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January 19, 2010

Katherine Kealoha, Esq.
Director
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235 South Beretania Street, Suite 702
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Dear Ms. Kealoha:

Subject: Finding of No Significant Impact (FONSI) for "Field Release of *Aroplectrus dimerus* for Biological Control of the Stinging Nettle Caterpillar (*Darna pallivitta*) in Hawaii"

The Hawaii Department of Agriculture has reviewed the draft EA. The draft EA was published in the Environmental Notice on April 23, 2008. A 30-day public comment period began on April 23, 2008, but no comments were received. The agency has determined that this project will not have significant environmental effects and has issued a FONSI.

We have enclosed a completed OEQC Publication Form and four copies of the final EA. Please call Dr. Neil Reimer at 973-9522, should you have any questions.

Sincerely,

Sandra Lee Kunimoto
Chairperson, Board of Agriculture

Field Release of *Aroplectrus dimerus* for Biological Control of the Stinging Nettle Caterpillar (*Darna pallivitta*) in Hawaii

Final Environmental Assessment
January 2010

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I. Project Description, Purpose, and Need

A. Description of proposed action:

The Hawaii Department of Agriculture (HDOA) proposes to introduce the natural enemy *Aroplectrus dimerus* into Hawaii as a biological control agent of *Darna pallivitta*, the nettle caterpillar (NC). Host specificity studies have been completed in the HDOA Insect Quarantine Facility and *A. dimerus* was found to attack only *D. pallivitta* and not any species of non-target insects tested. The release of this natural enemy is expected to result in long-term control of this invasive pest. The nettle caterpillar is a threat due to its stinging spines and appetite for a wide range of plants. The objective of this project is for the released natural enemy to reduce the *D. pallivitta* populations to low levels so that they are no longer a significant problem to human health and no longer an economic pest of plants.

B. Background information on the pest to be controlled:

Classification of target (host) organism

The pest species

Darna pallivitta is a new immigrant pest in Hawaii that was first found during September 2001 at a plant nursery on the east side of the Big Island (island of Hawaii). It was suspected of having entered the state on *Rhapis* palm seedlings imported from Taiwan. Since that time, it has become widespread on the Big Island. Populations have subsequently been discovered on the islands of Oahu and Maui during 2007. The moth dispersed to these islands through the inter-island movement of infested plants.

Darna pallivitta naturally occurs in China, Taiwan, Thailand, western Malaysia, Indonesia, and Java. Its host plants in these regions include *Adenostemma* sp., *Areca* sp., *Breynia* sp., coconut, *Ficus* sp., grasses, maize, and oil palm. It is considered only a minor pest of coconut palms in its natural range, probably due to the presence of natural enemies that do not occur in Hawaii (Holloway et al. 1987).

Life history of the target organism

Chayopas (1982) (unpublished and from Holloway et al. 1987) presented general life-history data of *D. pallivitta* from Thailand. A detailed study on the biology was done at the HDOA Insect Quarantine Facility (see Appendix 2 - Nagamine and Epstein 2007) and the information is summarized here.

Life stages: Eggs are elliptical, scale-like, and laid in masses, each egg measuring about 1.1 x 1.6mm. Duration is seven days. There were a variable number of larval instars, ranging from 8 to 11. Duration of all larval stages is summarized by the instar that cocooned; they were 49d, 56d, 63d, and 73d for individuals that reached 8^{th}, 9^{th}, 10^{th}, and 11^{th} instars, respectively. The cocoon stage averaged 19d with a range of 17-21d. Total duration, from egg to adult was 75d – 99d, depending on the number of larval instars stages. Adults of both sexes are externally similar except that females are larger and have filiform antennae while males have bipectinate antennae.

Adults do not feed.

Potential fecundity: Counts of ovarian eggs of one-day old females showed potential fecundity to be extremely high. Upon emergence, each female carried an average of 573 eggs, and of these, 202 were mature.

Reproductive attributes: Females laid an average of 479 eggs (range = 306-676) over their lifetime, with a hatching rate of 55%. Females can begin mating on the day of emergence and can deposit their highest number of eggs on the second day after emergence, averaging 229 (range = 124-339). From then on, the number of eggs declined over the six-day oviposition period, while the postoviposition period was 2.6 days. Female and male longevity averaged 9.7 and 11.0 days, respectively.

Pest status of nettle caterpillar

Stings from the caterpillar stage of the moth have become a human health problem in Hawaii as *D. pallivitta* becomes more widespread and established in different habitats. Plant nursery workers, as well as homeowners, hikers, and school children, must now be aware of the hazard of making contact with the venomous spines. Most commonly, people get stung while doing yard work, and only realize it after having intense itching and development of welts or blisters. Those that are hypersensitive seek treatment with a visit to the doctor or hospital emergency room. Reports of humans getting stung increase during the summer months when there is a natural population surge of *D. pallivitta*.

The caterpillar feeds on many different species of plants, many of which are commonly used as landscape plants around homes, gardens, resorts, and hotels. This polyphagous habit also makes *D. pallivitta* a potentially damaging pest because of the variety of plants and ideal climate in Hawaii. Kishimoto (2006) observed feeding damage on 57 species of plants, representing 54 genera in 26 families. These include weeds, ornamentals, crops, and indigenous plants. Some of the favored ornamental plants include the areca palm (*Dypsis lutescens*), rhapis palm (*Rhapis excelsa*), Phoenix palm (*Phoenix roebelenii*), Hawaiian ti (*Cordyline fruticosa*), mondo grass (*Ophiopogon japonicus*), and species of lilies and irises. Insecticides to control *D. pallivitta* are costly to nurseries and homeowners. The tourist industry is also potentially impacted if *D. pallivitta* becomes established on the ubiquitous coconut palm used in hotel landscape areas. Caterpillars that sting visitors would negatively impact the image of Hawaii.

C. Background information on the natural enemy to be released

Aroplectrus dimerus life history (see Appendix 1 for details and pictures)

The general life cycle study for *A. dimerus* was researched in the HDOA Insect Quarantine Facility. More extensive biology studies were not completed due to the presence of a larval disease in the *D. pallivitta* colonies. The disease, identified by Harry Kaya (University of California at Davis) as a cytoplasmic polyhedrosis virus (CPV), became entrenched in the HDOA Insect Quarantine Facility, such that *D. pallivitta* larvae could no longer be reared for more than one generation.

Aroplectrus dimerus is biparental, females and males both orange in color. It is a synovigenic species, i.e., females successively develop eggs to maturity throughout their reproductive life. It is an ectoparasitoid and gregarious in habit, typically 5-10 wasps developing from a single host larva, depending on its size. The female first stings the host larva to paralyze it, inserting its ovipositor usually at the edges of the smooth ventral side (belly). The *D. pallivitta* larva attacked by a wasp may flail wildly and regurgitate a brownish liquid. The female wasp deposits single eggs externally on the host larva, most commonly, laterally, embedded between segments. The host larva becomes totally immobilized within two days and remains adhered to the leaf substrate. The wasp larvae also hatch from their eggs on the second day and migrate to the belly of the host larva. They feed externally for six days and remain concealed under the host body. The dark fecal material is clearly seen in the gut as they reach maturity, and about one day prior to pupation, the waste product (meconium) is discharged as a brown, worm-like matter. The wasp pupae mature in five days and the adults emerge. The total life cycle is 13days; egg (2d), larva (6d), and pupa (5d).

Natural geographic range of *Aroplectrus dimerus*

Larry Nakahara (former HDOA Plant Pest Control Branch Manager) discovered *Aroplectrus dimerus* parasitizing *D. pallivitta* larvae at a plant nursery in Tien-wei, Taiwan on October 8, 2004. Tien-wei is located in central Taiwan and has a subtropical climate. In the scientific literature, this parasitoid is also recorded from China (Zhu et al. 2002), India (Singh et al. 1988), and the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999).

Host range of *Aroplectrus dimerus* (see Appendix 1 for details)

There were no detailed studies in the scientific literature for *A. dimerus*, therefore, host specificity tests were done in the HDOA Insect Quarantine Facility, the objective of which was to determine if this parasitoid would attack any non-target insects. In Hawaii, there are no other species of insects related to the nettle caterpillar (species in the family Limacodidae), and there are no species in its superfamily Zygaenoidea, hence, there were no Hawaiian species closely related taxonomically. Of the 25 Lepidoptera species tested, which represented 13 families, four are beneficial species (two currently used for weed biocontrol and two still under study in the quarantine), two are Hawaiian endemics, and 19 are immigrant pests.

Host specificity evaluations were based on no-choice tests, 20 larvae of each Lepidoptera species being exposed to parasitoid females. *Aroplectrus dimerus* did not deposit any eggs on any of the 500 total larvae tested, hence, there was no parasitoid emergence from the non-target Lepidoptera species.

These studies demonstrated that the natural enemy *S. dimerus* will not attack other insects in Hawaii and is host specific to the nettle caterpillar.

Host range list

In the scientific literature, *A. dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987,); these are *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P.*

zelaznyi Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988). None of these species are present in Hawaii.

Parasites/hyperparasites
There are no records of parasites or hyperparasites attacking *A. dimerus.*

Status as hyperparasite
There are no records of *A. dimerus* attacking other parasitoids.

Locations of rearing facilities and release sites
The HDOA Insect Quarantine Facility is located at 1428 South King Street, Honolulu, Hawaii. If *A. dimerus* is approved for release from quarantine as a biocontrol agent, mass propagation of the wasp will be done in the HDOA Insect Rearing Facility at the same location. *A. dimerus* will be shipped to the other Hawaiian islands for release where needed.

Number/quantity to be released
Releases will continue to be made until the insect becomes established. Numbers released per month cannot be predicted at this time.

Timing of release
No particular timing of releases is made. Areas of high *D. pallivitta* populations will have preference for inoculative releases of *A. dimerus.*

Location of voucher specimens
Aroplectrus dimerus Lin (Hymenoptera: Eulophidae) was identified by Chao-dang Zhu on December 6, 2004. Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China, compared the Taiwan specimens with those at the Natural History Museum (London) and made the identification. Voucher specimens are deposited in the collections at the Natural History Museum (London), National Museum of Natural History (Washington D.C.), the National Museum of Natural Science (Taichung, Taiwan), and the Hawaii Department of Agriculture (Honolulu, Hawaii).

II. Alternatives to the Proposed Action

The actions being considered in this EA are (1) no Action (i.e., the natural enemy would not be released) or (2) release of *A. dimerus*. The no action alternative will allow the continued spread and establishment of *D. pallivitta* throughout the state of Hawaii, with the increasing use of insecticides to control this pest. An alternative to the release of the natural enemy would be control through spraying of chemical pesticides on host plants of the caterpillar to control the pest. This alternative would be more costly, non-sustainable, and have adverse environmental consequences due to the use of pesticides. The release of *A. dimerus* would result in biological control of *D. pallivitta* and eliminate the need for chemical use.

III. Environmental Impacts of the Proposed Action and Alternatives

Expected environmental impacts of the proposed release:
Environmental impacts associated with the no action alternative of not issuing permits for release will result in the further spread of *D. pallivitta* throughout the Hawaiian Islands and an increase in health-related caterpillar stings and caterpillar feeding damage to native and introduced plants. The use of a natural enemy is believed to be the only long term solution to the problem and would suppress *D. pallivitta* population densities to levels where caterpillars would no longer require chemicals for control.

Potential impacts on human environment
There will be no impact of the release of *A. dimerus* on the human environment in Hawaii. This parasitoid does not harm humans, animals, or plants. It will only attack *D. pallivitta* in Hawaii.

Literature search for other host records
In the scientific literature, *A. dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999); these are *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P. zelaznyi* Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988). None of these hosts or any related insects occur in Hawaii.

Host specificity in country of origin
No host specificity testing was done in the country of origin. Thorough tests conducted in the HDOA Insect Quarantine Facility showed that *A. dimerus* did not attack any of the 25 tested species of Lepidoptera which occur in Hawaii.

Interactions with established biocontrol agents
There have been no other biocontrol agents released for *D. pallivitta* in Hawaii.

Potential impact on T&E species
Host specificity testing done in the HDOA Insect Quarantine Facility showed that *A. dimerus* did not attack any of the 25 species of Lepidoptera tested, and there is no expected impact on any threatened or endangered insect species anticipated.

Impact to related non-target potential hosts
In Hawaii, there are no other species in the family Limacodidae except *D. pallivitta*, and there are no species represented in its superfamily Zygaenoidea, hence, there are no Hawaiian species that are taxonomically closely related.

