groups of Epipsocetae (Casasola-González & García-Aldrete, 2002; Yoshizawa personal observation). However, the phylogenetic placement of Psocetae is far from stable. Further detailed study of Psocidae and the establishment of a stable higher level classification of Psocomorpha are critical to understanding the origin and diversification of the family.

In conclusion, based on the present morphological and molecular analyses, the classification scheme as shown in Table 4.3 and Fig. 4.4.5 is proposed here for the family Psocidae. It is evident from the present study that an independent genus should be established for the "Blaste" lunulata group. However, we postpone this action for two reasons. First, we only examined a single undescribed species of the group in this study. Second, judging from the literature, other Australian "Blaste" are also quite distinctive from the "typical" members of the genus (e.g., New, 1974; Smithers, 1984), and an official nomenclatural act should also consider those heterogeneous species.

ACKNOWLEDGMENTS

We thank Stephen Cameron and David Morris for offering DNA samples and David Britton for loan of specimens stored in the Australian Museum. This paper was partly supported
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LITERATURE CITED


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Table 4.1. Specimens examined

"Blaste" sp. (lunulata species group): morphology & molecular

*Clematostigma maculiceps* (Enderlein, 1903): morphology

*Clematostigma* sp. KY418 (Brisbane, Australia): morphology & molecular

*Kaindipsocus splendidus* Lienhard, 2008 (= *Kaindipsocus* sp.: Yoshizawa & Johnson, 2008):
  morphology & molecular

*Kaindipsocus* sp. KY379 (Cameron Highland, Manalysis): molecular (available by females only)

*Lasiopsocus dicellyus* : morphology

*Tanystigma latimentula*: morphology

*Tanystigma* sp. 1: morphology & molecular

*Tanystigma* sp. 2: morphology & molecular
Table 4.2. GenBank accession numbers for sequence data taken from Kaindipsocinae (see Yoshizawa & Johnson, 2008 for other sequences). "—" indicates missing data. (*) indicates pending accession numbers: filled upon acceptance.

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Table 4.3. Higher level systematics of Psocidae newly proposed here. For tribes and genera of Amphigetontinae and Psocinae, see Yoshizawa & Johnson (2008)

Family Psocidae

Subfamily Kaindipsocinae

Tribe Kaindipsocini

"Blaste lunulata" species group

Kaindipsocus

Tribe Clematostigmini new

Tanystigma

Lasiopsocus

Clematostigma

Subfamily Amphigerontiinae

Subfamily Psocinae
Fig. 4.1. Male terminalia, lateral view. A. *Glossobaste amamiensis*, B. "Blaste" sp. (*lunulata* species group), C. *Tanystigma* sp. 2, D. *Tanystigma latimentula*, E. *Lasiopsocus dicellyus*, F. *Clematostigma* sp. KY379. Circles indicate the clunium-hypandrium fusion/articulation. An arrow in F indicates the clunium-8th sternum fusion which is not homologous with those indicated by circles.
Fig. 4.2. Male terminalia, dorsal view. A. *Glossoblaste amamiensis*, B. "Blaste" sp. (*lunulata* species group), C. *Tanystigma* sp. 2, D. *Tanystigma latimentula*, E. *Lasiopsocus dicellyus*, F. *Clematostigma* sp. KY379.
Fig. 4.3. Male epiproct, posterior view. A. "Blaste" sp. (lunulata species group), B. Tanystigma sp. 2, C. Tanystigma latimentula, D. Lasiopsocus dicellyus, E. Clematostigma sp. KY379.
Fig. 4.4. Phallosome, ventral view. A. *Glossoblaste amamiensis*, B. "Blaste" sp. (lunulata species group), C. *Tanystigma* sp. 2, D. *Tanystigma latimentula*, E. *Lasiopsocus dicellyus*, F. *Clematostigma* sp. KY379.
Fig. 4.5. The ML tree estimated from the data set including all taxa. Branch lengths are proportional to ML estimated branch lengths. The numbers above the branches are Bayesian posterior probability/ML bootstrap/MP bootstrap support values and those below the branches are ML bootstrap support from the data set excluding *Tanystigma* sp. 2. con indicates the constrained branches (see Materials and Methods).