Potential of *Aroplectrus dimerus* to act as a hyperparasite
There are no records in the scientific literature of *A. dimerus* acting as a hyperparasitoid. The development of the parasitoid on the host larva is highly synchronized very host specific, such that it could not develop as a hyperparasitoid on other insects.

Potential of *Aroplectrus dimerus* to attack non-targets in the mainland U.S.

According to limacodid specialist Marc Epstein there are subtropical limacodids in the mainland United States (southern California, Florida, Mississippi, Alabama, Georgia, Texas, etc.). These limacodids have not been tested as potential hosts of *A. dimerus*. The HDOA, however, believes the risk would be minimal for this parasitoid from Hawaii to be accidentally exported and become established on the mainland U.S. *A. dimerus* is host specific to the nettle caterpillar and could only arrive on the U.S. mainland in parasitized NC. The risk of this happening would be extremely low unless plants with heavy infestations of the caterpillar were to be exported. This is very unlikely with the current export inspections and certifications in place to insure that plant material arriving on the mainland from Hawaii is not infested with agricultural pests.

IV. Environmental Assessment Process and Environmental Permits

A. Basis for Environmental Assessment

This Environmental Assessment was prepared in accordance with Chapter 343, Hawaii Revised Statutes (HRS) by the proposing agency. The EA was triggered because state funding was used by HDOA in the research conducted. As the release will impact a significant agricultural pest, the Hawaii Department of Agriculture is acting as the approving agency in accordance with Chapter 343.

A draft environmental assessment (EA) was prepared by the proposing agency and posted in The Environmental Notice of the Office of Environmental Quality Control on April 23, 2008. No comments on the draft EA were received by the Proposing Agency or the Determining Agency during the 30 day comment period.

B. Environmental Permits

The proposed action requires permits from United States Department of Agriculture Plant Protection and Quarantine (USDA/PPQ) and the Hawaii Board of Agriculture (BOA).

Conditions for the environmental release of *A. dimerus* have been established by the Hawaii Board of Agriculture under the provisions of HRS Chapters 141 (Department of Agriculture) and 150A (Plant and Non-Domestic Animal Quarantine).

Conditions for the importation of *A. dimerus* into the HDOA Insect Containment Facility have been obtained from USDA/PPQ. USDA/PPQ permit conditions for the release of *A. dimerus* from the facility into the environment will be obtained once the federal EA is finalized and if a FONSI declared.

V. Listing of Agencies and Persons Consulted

A. Public Meetings

This proposed action, to release *A. dimerus* for control of the nettle caterpillar, has gone through

a public notification process through the Board of Agriculture permitting process. This is in accordance with Chapter 92 (Public Agency Meetings and Records), HRS, commonly referred to as the Sunshine Law. As part of this process the public was notified and had the opportunity to attend, comment and testify on this proposed release at the Plants and Animals Committee meeting and at a Board of Agriculture meeting. No comments opposed to this action were presented at these public meetings.

B. List of Consulted Parties

Following is a list parties, agencies, and individuals that were consulted:

- Dr. Marc Epstein, insect systematist and an authority on the family Limacodidae, formerly of the Smithsonian Institution, now with the California Department of Food and Agriculture, Sacramento, California.
- Dr. Cheng-Shing Lin, Curator of Entomology at the National Museum of Natural Science, Taichung, Taiwan.
- Dr. Chao-dang Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China.
- United States Fish and Wildlife Service, Ecological Services, Dr. Patrick Leonard
- United States Department of Agriculture, Plant Protection and Quarantine
- Hawaii Board of Agriculture
- Hawaii Invasive Species Council
- Coordinating Group on Alien Pest Species (CGAPS)
- Department of Business, Economic Development & Tourism, Small Business Regulatory Review Board
- Dr. Lorna Arita-Tsutsumi, entomologist, University of Hawaii, Hilo
- Dr. Peter Follet, entomologist, USDA Agricultural Research Service
- Dr. Frank Howarth, entomologist, Bishop Museum
- Dr. Arnold Hara, entomologist, University of Hawaii, Manoa
- Dr. Ronald Mau, entomologist, University of Hawaii, Manoa
- HDOA Plants and Animals Committee
    - Dr. Roy Nishimoto, University of Hawaii, Manoa
    - Dr. Mindy Wilkinson, Department of Land and Natural Resources
    - Lyle Wong, Department of Agriculture
    - Dr. Chris Kelly, University of Hawaii, Manoa
    - Dr. Sarah Park, Hawaii Department of Health
    - Dr. Genevieve Salmonson, Office of Environmental Quality Control, Dept. of Health

VI. Findings and Reasons

Chapter 11-200-12, HRS, outlines those factors agencies must consider when determining whether and action has potential for a significant effect.

1) Involves an irrevocable commitment to loss or destruction of any natural or cultural resources.
The NC is not a natural or cultural resource. In fact, NC is detrimental to natural and cultural resources such as native plants and plants used for leis and in traditional hula. Control of NC will be beneficial to natural and cultural resources.

2) Curtails the range of beneficial uses of the environment.
The proposed action will not curtail beneficial uses of the environment. In fact, it will cause a decline in the population of NC which currently stings Hawaii residents. A decline in NC densities will allow Hawaii residents to enjoy the environment with less likelihood of receiving stings from this caterpillar.

3) Conflicts with the state's long-term environmental policies or goals and guidelines as expressed in Chapter 344, HRS, and any revisions thereof and amendments thereto, court decisions, or executive orders.
The proposed action does not conflict with the state's environmental policies or goals and guidelines as expressed in Chapter 344, HRS. The proposed action is in harmony with these guidelines as it will protect native flora from this damaging insect and enhance environmental experiences by decreasing populations of a stinging insect in the environment.

4) Substantially affects the economic or social welfare of the community or state.
The proposed action will not negatively affect the economic or social welfare of the state. Control of NC by the natural enemy will result in an economic and social benefit for the nursery industry, landscapers, resorts, hotels, and homeowners as they will be able to decrease and in many cases eliminate their use of pesticides to control NC and they and their stakeholders will see a decrease in stinging from the NC.

5) Substantially affects public health
The proposed action will have a positive benefit on public health. Decreases in the NC population on landscape, agriculture, and other plants will result in a decrease in sting incidents from this caterpillar.

6) Involves substantial secondary impacts, such as population changes or effects on public facilities.
No secondary impacts on population changes or public facilities are expected from the control of this insect.

7) Involves a substantial degradation of environmental quality.
No substantial degradation of environmental quality is expected from the release of this natural enemy. In fact, environmental quality should improve due to decreases in this plant feeding insect.

8) Is individually limited but cumulatively has considerable effect upon environment or involves a commitment for larger actions

The proposed action is limited to controlling a significant pest to human health and certain plant species. No cumulative negative effect on the environment is anticipated

9) Substantially affects a rare, threatened or endangered species, or its habitat.
The proposed action is not anticipated to substantially affect rare, threatened or endangered species or their habitat. The natural enemy is host specific to the NC in Hawaii. No native, rare, threatened or endangered insects are closely related to its host. Studies by HDOA demonstrated that the natural enemy will not attack other insect species.

10) Detrimentally affects air or water quality or ambient noise levels.
The proposed action, to release an insect natural enemy, is not anticipated to affect air or water quality or ambient noise levels. Reductions in NC populations may have a positive local impact on water quality with the decrease in the use of pesticides.

11) Affects or is likely to suffer damage by being located in an environmentally sensitive area such as a flood plain, tsunami zone, beach, erosion-prone area, geologically hazardous land, estuary, fresh water, or coastal water.
The proposed action is not anticipated to have any impact on the environmentally sensitive areas.

12) Substantially affects scenic vistas and view planes identified in county or state plans or studies.
The proposed action is not anticipated to affect scenic vistas or view planes.

13) Requires substantial energy consumption.
No substantial energy consumption will be required for this proposed action.

Issues of Uncertainty

Uncertainty regarding the consequence of a subject action requires evaluation as part of an EA. In the case of the proposed project, questions regarding uncertainty were expressed during the consultations.

One concern related to the uncertainty that *A. dimerus* may attack non-target insects. The commenters were satisfied with the evidence in Appendix 1 in which host specificity studies were conducted on related insects that are known to occur in Hawaii and none served as viable hosts for this natural enemy. In addition, all literature on *A. dimerus* demonstrates that the natural enemy has a very narrow host range within a group of moths which are taxonomically related to the nettle caterpillar. No moths in this taxonomic grouping occur in Hawaii. Historically, attacks on non-target hosts by introduced insect biological control natural enemies have not occurred with natural enemies released after 1975. All releases after this date underwent modern host specificity analysis and were reviewed by three expert committees.

Another potential uncertainty relates to the degree to which *A. dimerus* will parasitize nettle caterpillar. In other words, will this release result in effective reductions in nettle caterpillars. This is difficult to predict. What is known is that A. dimerus is known to be the primary parasite

attacking nettle caterpillar in Taiwan and that NC is uncommon and extremely difficult to find. When infestations are found, they are at very low densities and always heavily parasitized by *A. dimerus*. Based on infestations in Taiwan, and the history of similar biocontrol projects involving similar natural enemies, it appears likely that the effort will be effective.

In summary, there is no action that has consequences that are completely predictable, and thus there is uncertainty associated with any proposed action, including this one. Uncertainty must be weighed against potential benefits of an action and adverse impacts that are likely to occur if an action is not undertaken. In this case, there is a consensus among biologists in Hawai'i that nettle caterpillar is deleterious to the public, landscaping, tourism, agriculture, and the native flora. The uncertainty associated with biocontrol of nettle caterpillar appears to be low, due to the rigorous testing of this biocontrol agent and the general success of biocontrol projects in Hawai'i. Balanced against the certainty of the damage posed by nettle caterpillar, the levels of uncertainty associated with the proposed action appear acceptable.

VII. Final Determination

The Hawaii Department of Agriculture has reviewed the draft EA. The draft EA was published in the Environmental Notice on April 23, 2008. A 30-day public comment period began on April 23, 2008 but no comments were received. The agency has determined that this project will not have significant environmental effects and has issued a FONSI.

VII. References

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VII. Appendices

Appendix 1 - Nagamine, W.T., J.Y. Yalemar, and L.M. Nakahara. 2007. Host specificity testing for *Aroplectrus dimerus* Lin (Hymenoptera: Eurytomidae), a biological control agent of the nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae).

Appendix 2 - Nagamine, W.T. and M.E. Epstein. 2007. Chronicles of *Darna pallivitta* (Moore 1877) (Lepidoptera: Limacodidae): biology and larval morphology of a new pest in Hawaii. Pan-Pacific Entomologist 83(2):120-135.

HAWAII DEPARTMENT OF AGRICULTURE
Honolulu, Hawaii

Host Specificity Testing for *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae), a biological control agent of the nettle caterpillar, *Darna pallivitta* (Moore) (Lepidoptera: Limacodidae).

Walter Nagamine, Juliana Yalemar, and Larry Nakahara
Biological Control Section, Plant Pest Control Branch

Introduction

Nature of the problem

The nettle caterpillar, *Darna pallivitta* (Moore), is a new immigrant pest to Hawaii that was first noticed in September 2001 after workers at a nursery on the east side of the island of Hawaii (Big Island) were being "stung" by a caterpillar while handling rhapis palms (*Rhapis* sp.). It was suspected of having entered the state on palm seedlings legally imported from Taiwan. Immediately after its detection, an eradication attempt with pesticides was made but proved unsuccessful. In January 2002, surveys showed its establishment on three surrounding farms where the larvae were found feeding on coconut palm (*Cocos nucifera* L.), areca palm (*Chrysalidocarpus lutescens* Wendl), rhapis palm, Hawaiian ti (*Cordyline terminalis* Kunth), and *Dracaena* sp.

Darna pallivitta is now well established in the Hilo area on the east side of the Big Island and has slowly moved from the original infestation site southward into the Puna District. It was discovered in Kona on the west side of the island during September 2006, and at Kohala in the north side during February 2007, both of these infestations likely resulting from movement of infested plants. During June 2007, an infestation at a nursery on Oahu Island was discovered after workers were being stung while handling areca palm plants. The source of this infestation was believed to be the importation of palms about a year earlier from a nursery located on the east side of the Big Island, where *D. pallivitta* is firmly established. A similar scenario occurred on Maui island during July 2007, where a new infestation was found in an area nearby to plant nurseries.

The polyphagous habit of *D. pallivitta* increases its pest potential in Hawaii. Field observations of feeding damage include both weedy and ornamental plants commonly grown in residences and agriculture (Kishimoto 2006). Damage to ornamental plants, including the many palm species grown in Hawaii, could result in economic losses to the nursery industry and homeowners. Also potentially threatened by larval feeding are endemic plant species. Of medical importance are the stinging spines of the larva, which cause dermatitis on contact with the skin. Reports of people being stung by *D. pallivitta* larvae typically increase during the summer months due to population surges. Outbreaks in residential communities result in homeowners getting stung while working in the yard. Symptoms vary, but include itching,

welts, and blisters, depending on a person's sensitivity. Doctor and emergency room visits for caterpillar stings have been recorded.

D. pallivitta occurs in China, Taiwan, Thailand, western Malaysia, Indonesia, and Java, and its host plants in those regions include *Adenostemma* sp., *Areca* sp., *Breynia* sp., coconut, *Ficus* sp., grasses, maize, and oil palm. It is considered only a minor pest of coconut palms in its natural range, probably due to the presence of natural enemies that do not occur in Hawaii (Holloway et al. 1987).

Biological control program

The Nettle Caterpillar Project was a joint project between the Hawaii Department of Agriculture (HDOA) and the University of Hawaii (Dr. Arnold Hara, UH-Manoa). The first effort to search for *D. pallivitta* natural enemies was a collaboration with Dr. Dantje Sembel of Sam Ratulangi University located in Manado, North Sulawesi, Indonesia during 2003. Sembel, an entomologist and Professor in the Faculty of Agriculture, has vast experience in coconut pests at the Coconut Research Center in Manado. His collections of three limacodid species (*Pectinarosa alastor* Tams, *Thosea monoloncha* Meyrick, and *Darna catenatus* Snellen) from Sulawesi Island yielded a parasitoid, *Nesolynx* sp. (Hymenoptera: Eulophidae) attacking the cocoon stage of *Darna catenatus* Snellen. However, testing in the HDOA Insect Quarantine Facility showed this parasitoid to be a generalist as it developed on two species of fly pupae.

The second attempt to collect potential biocontrol agents was made by Kenneth Teramoto, Chief of the HDOA Biological Control Section, in collaboration with Dr. Banpot Napompeth of the National Biological Control Research Center in Thailand during June 2004. Teramoto and Napompeth searched central and southern Thailand and found the limacodid *Parasa lepida* Cramer being attacked by a braconid wasp, however, the parasitoids died before shipment to Hawaii.

Further exploration was conducted in Taiwan during October 2004 by Larry Nakahara, former manager of the HDOA Plant Pest Control Branch. Cooperation and critical field assistance in Taiwan was provided by Jai-Hsueh "Michelle" Lin, staff of the Taiwan Agricultural Research Institute (TARI), nurserymen from Tien-wei Village (central Taiwan) and Ping-tung County (southern Taiwan), and entomology students from Ping-tung University.

The major discovery of the *D. pallivitta* species was made by Nakahara at a Tien-wei nursery on October 8, 2004. There he also found parasitized larvae on ti plants (*Cordyline terminalis*), rhapis palms, and miniature coconut palms. Adult wasps began emerging from the parasitized caterpillars three days later. Collections of live, unparasitized *D. pallivitta* larvae were also made at two Ping-tung nurseries and these caterpillars were used for propagation of the parasitoid from Tien-wei. One shipment of parasitoids was sent by Nakahara to Hawaii for study in the HDOA Insect Quarantine Facility.

The parasitoid from Taiwan was identified as *Aroplectrus dimerus* Lin (Hymenoptera: Eulophidae) by Dr. Chao-dang Zhu on December 6, 2004. Zhu, a eulophid specialist at the Institute of Zoology, Chinese Academy of Sciences, Beijing, Peoples Republic of China, compared the Taiwan specimens with those at the Natural History Museum (London, UK) and

made the identification. Specimens of *A. dimerus* are deposited there and also in the collections at the National Museum of Natural History (Washington D.C.), the National Museum of Natural Science (Taichung, Taiwan), and the Hawaii Department of Agriculture (Honolulu, Hawaii).

In the scientific literature, *A. dimerus* has been recorded attacking six limacodid species in the Philippines (Cock et al. 1987, Philippine Coconut Authority 1999); these are *Darna mindanensis* Holloway, *Penthocrates albicapitata* Holloway, *P. rufa* Holloway, *P. rufofascia* Holloway, *P. styx* Holloway, and *P. zelaznyi* Holloway. In India, the limacodid *Parasa bicolor* Walker is also a recorded host (Singh 1988).

There was no detailed biology in the scientific literature for *A. dimerus*, therefore, host specificity and life cycle studies were conducted in the HDOA Insect Containment Facility (ICF) with the results presented in this report.

Materials and Methods

Nettle caterpillar propagation

Darna pallivitta larvae were reared in screened cages (42 x 42 x 62 cm) and fed leaves of Hawaiian ti or iris (*Tritonia crocosmiiflora*). After cocooning (pupating) and emergence of adults, about five female and five male moths were collected from the stock cage and placed in a wide-mouth, one-gallon glass jar for mating and egg-laying. A bouquet of ti or iris leaves, made with a strip of cotton wrapped around the petioles and snugly inserted into a small narrow-necked bottle or florist's vial, was placed into the jar. The mouth of the jar was covered with organdy cloth and secured with rubber bands. Moths usually laid eggs on the glass and not on the leaves. Newly hatched larvae crawled from the glass onto the leaves to feed. As they matured, the entire bouquet was transferred to a screened cage for continued feeding and development. A larval disease, identified by Dr. Harry Kaya (University of California at Davis) as a cytoplasmic polyhedrosis virus (CPV), later became entrenched in the HDOA Insect Containment Facility, such that *D. pallivitta* could no longer be reared for more than one generation. Despite decontamination efforts, the virus could not be eliminated from the lab. Instead, the HDOA Big Island Insectary, which was able to better manage the CPV disease, shipped *D. pallivitta* larvae to Oahu on a regular basis for propagation of the parasitoid.

Parasitoid propagation

Aroplectrus dimerus was reared in a one-gallon glass jar (previously described) containing 15 mid- to late-instar (ca. L6-L10) larvae and five mated female parasitoids. Honey was dotted inside the jar as a food source for the wasps. After a 7-day exposure period, the female wasps were removed. A new generation of adult wasps began emerging 13 days after initial exposure.

Life cycle study

The general life cycle for *A. dimerus* was determined, however, detailed studies were not possible due to the presence of the CPV disease in the *D. pallivitta* larval colonies.

Host specificity testing

In Hawaii, there are no other species in the family Limacodidae except *D. pallivitta*, and there are no species represented in its superfamily Zygaenoidea (Dalceridae, Epipyropidae, Lacturidae, Megalopygidae, and Zygaenidae), hence, there were no Hawaiian species closely related taxonomically. Twenty-five Lepidoptera species (Table 1), representing 13 families, were tested to determine if the parasitoid *A. dimerus* would attack any non-target species. These included four beneficial species (two currently used for weed biocontrol and two still under study), two Hawaiian endemics, and 19 immigrant pests. For some species, field-collected larvae were used for testing if they were found in abundance. For others, field-collected eggs, larvae, or adults were then propagated in the lab for one or more generations to increase their numbers for testing. A few species were already being lab-reared for other projects and were readily available.

All host specificity testing for *A. dimerus* was conducted in the HDOA Insect Containment Facility. Host specificity evaluations were based on no-choice tests. Ten larvae of a Lepidoptera test species were placed in a one-gallon glass jar (previously described) with their food source and exposed to five *A. dimerus* females for a 24-hour period. The respective larval food sources were pods, flowers, or leaf bouquets, placed in the jar and replenished as necessary. The control replicate was done in the same way but with 10 *D. pallivitta* larvae and a bouquet of iris leaves as a food source. Honey was dotted in the jar for the wasps.

After the exposure period, each test larva was removed from the jar and the number of parasitoid eggs counted on its body using a dissecting microscope. The 10 test larvae were then placed in another jar with their respective food source to continue to feed until moth or parasitoid emergence occurred. The same procedure was followed with the 10 control (*D. pallivitta*) larvae, however, because of their long life cycle (ca. 10 weeks), the larvae were only held for parasitoid emergence (about 3 weeks). Parasitoids were used only once during testing, and their ages were the same for a test and control replicate, but may have varied among replicates of different species. Two replicates of 10 larvae each were conducted for each Lepidoptera species, for a total of 20 larvae tested per species.

Results

Life history

Aroplectrus dimerus is biparental, females and males both orange in color. It is a synovigenic species, i.e., females successively develop eggs to maturity throughout their reproductive life. It is an ectoparasitoid and gregarious in habit, typically 5-10 wasps developing from a single host larva, depending on its size. The female first stings the host larva to paralyze it, inserting its ovipositor usually at the edges of the smooth ventral side (belly) (Fig. 7). The *D. pallivitta* larva attacked by a wasp may flail wildly and regurgitate a brownish liquid (Fig. 8). The female wasp deposits single eggs externally on the host larva, most commonly laterally embedded between segments (Fig. 9). The host larva becomes totally immobilized within two days and remains adhered to the leaf substrate. The wasp larvae hatch from the eggs, also in two days, and migrate to the belly of the host larva (Fig. 10). They feed externally for six days and remain concealed under the host body (Fig. 11). The dark fecal material is clearly seen in the wasp larvae as they reach maturity, and about one day prior to pupation, the waste product (meconium) is discharged as a brown, worm-like matter (Fig. 12). The wasp pupae mature in five days (Fig. 13) and the adults then emerge. The total life cycle is 13 days; egg (2d), larva (6d), and pupa (5d).

Host specificity tests

Results of host specificity tests for *A. dimerus* are shown in Table 2. In no-choice tests, *A. dimerus* females did not deposit any eggs on any larvae of the 25 non-target Lepidoptera species tested, hence, there was no parasitoid emergence. All test larvae examined under a dissecting microscope also showed no evidence of injury due to ovipositional probing and there were no indications of larval regurgitation in the jar due to attack by an *A. dimerus* female. The female parasitoids also appeared to have no specific attraction to three larval species (*Agraulis vanillae*, *Nyctemera apicalis,* and *Secusio extensa*) that have long setal hairs somewhat similar to *D. pallivitta.*

Parasitism was recorded in all control (*D. pallivitta*) replicates for all Lepidoptera species tested. Analysis by One Way ANOVA showed a significant difference ($P \leq 0.05$) for parasitism among all non-target Lepidoptera species compared with their controls. The number of *D. pallivitta* larvae parasitized for a pair of replicates (N=20 larvae) ranged from 40-85%, with an average of 4.7 wasps emerging per parasitized larva.

Conclusion

The collection of *D. pallivitta* on palm plants at a Taiwan nursery by L. Nakahara in 2004 was a major finding in itself because of its minor pest status in its homeland. The second crucial discovery was of the eulophid wasp *A. dimerus* parasitizing *D. pallivitta* caterpillars. Natural enemies found in the native range of a pest are more likely to have evolved with its host and therefore have greater specificity. Our studies showed that *A. dimerus* did not attack any of the 25 Lepidoptera species tested. In total, 500 test larvae were exposed to the parasitoid with none being parasitized.

The development of *A. dimerus* is well-adapted to its host. The complete immobilization of the paralyzed *D. pallivitta* larva, timed with the hatching and migration of the wasp larvae to the host's belly, suggests a highly synchronized process. The maturing of the wasp larvae under the smooth belly of the host provides added protection. The specialized development of *A. dimerus* appears suited to the slug-like morphology of *D. pallivitta* larvae and would preclude its growth on any other type of caterpillars. Hawaii does not have any other species in the family Limacodidae nor in its superfamily Zygaenoidea. We conclude that the parasitoid *A. dimerus* is not a threat to non-target Lepidoptera species in Hawaii.

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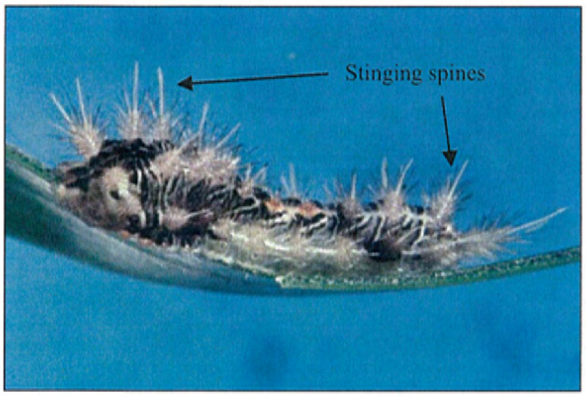

Fig.1. The nettle caterpillar, *Darna pallivitta*. Lateral view shows the venomous spines.

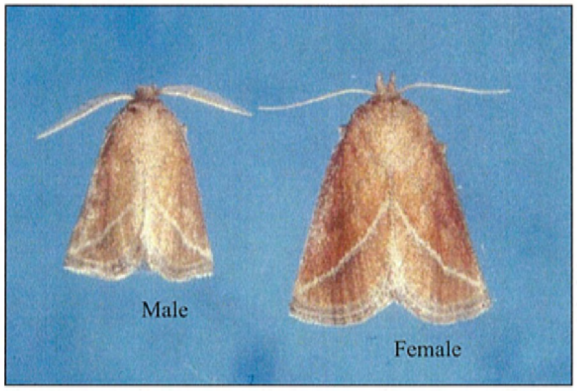

Fig.2. *Darna pallivitta* moths.

Fig. 3. Only midribs remain on a coconut leaf stripped by *D. pallivitta* larvae.

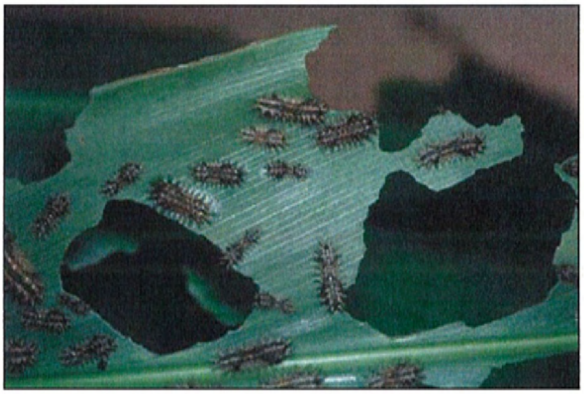

Fig. 4. Leaves of the Hawaiian ti plant are a favorite food of *D. pallivitta* larvae.

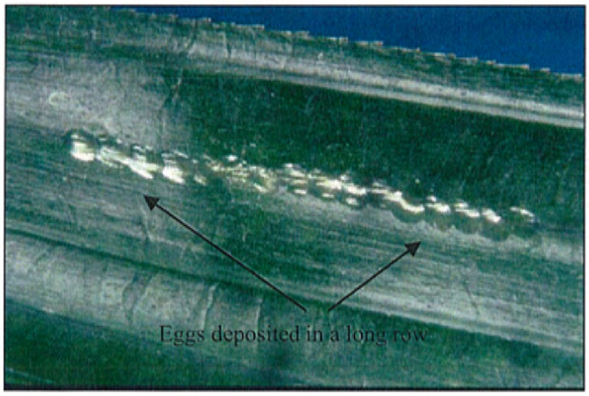

Fig. 5. *D. pallivitta* eggs appear as a silvery sheen on a leaf and are easily overlooked.

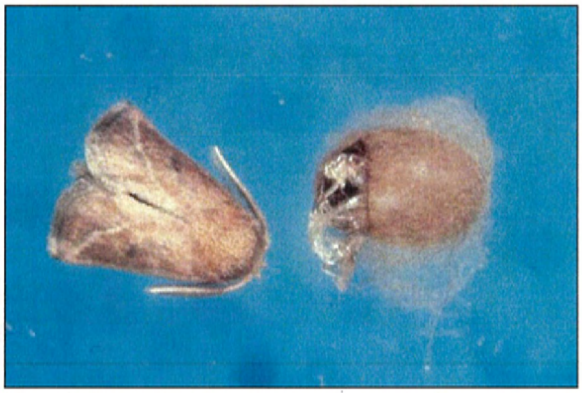

Fig. 6. *D. pallivitta* moth emerges from the cocoon (right) by popping open a cap.

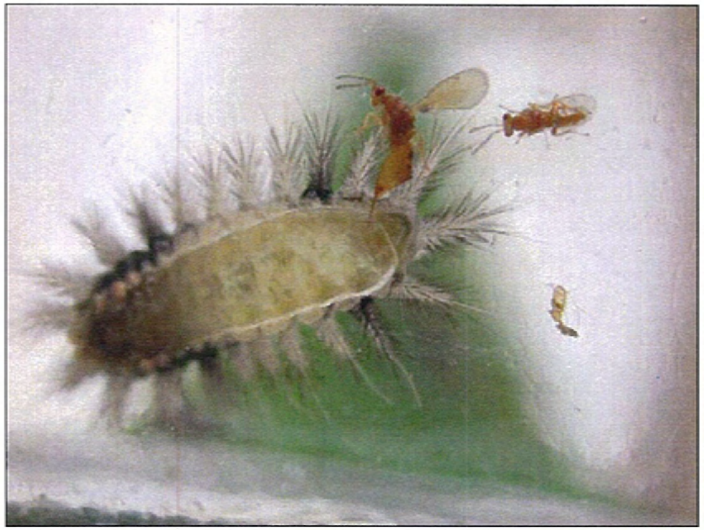

Fig. 7. An *Aroplectrus* female "stings" a *Darna* larva on its fleshy underside "belly" to immobilize it before laying eggs on its host (ventral view, seen through glass jar).

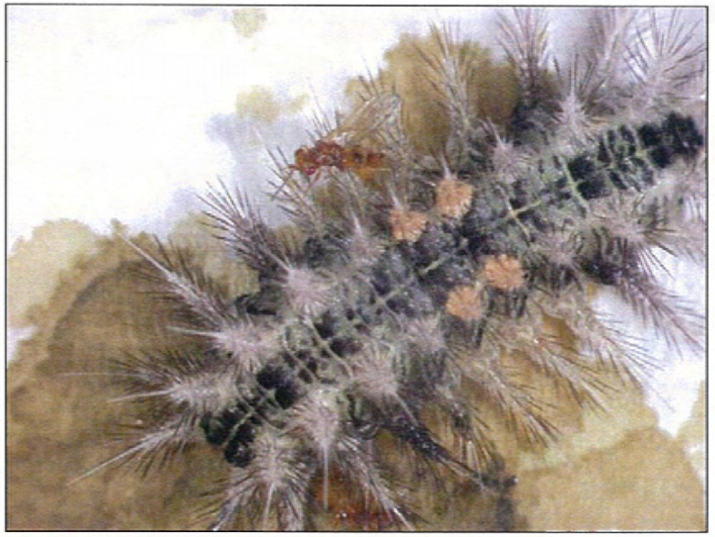

Fig. 8. A *Darna* larva will sometimes flail and regurgitate a brownish liquid while being attacked by an *Aroplectrus* wasp.

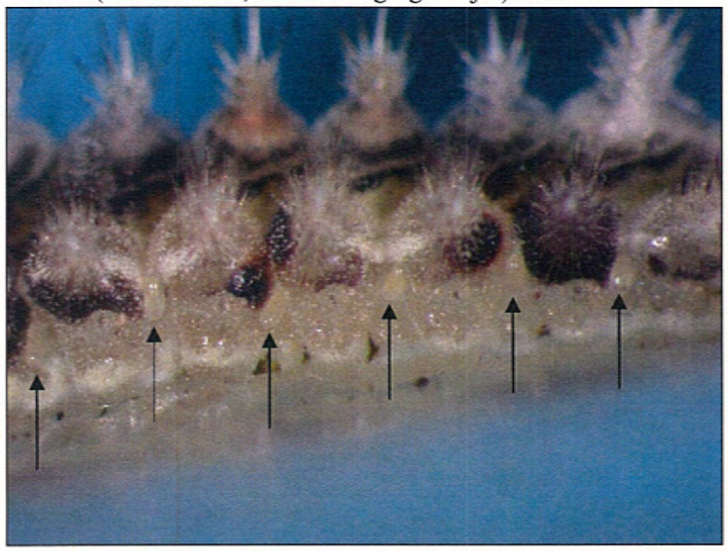

Fig. 9. *Aroplectrus* eggs are typically deposited on the sides of the *Darna* larva, in between the body segments.

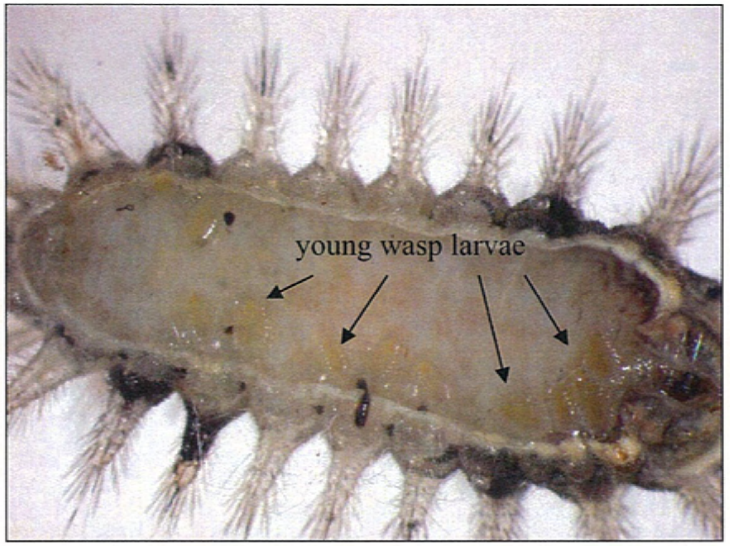

Fig. 10. Newly hatched *Aroplectrus* larvae migrate to the "belly" and begin feeding externally (ventral view, seen through glass jar).

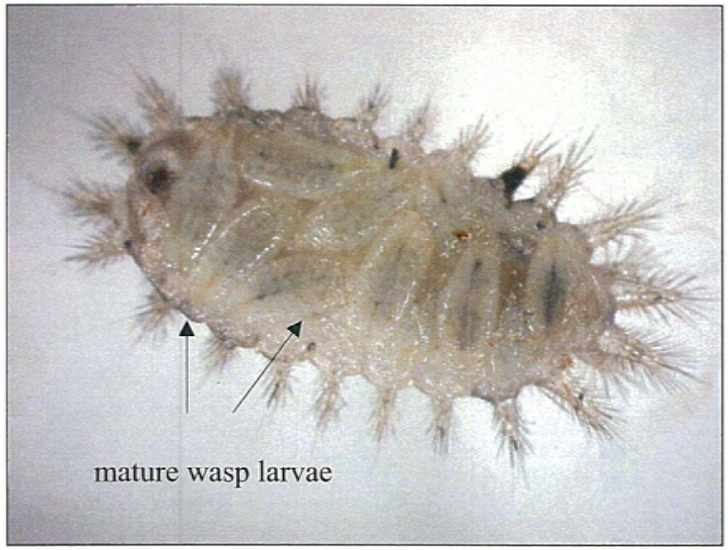

Fig. 11. Mature *Aroplectrus* larvae occupy the entire belly of the *Darna* larva (ventral view, seen through glass jar).

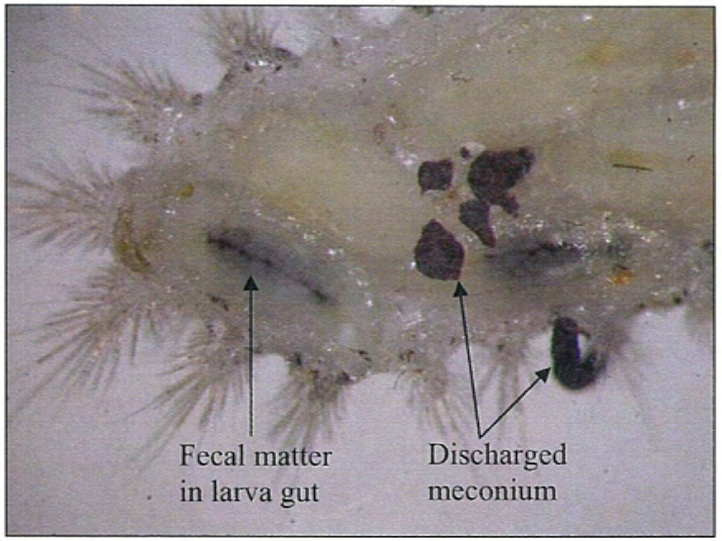

Fig. 12. Brownish meconium is discharged by *Aroplectrus* larvae prior to pupating (ventral view, seen through glass jar).

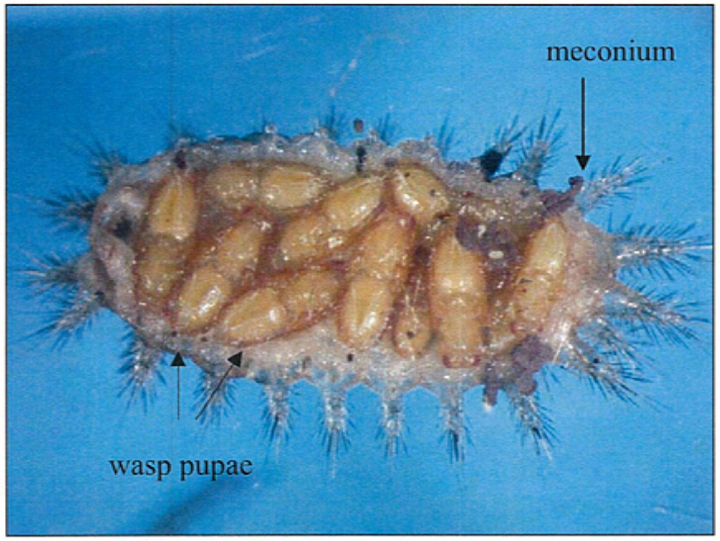

Fig. 13. *Aroplectrus* pupae are amber-colored prior to wasp emergence (ventral view, seen through glass jar).

Fig. 14. A parasitized larva is shriveled and normally stuck to a leaf with parasitoid meconium extruding from under the body.

Table 1. Non-target lepidopterous species used in host specificity tests for the parasitoid *Aroplectrus dimerus*.

Family, species name	Status	Source and host plant
Arctiidae		
***Nyctemera apicalis* Walker**[1] a leaf-feeder	Beneficial	Lab-reared, fireweed leaves, *Senecio madagascariensis*
***Secusio extensa* (Butler)**[1] a leaf-feeder	Beneficial	Lab-reared, fireweed leaves, *Senecio madagascariensis*
Choreutidae		
***Choreutis* sp.** a leaf-tier	Pest	Field-collected, weeping fig leaves, *Ficus benjamina*
Crambidae		
***Diaphania nitidalis* Cramer** pickleworm	Pest	Lab-reared, cucumber flowers/fruit, *Cucumis sativa*
***Omiodes blackburni* (Butler)** coconut leaf roller	Endemic	Lab-reared, coconut leaves, *Cocos nucifera*
***Udea stellata* (Butler)** a leaf-feeder	Endemic	Lab-reared, mamaki leaves, *Pipturis albidus*
Ethmiidae		
***Ethmia nigroapicella* (Sallmuller)**, kou leafworm	Pest	Field-collected, kou leaves, *Cordia subcordata*
Geometridae		
***Anacamptodes fragilaria* (Grossbeck)**, koa haole looper	Pest	Field-collected, koa-haole leaves, *Leucaena leucocephala*
***Macaria abydata* Guenee** koa haole moth	Pest	Field-collected, koa-haole leaves, *Leucaena leucocephala*
Lycaenidae		
***Lampides boeticus* (Linnaeus)** bean butterfly	Pest	Field-collected, rattlepod beans, *Crotalaria* sp.
Noctuidae		
***Achaea janata* (Linnaeus)** croton caterpillar	Pest	Field-collected, castor bean leaves, *Ricinus communis*
***Agrotis* sp.** a cutworm	Pest	Lab-reared, cotton leaves, *Gossypium hirsutum*
***Anomis flava* (Fabricius)** hibiscus caterpillar	Pest	Lab-reared, cotton leaves, *Gossypium hirsutum*
***Heliothis virescens* (Fabricius)** tobacco budworm	Pest	Field-collected, love-in-a-mist flowers, *Passiflora foetida*

Family, species name	Status	Source and host plant
Noctuidae, continued		
***Pandesma anysa* Guenee** a leaf-feeder	Pest	Field-collected, opiuma leaves, *Pithecellobium dulce*
***Spodoptera mauritia* (Boisduval)**, lawn armyworm	Pest	Lab-reared, undetermined grass species
Nymphalidae		
***Agraulis vanillae* (Linnaeus)** passion vine butterfly	Pest	Field-collected, passion vine leaves, *Passiflora edulis*
***Vanessa cardui* (Linnaeus)** painted lady	Pest	Field-collected, cheeseweed leaves, *Malva parviflora*
Pieridae		
***Pieris rapae* (Linnaeus)** imported cabbageworm	Pest	Field-collected, broccoli leaves, *Brassica oleracea*
Plutellidae		
***Plutella xylostella* (Linnaeus)**, diamondback moth	Pest	Field-collected, broccoli leaves, *Brassica oleracea*
Pyralidae		
***Hellula undalis* (Fabricius)** imported cabbage webworm	Pest	Field-collected, mustard cabbage leaves, *Brassica juncea*
Sphingidae		
***Daphnis nerii* (Linnaeus)** oleander hawk moth	Pest	Field-collected, oleander leaves, *Nerium oleander*
Tortricidae		
***Croesia zimmermani* Clarke** a biocontrol agent	Beneficial	Field-collected, blackberry leaves, *Rubus argutus*
***Cryptophlebia ombrodelta* (Lower)**, litchi fruit moth	Pest	Field-collected, undetermined legume species
***Episimus utilis* Zimmerman** a biocontrol agent	Beneficial	Field-collected, x-mas berry leaves, *Schinus terebinthifolius*

[1] Potential biological control agents being studied in HDOA Insect Quarantine Facility.

Table 2. Results of no-choice host specificity tests for the parasitoid *Aroplectrus dimerus* using 25 non-target Lepidoptera species and *Darna pallivitta* as the control. Two replicates of 10 Lepidoptera larvae each were conducted for each test species (N=20).

Family and species name	**No. parasitoid eggs deposited on larvae (mean ± SEM)**	**No. larvae parasitized**	**No. parasitoids emerging**	**No. moths of test sp. emerging**
Arctiidae				
Nyctemera apicalis	0	0	0	20 (100%)
D. pallivitta (control)	3.0 ± 0.9	10 (50%)	49 (42♀, 7♂)	-
ANOVA	F=11.9 DF=1,39 P=0.0014			
Secusio extensa	0	0	0	15 (75%)
D. pallivitta (control)	5.1 ± 1.1	13 (65%)	94 (61♀, 33♂)	-
ANOVA	F=19.7 DF=1,39 P=0.0001			
Choreutidae				
***Choreutis* sp.**	0	0	0	15 (75%)
D. pallivitta (control)	4.1 ± 0.9	12 (60%)	73 (52♀, 21♂)	-
ANOVA	F=21.0 DF=1,39 P=0.0000			
Crambidae				
Diaphania nitidalis	0	0	0	20 (100%)
D. pallivitta (control)	3.4 ± 0.7	14 (70%)	67 (52♀, 23♂)	-
ANOVA	F=21.0 DF=1,39 P = 0.0000			
Omiodes blackburni	0	0	0	14 (70%)
D. pallivitta (control)	4.5 ± 1.2	11 (55%)	87 (62♀, 25♂)	-
ANOVA	F=14.4 DF=1,39 P=0.0005			
Udea stellata	0	0	0	20 (100%)
D. pallivitta (control)	5.1 ± 1.1	12 (60%)	38 (26♀, 12♂)	-
ANOVA	F=19.5 DF=1,39 P=0.0001			

Family and species name	No. parasitoid eggs deposited on larvae (mean ± SEM)	No. larvae parasitized	No. parasitoids emerging	No. moths of test sp. emerging
Ethmiidae				
Ethmia nigroapicella	0	0	0	15 (75%)
D. pallivitta (control)	3.5 ± 0.8	13 (65%)	16 (10♀, 6♂)	-
ANOVA	F=18.5 DF=1,39 P=0.0001			
Geometridae				
Anacamptodes fragilaria	0	0	0	0 (0%)[1]
D. pallivitta (control)	4.9 ± 0.9	17 (85%)	36 (18♀, 18♂)	-
ANOVA	F=30.3 DF=1,39 P=0.0000			
Macaria abydata	0	0	0	4 (20%)
D. pallivitta (control)	3.6 ± 0.9	12 (60%)	27 (20♀, 7♂)	-
ANOVA	F=17.7 DF=1,39 P=0.0002			
Lycaenidae				
Lampides boeticus	0	0	0	20 (100%)
D. pallivitta (control)	5.5 ± 1.0	16 (80%)	45 (28♀, 17♂)	-
ANOVA	F=32.4 DF=1,39 P=0.0000			
Noctuidae				
Achaea janata	0	0	0	19 (95%)
D. pallivitta (control)	4.7 ± 1.0	14 (70%)	88 (54♀, 34♂)	-
ANOVA	F=21.9 DF=1,39 P = 0.0000			
***Agrotis* sp.**	0	0	0	1 (5%) [1]
D. pallivitta (control)	3.7 ± 1.2	11 (55%)	49 (22♀, 29♂)	-
ANOVA	F=8.66 DF=1,39 P=0.0055			

Family and species name	No. parasitoid eggs deposited on larvae (mean ± SEM)	No. larvae parasitized	No. parasitoids emerging	No. moths of test sp. emerging
Anomis flava	0	0	0	20 (100%)
D. pallivitta (control)	4.8 ± 0.9	13 (65%)	91 (61♀, 30♂)	-
ANOVA	F=30.1 DF=1,39 P=0.0000			
Heliothis virescens	0	0	0	13 (65%)
D. pallivitta (control)	5.1 ± 1.4	9 (45%)	60 (38♀, 22♂)	-
ANOVA	F=12.6 DF=1,39 P=0.0010			
Pandesma anysa	0	0	0	18 (90%)
D. pallivitta (control)	5.3 ± 1.1	14 (70%)	101 (70♀, 31♂)	-
ANOVA	F=24.6 DF=1,39 P=0.0000			
Spodoptera mauritia	0	0	0	19 (95%)
D. pallivitta (control)	4.2 ± 0.9	14 (70%)	80 (67♀, 13♂)	-
ANOVA	F=23.7 DF=1,39 P=0.0000			
Nymphalidae				
Agraulis vanillae	0	0	0	1 (5%) [1]
D. pallivitta (control)	4.5 ± 0.9	14 (70%)	76 (46♀, 30♂)	-
ANOVA	F=26.0 DF=1,39 P=0.0000			
Vanessa cardui	0	0	0	19 (95%)
D. pallivitta (control)	4.4 ± 1.0	12 (60%)	68 (45♀, 23♂)	-
ANOVA	F=19.0 DF=1,39 P=0.0001			
Pieridae				
Pieris rapae	0	0	0	19 (95%)
D. pallivitta (control)	4.4 ± 1.0	13 (65%)	68 (39♀, 29♂)	-
ANOVA	F=17.7 DF=1,39 P=0.0002			

Family and species name	No. parasitoid eggs deposited on larvae (mean ± SEM)	No. larvae parasitized	No. parasitoids emerging	No. moths of test sp. emerging
Plutellidae				
Plutella xylostella	0	0	0	20 (100%)
D. pallivitta (control)	3.5 ± 1.3	8 (40%)	44 (28♀, 16♂)	-
ANOVA	F=7.67 DF=1,39 P=0.0086			
Pyralidae				
Hellula undalis	0	0	0	10 (50%)
D. pallivitta (control)	4.8 ± 1.0	12 (60%)	56 (33♀, 23♂)	-
ANOVA	F=21.6 DF=1,39 P=0.0000			
Sphingidae				
Daphnis nerii	0	0	0	18 (90%)
D. pallivitta (control)	4.6 ± 0.9	15 (75%)	67 (6♀, 61♂)	-
ANOVA	F=28.2 DF=1,39 P=0.0000			
Tortricidae				
Croesia zimmermani	0	0	0	19 (95%)
D. pallivitta (control)	2.6 ± 0.8	8 (40%)	13 (9♀, 4♂)	-
ANOVA	F=9.63 DF=1,39 P=0.0036			
Cryptophlebia ombrodelta	0	0	0	20 (100%)
D. pallivitta (control)	3.0 ± 0.9	11 (55%)	33 (24♀, 9♂)	-
ANOVA	F=10.6 DF=1,39 P=0.0024			
Episimus utilis	0	0	0	20 (100%)
D. pallivitta (control)	3.9 ± 0.5	17 (85%)	42 (23♀, 19♂)	-
ANOVA	F=51.0 DF=1,39 P=0.0000			

[1] Disease or undetermined cause prevented larvae from completing development.

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Chronicles of *Darna pallivitta* (Moore 1877) (Lepidoptera: Limacodidae): biology and larval morphology of a new pest in Hawaii

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Abstract. The biology of *Darna pallivitta* (Moore 1877), an Asian species of nettle caterpillar recently introduced to the island of Hawaii, is described from this island population. The species is highly polyphagous and has stinging caterpillars commonly associated with Limacodidae. Information on mating, oviposition, potential fecundity, duration and number of instars, cocooning, pupation, and total development time are included. The female at eclosion was found to have 573.5 ± 184.1 eggs, of which 201.5 ± 53.5 were mature. Similar to other spiny species of Limacodidae, *D. pallivitta* was found to not feed until second instar. The duration of immature stages were as follows: egg (7.0 d), larval (53.0 ± 6.9 d), and cocoon (19.1 ± 1.0 d). First and second instars are described for the first time for a *Darna* species. In a family known for heteromorphic larvae, this is the first known example of a limacodid species with elongate first instar tubercles, which later develop into spiny, urticating scoli rather than becoming smooth bodied or retaining tubercles that become hairy. Caterpillars from 6th instar or later have a delayed phenotypic expression of SD2 verrucae closely associated with spiracles on A2 to A7. Larval stages varied from 8–11 instars with a total larval duration of 45–72 d. Eleven instars equal the maximum reported for Limacodidae. Pupation took place on the fifth day after cocooning, which is a brief period compared to other members of the family. The total time from egg hatch to adult eclosion was 80.0 ± 7.1 d. Adult life span was found to be 11.0 ± 1.3 d in females and 9.7 ± 1.1 d in males.

Key Words. Lepidoptera, Limacodidae, *Darna pallivitta*, number of instars, fecundity, larval morphology, invasive species to Hawaii.

INTRODUCTION

The nettle caterpillar, *Darna pallivitta* (Moore 1877), is a new immigrant pest to Hawaii that was first noticed in September 2001 after workers at a nursery on the island of Hawaii were being "stung" by a caterpillar while handling rhapis palms (*Rhapis* sp.)(Conant et al. 2001). It was suspected of having entered the state on potted palm plants legally imported from Taiwan. Immediately after its detection, an eradication attempt with pesticides was made but proved unsuccessful. In January 2002, surveys showed its establishment on three surrounding farms where the larvae were found feeding on coconut palm (*Cocos nucifera* L.), areca palm (*Chrysalidocarpus lutescens* Wendl.), rhapis palm, Hawaiian ti (*Cordyline terminalis* Kunth), and *Dracaena* sp.

Darna pallivitta is now well established on the east side of the Big Island (island of Hawaii) and has slowly moved from the original infestation site southward during 2004 and 2005. The polyphagous habit of *D. pallivitta* increases its pest potential in Hawaii. Field observations of feeding damage include both weedy and ornamental plants commonly grown in residences and agriculture. Damage to ornamental plants could result in economic losses to the nursery industry. Also potentially threatened

by larval feeding are endemic plants and palm species, including the ubiquitous coconut palm. Of medical importance are the stinging spines of the larva, which cause dermatitis on contact with the skin. Reports of humans being "stung" by *D. pallivitta* larvae increased during an outbreak in a residential community during 2005 (P. Conant, personal communication). The combination of stately trees such as the coconut palm becoming unsightly or removed due to defoliation and the annoyance by medical problems from caterpillar spines has a potentially damaging impact on Hawaii's visitor industry.

Marc E. Epstein (at the time with the Smithsonian Institution, Washington D.C.) and Cheng-shing Lin (National Museum of Natural History, Taiwan) identified the original Hawaiian specimens of *D. pallivitta*. According to Holloway et al. (1987), *D. pallivitta* occurs in China, Taiwan, Thailand, western Malaysia, Indonesia, and Java, and its host plants in those regions include *Adenostemma* sp., *Areca* sp., *Breynia* sp., coconut, *Ficus* sp., grasses, maize, and oil palm. It is considered only a minor pest of coconut palms in its natural range, probably due to the presence of natural enemies that do not occur in Hawaii. Chayopas (1982) (unpublished and from Holloway et al. 1987) presented the following life-history data of *D. pallivitta* from Thailand for the mean duration of developmental stages (and their ranges): egg 4.8 d (4–5), larva 40.1 d (40–53), pupa 13.3 d (11–15), which likely represents the entire cocoon period as discussed further on, and adult 5.3 d (3–7) [note: the larval mean duration appears to be miscalculated, as it is too low based on the given range].

Given the potential for the spread of this species in Hawaii and beyond, we undertook a study to provide basic knowledge of the biology of *D. pallivitta* in the hope that it will aid in the development of monitoring and integrated control strategies. Previously there has been no detailed information on the biology, including life history and larval morphology, of this species in the literature other than the aforementioned unpublished information by Chayopas. We provide a description of the life history of *D. pallivitta*, which includes an unexpected morphological and developmental find: the first known example of SD2 verrucae in spiny limacodid caterpillars, which is not phenotypically expressed until later instars. Furthermore, *D. pallivitta* is presently the only limacodid caterpillar known to develop spiny scoli or verrucae from elongate tubercles found on the first instar; in subsequent instars these tubercles are normally transformed into simple hairlike setae or remain, becoming hairy rather than spiny (Epstein 1996).

Materials and Methods

Biological studies of *D. pallivitta* were conducted at the Hawaii Department of Agriculture (HDOA) Quarantine Laboratory in Honolulu (Oahu Island) during 2002. A colony was established from 26 cocoons received from the HDOA Hilo Insectary (Big Island) during March 2002. Cocoons were placed in a cage (42 × 42 × 62 cm) with Lumite™ screen (amber, 52 × 52 mesh, SI Corporation, Gainesville, GA) for emerging adults to mate and oviposit. Leaf bouquets of three plant species, coconut palm (*Cocos nucifera* L.), areca palm (*Chrysalidocarpus lutescens* Wendl.), and Hawaiian ti (*Cordyline terminalis* (L.)), were initially offered to larvae for feeding; however, only *C. terminalis* was ultimately used for maintaining the laboratory colony due to its larger leaf size and greater availability. Rearing conditions for all studies were 24.6 ± 1.5°C, 70.6 ± 5.2% RH, and 12:12 (L:D) photoperiod.

The life cycle was determined by observing 25 larvae, each isolated in a disposable petri dish (100 × 15 mm) lined with a #1 filter paper (9 cm round). These were reared individually and monitored almost daily to determine the number of instars. A petri dish was emptied of frass each time a larva was checked. Each new instar was determined by the presence of a molted skin, but in some cases, a molted skin was not found because the larva ate it. If this occurred, molting was based on two observations: the color change in the larva from dark to light and the absence of frass, which indicated that no feeding took place the day before molting. Due to their retractile heads (Epstein 1996) and feeding on cast skin, head capsules of the various larval instars could not be accurately measured. Larvae were fed the leaflets of areca palm throughout the study. A leaflet about 15–20 cm long was wrapped with a wet 1 cm wide strip of cotton at about 2 cm from its base and then snugly plugged into a small water-filled 1-dram glass shell vial (9 mm outer dimension × 30 mm length). This "bouquet" was placed in the petri dish so that the leaflet curled around the inside and was replaced when it began drying out (about 3 days) or was consumed. Precaution was necessary to avoid the stinging spines of the larva beyond the first few instars (Figs. 4 and 6).

All measurements for eggs, cocoons, and adult forewing length (= tegula to wing apex) were done under a Wild M8 dissecting microscope fitted with an ocular micrometer (0.02 mm units) at 25× or 50× magnification. Measured eggs were obtained from the laboratory colony deposited on *C. terminalis* leaves. Age of pupation within the cocoon was determined by cutting an oval, lengthwise observation window on one side of the shell (Fig. 21). A water-soluble glue (Lepage's, Gloucester, MA) used for pinning insects was carefully dabbed around the edges of the opening with a brush, followed by firm placement of a #2 glass cover slip (15 mm round) to complete the seal. In previous dissections of cocoons of known ages, pupation was found to occur at least 5 days following construction; therefore, windows were made when the cocoons were 3 days old. A total of 12 cocoons, three each that cocooned on 4 successive days, were "windowed" for observation and then checked daily for pupation.

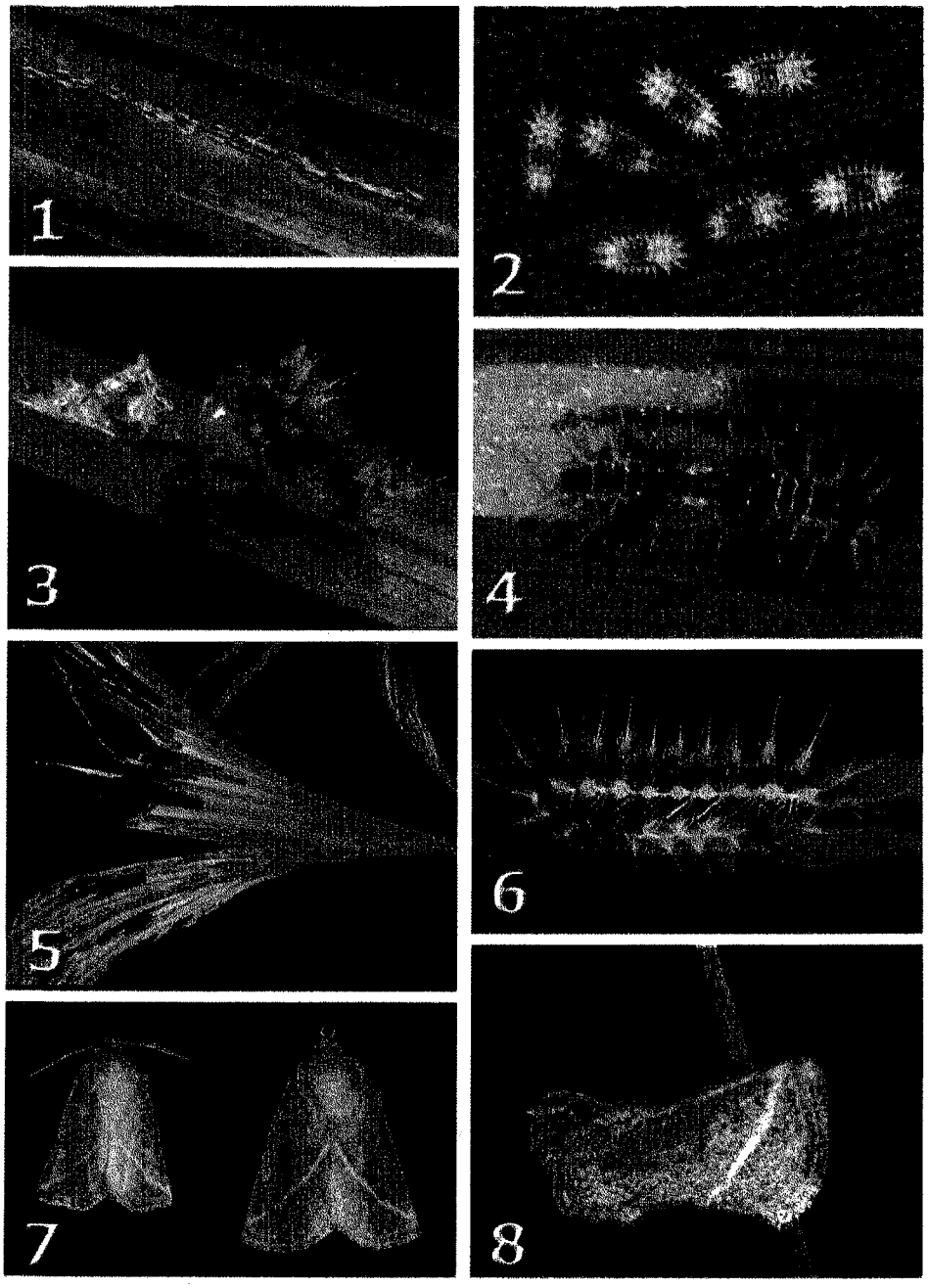
Mating and fecundity studies were conducted by pairing a one-day old female and a male adult in a wide-mouth glass gallon jar with an organdy cloth cover secured by rubber bands. Although intake of fluids is reported for adult Limacodidae (Epstein 1996), individuals were not given liquid prior to mating or oviposition. The entire jar was placed horizontally on a wooden rack. Each day, the adult pair was transferred to a new jar. Ten pairs were monitored daily for oviposition and longevity. The number of eggs deposited in the jar of the previous day was counted from the outside of the glass. As larvae hatched, they were removed from the jar with a fine-tipped brush and counted. This procedure was repeated until hatching ceased.

Counts of ovarian eggs to determine potential fecundity were done by dissecting ten one-day old females and removing their ovaries. The ovaries were placed in a glass petri dish (100 × 15 mm) with water and then the ovarioles "untangled" with a teasing needle. Counts of mature and immature eggs were done for each ovariole under a Wild M8 dissecting microscope. Eggs at the basal end of the ovariole, closest to the common oviduct, were considered to be mature, whereas others that showed a subtle change to a smaller size in the direction of the distal end of the ovariole were deemed immature.

RESULTS

Life Stages. Egg: Females mostly deposited the flat, scalelike eggs in masses, sometimes in a long line (Fig. 1), or singly. In the laboratory, females laid eggs on the cage interior and on the bouquets of host leaves. Egg masses were typically the width and length of two or three eggs with successive eggs overlapping; they appear as a translucent sheen on the leaf surface that is easily overlooked, blending in with the leaf color. Each egg is elliptical and measures about 1.1 × 1.6 mm (Table 3). Newly deposited eggs are yellow and within several days the embryo can be seen within as it develops. Duration of the egg stage was 7 days (Table 1).

Larva: All instars of *D. pallivitta* have the basic limacodid form with retractile head, small thoracic legs (Fig. 14), smooth elastic cuticle and sucker disks on the ventrum without crochets (Figs. 9–10), and an anal proleg with shagreened cuticle (Fig. 18) (Epstein 1996). The 1st instar is a nonfeeding stage that lasts two days (Figs. 2 and 9); it is pale yellow with a darker red-brown center and is gregarious (Fig. 2) in the sense that it hatches from clusters of eggs. The head has a fishtail-shaped spinneret, which is found most limacodids, particularly in the 1st instar, and in dalcerids (Epstein 1996); this shape is maintained throughout all instars (Figs. 11–12). The labrum has comblike spinules along the anterior margin (Fig. 11). The prothorax has two hairlike L (= lateral) setae anteroventral to the spiracle, while the other primary setae throughout this segment are of the same type, characteristic of limacodid caterpillars in all instars (Fig. 9)(Epstein 1996). The meso- and metathoracic segments (= T2 & T3) and abdominal segments A2 to A8 have two pairs of elongate tubercles on each segment, which correspond to one D (= dorsal) and SD (= subdorsal) seta on each side; these appear to be nonurticating and are branched at the apex (Fig. 9). Tubercles in limacodids are fleshy setae normally found only on 1st instars and are either simple or once divided at the apex (see discussion further on and Epstein 1996 for the homologies of these tubercles). On segment A1 the SD tubercle is missing and has a spiracle in its place, which is situated more dorsad than the line of spiracles to the posterior (Fig. 9). This condition is typical of known spiny limacodid caterpillars and persists throughout all instars. On A9 there is only one tubercle on each side; similar examples in other spiny caterpillars were considered to be a D tubercle by Dyar (1899), however it probably represents a fusion and reduction of D and SD tubercles (note: D and SD tubercles were referred to as subdorsal and lateral by Dyar 1899). The L setae of T2 and T3 and the abdomen have a hairlike dorsal member and a reduced, fungiform ventral member. *Darna pallivitta* is typical of limacodid species with spiny caterpillars in having a large increase in the number of spines, particularly between the 1st and 2nd instars (compare Figs. 9 and 10). The 2nd instar (Fig. 10) is the first feeding stage. Tubercles from the previous instar are transformed into spiny scoli, each with a broad and more elongate central spine that appears to be urticating. These scoli are surrounded by five or more spines on the D row and have four that are urticating and one that is a tactile seta along the bottom on the SD row. The more ventral of the two L setae on T2, T3 and the abdomen becomes hairlike rather than fungiform, as in the 1st instar, although it is shorter than its dorsal counterpart. Another change that occurs with the 2nd instar is the appearance of skin spines throughout the lateral and dorsal surfaces; these become capitate in later instars (Fig. 16). In subsequent instars the spines on the scoli increase in number while the caterpillars develop a variegated pattern of black patches and lighter orange brown in contrast to the



Figures 1–8. Life history and biology of *Darna pallivitta* (photographs by W. Nagamine). Fig. 1. Eggs. Fig. 2. First instars soon after hatching. Fig. 3. Newly molted 3rd instar and cast skin (note: head is fully exposed). Fig. 4. Fifth instar feeding on one leaf surface. Fig. 5. Early instar feeding damage on coconut leaf from early instars above and late instars below. Fig. 6. Stinging late-instar. Fig. 7. Adult male (L) and female (R). Fig. 8. Live adult resting posture (ventrum above).

light yellow or sometimes pink scoli (Fig. 6). The dark patterning does not extend below the SD scoli on A3 to A6; it produces a dumbbell appearance when viewed laterally or dorsally (Figs. 3–4). By the 6th instar the scoli are noticeably more elongate and nearly equal in size on T2, T3, A1, A2, A7 and A8 of the D row, the A9

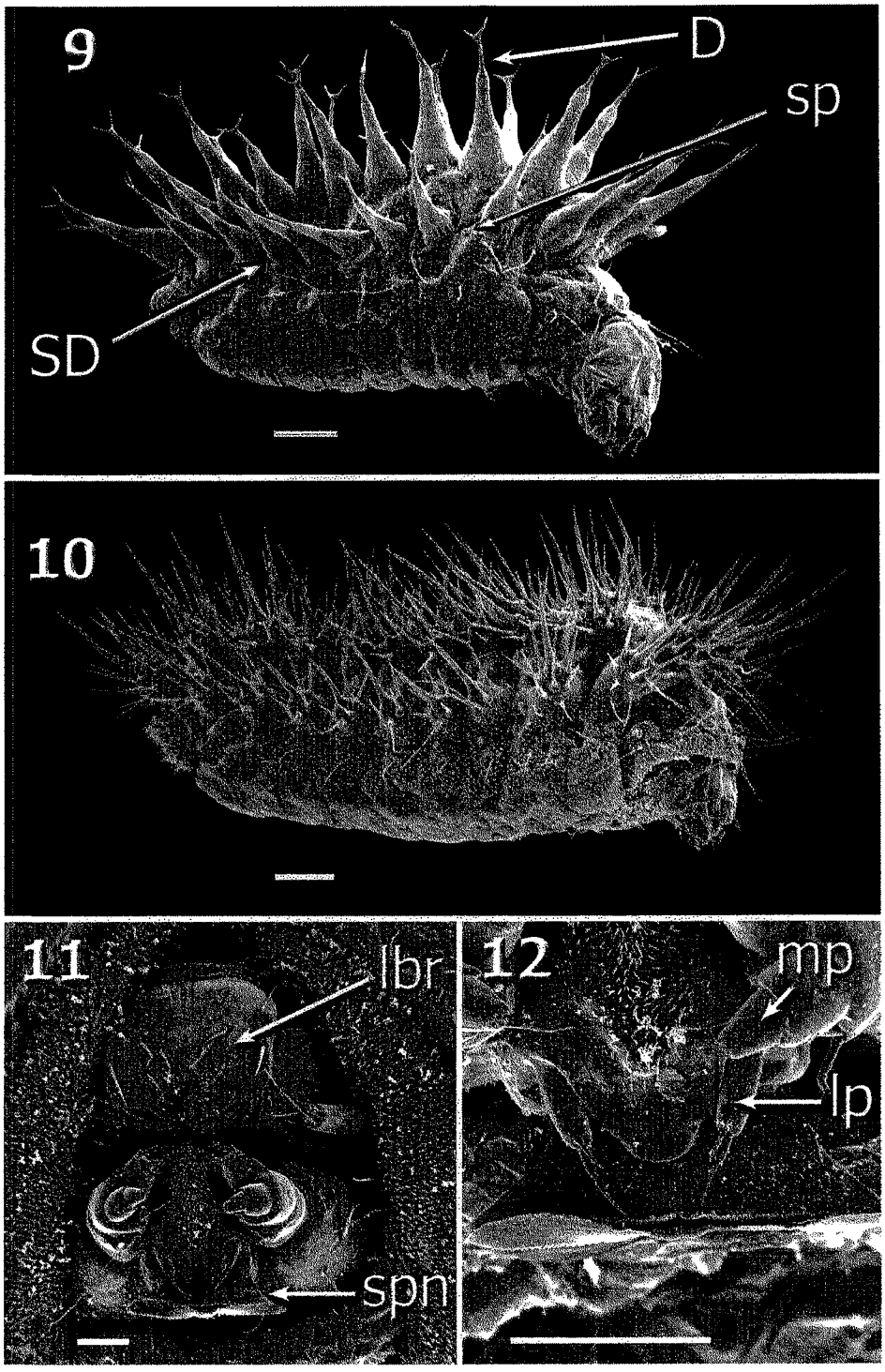
Table 1. Duration of developmental stages of *Darna pallivitta* reared individually on excised leaflets of areca palm.

Stage	N	Mean ± SEM (days)	Range (minimum to maximum days)
Egg	25	7.0 ± 0	7
Larva			
Instar 1	25	2.0 ± 0	2
Instar 2	25	5.8 ± 0.8	5–7
Instar 3	25	6.3 ± 0.5	6–7
Instar 4	25	6.0 ± 0.7	5–7
Instar 5	25	6.0 ± 0.6	6–8
Instar 6	25	6.9 ± 0.3	6–7
Instar 7	25	7.2 ± 0.9	6–9
Instar 8	25	8.2 ± 1.0	7–11
Instar 9	13	8.5 ± 1.3	7–11
Instar 10	5	8.2 ± 1.1	7–9
Instar 11	1	9.0	9
All larval instars	25	53.0 ± 6.9	45–72
Pupa	12	5.0 ± 0	5
Cocoon	25	19.1 ± 1.0	17–21
All stages	25	80.0 ± 7.1	72–99

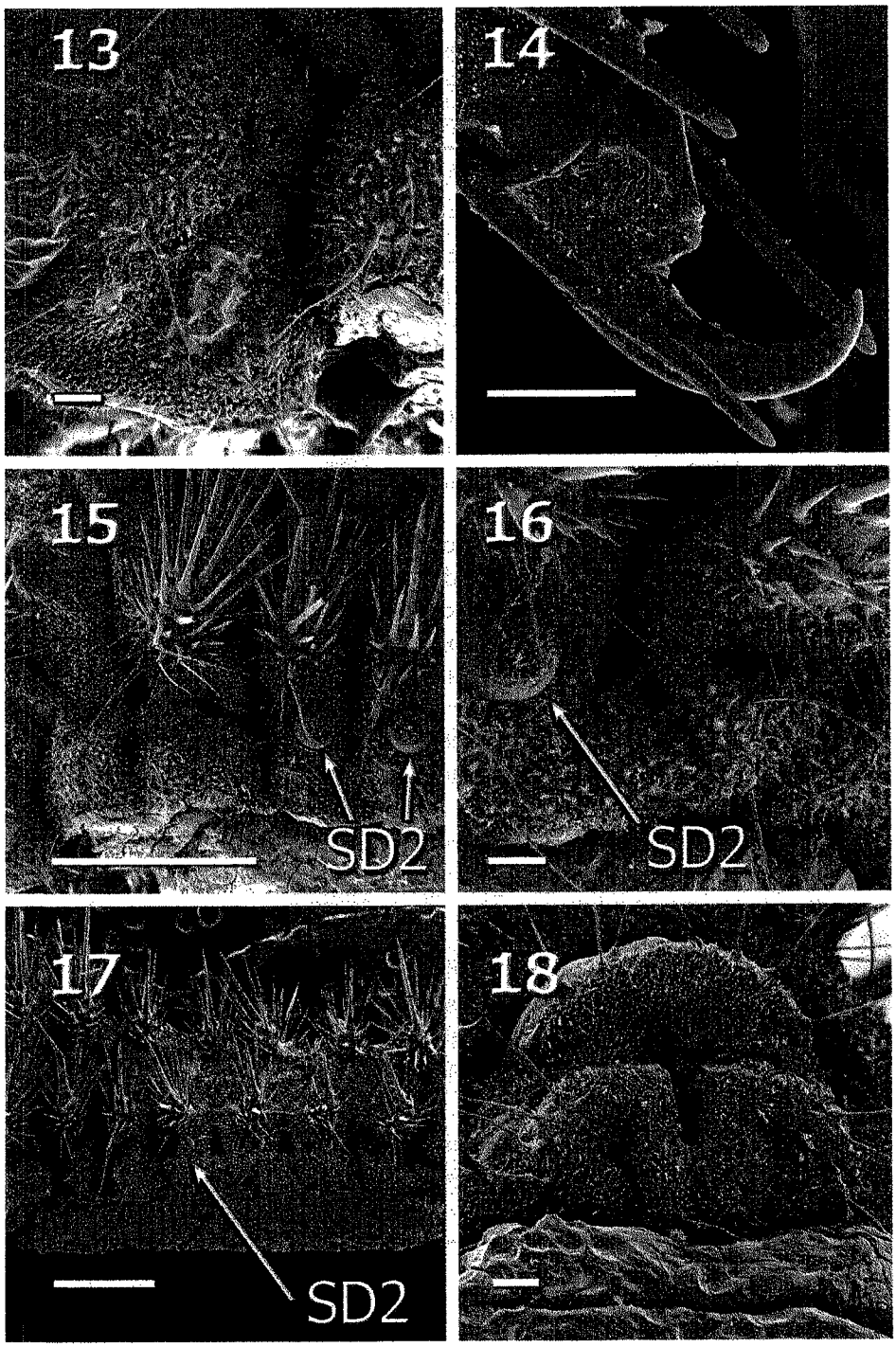
scolus, and on all segments of the SD row except on A1, where they are absent; those on the D row of A3 to A6 are short compared to all others (Fig. 6). All of these scoli develop dark spines except for the more elongate, pale colored central spine, which is always slightly longer than the scolus that bears it. The dorsum has a light colored medial stripe from the anterior to posterior bordered by dark patches that are broader between scoli on each side. The flanks have oblique stripes from the dorsal margin of the SD scolus to the posterior of the corresponding D scolus above it (Fig. 6). The 6th instar marks the first appearance of SD2 verrucae, which are partially sunken in the surrounding rough cuticle anterior to the spiracles on A2 to A4. Each is a wart ca. 2× the diameter of the proximal spiracle and has a dense vertical band of tiny spines (Figs. 15–17). In subsequent instars these verrucae are found in the same position on each segment from A2 to A7 (Fig. 17) (see discussion of homology further on); on T1 (Fig. 13), A1 (Fig. 15), and T8 (Fig. 16) they are absent.

The caterpillars feed on one surface of leaf mesophyll in short tracks parallel to the midrib through the 4th or 5th instar (Fig. 4). Later instars feed through the entire leaf except the midrib (Fig. 5). Each instar feeds until about two days before molting, at which time the larva becomes sessile and darkens to a grayish black. Upon molting (Fig. 3), the larva usually eats its cast skin, including the head capsule, before feeding on leaf tissues. In the laboratory the caterpillars were voracious eaters and there were a variable number of larval instars, ranging from 8 to 11 (Table 1). Duration of all larval stages is summarized by the instar that cocooned (Table 2); they are 49 d, 56 d, 63 d, and 73 d for individuals that reached 8th, 9th, 10th, and 11th instars, respectively.

Cocoon: There are more reliable indicators that cocooning (i.e., formation of the cocoon) is imminent than the instar number, which varies from 8 to 11 (Table 2). These include, first, the ventral side (belly) turning orange from its normal pale color and, second, about a day before cocooning, the larva expelling its last fecal pellet



Figures 9–12. Early instars and head of *D. pallivitta* (scanning electron micrographs by S. Kinnee; scale bar = 100 μm). Fig. 9. First instar (D = dorsal tubercle; SD = subdorsal tubercle). Fig. 10. Second instar (note: increase in spines). Fig. 11. Spinneret (spn) and labrum (lbr). Fig. 12. Spinneret, labial (lp) and maxillary (mp) palpi.



Figures 13–18. Selected morphology of 8th instar of *D. pallivitta* (scanning electron micrographs by S. Kinnee; scale bar = 100 μm unless indicated). Fig. 13. Prothoracic spiracle (note: absence of SD2 verruca). Fig. 14. Tarsal claw of thoracic leg (scale bar = 20 μm). Fig. 15. Lateral aspect of thoracic and abdominal segments A1-3; verrucae SD2 on A2 and A3 (scale bar = 1 mm). Fig. 16. Detail of SD2 verruca on A7 (note: A8, right, has no SD2 verruca). Fig. 17. SD2 verrucae on A2 through A7 (scale bar = 1 mm). Fig. 18. Anal proleg with remainder of anal segment (above) and smooth textured ventrum of A9 (below).

Table 2. Duration of larval instar that cocooned for *D. pallivitta*.

Instar that cocooned	N	Mean ± SEM (days)				Adults emerging
		Egg	All larval instars	Cocoon	All stages	
8	12	7.0 ± 0	49.2 ± 2.2	19.1 ± 1.0	75.3 ± 1.9	4♂♂, 8♀♀
9	8	7.0 ± 0	56.6 ± 2.1	18.8 ± 0.9	82.4 ± 2.3	5♂♂, 3♀♀
10	4	7.0 ± 0	63.8 ± 1.3	20.0 ± 0.8	90.8 ± 1.3	1♂♂, 3♀♀
11	1	7.0	73.0	19.0	99.0	1♂♂

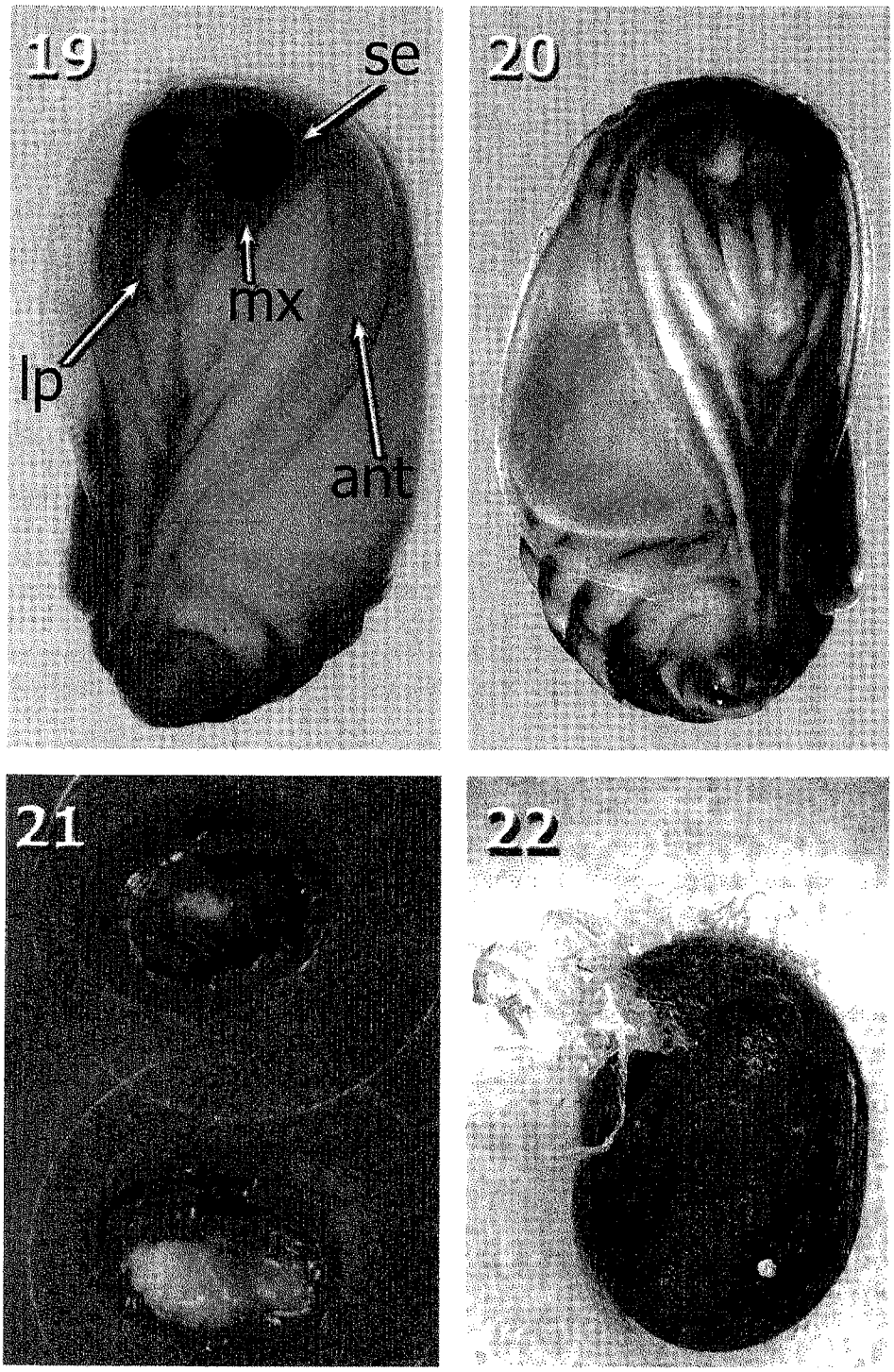
along with a clear, slightly viscous liquid. The prepupa then takes on a "C-shaped" form by wetting itself down, spinning brown silk around itself, and eventually forming a hardened brown outer shell (for a review and description of limacodid cocoon construction see Epstein 1996). The cocoon stage averaged 19 d with a range of 17–21 d. The female cocoon is slightly larger than that of the male (Table 3).

Pupation and duration: Pupation occurs within the cocoon on day 5 after cocooning; the pupa is typical of limacodids in having legs, labial palpi, antennae and wings that are free (Figs. 19–21). The maxillae, which are laterad of the labial palpi are shorter than half the length of these palpi and do not have a lateral extension, which commonly occur in limacodids, although not universally (Epstein 1996). The sculpted portion of the eye is found on the anterolateral margin of the eye, holding the front legs in place between the femoral and tibial junction. Pupae can be most easily sexed by the width of the antenna, which is equal to that of the eye in the basal third (= male) or narrower (= female) (Figs. 19–20). The shriveled larval skin and attached head capsule can be found within the cocoon after adult emergence. The pupa pushes out a cap on one end of the cocoon to emerge (Fig. 22). The duration from egg to adult was 75 d–99 d, depending on the number of larval instars stages (Table 2).

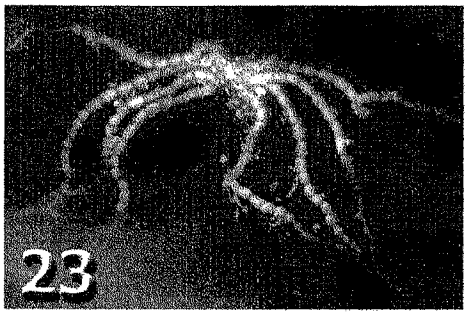
Adult: Adults of both sexes are externally similar except that females are larger, as indicated by forewing length (Table 3), and have filiform rather than bipectinate antennae (Fig. 7) typical of many Limacodidae. The rust-colored forewing is divided by a whitish diagonal line that runs from the midpoint of the inner margin to the

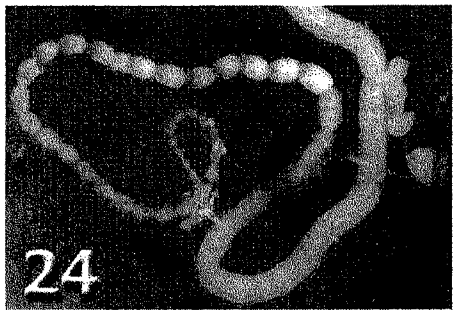
Table 3. Measurements of immature stages and adults of *Darna pallivitta*.

Stage	N	Dimensions (mm)	
		Mean ± SEM	Range
Egg:	10		
Width		1.1 ± 0.07	0.94–1.14
Length		1.6 ± 0.11	1.38–1.74
Cocoon:			
Female - width	11	6.56 ± 0.38	5.81–6.97
- length		8.45 ± 0.54	7.97–9.63
Male - width	14	6.42 ± 0.22	5.98–6.64
- length		7.88 ± 0.31	7.3–8.33
Adult forewing length:			
Female	11	11.89 ± 0.46	10.96–12.45
Male	14	10.51 ± 0.28	9.96–10.96



Figures 19–22. Pupae and cocoons of *D. pallivitta* (images by S. Kinnee unless otherwise indicated). Fig. 19. Male pupa (lp = labial palpus; mx = maxilla; se = sculpted eyepiece; ant = antenna). Fig. 20. Female pupa. Fig. 21. Male cocoons with coverslip windows for pupal observation (prepupa above and pupa below) (photo by W. Nagamine). Fig. 22. Open cocoon with pupal exuvia.





Figures 23–24. Ovarioles of *Darna pallivitta*. Fig. 23. Ovarioles removed from female. Fig. 24. Closeup of developing eggs in ovarioles.

apex (Fig. 7); the ventral surface and the hindwing are a lighter brown. Each sex has labial palpi that are visible beyond the head when viewed from above, short maxillary palpi that are obscured by the labial palpi, and the proboscis is absent. The lack of a proboscis correlates with observations that the female did not attempt to drink from a wetted cotton wick with or without sugar water. Mating started on the first day after emergence, sometimes during the daytime; however it is not known how long the sexes remain in copula. Forewing lengths of both sexes are compared as an indicator of size in Table 3. This species has a typical limacodid adult resting posture of holding the wings below the body and sometimes a moth was observed to grasp a vertical stem with its hind legs while its body hung in horizontal position (Fig. 8).

Fecundity. Potential fecundity: Counts of ovarian eggs (Figs. 23–24) of one-day old females showed potential fecundity to be extremely high (Table 4). Upon emergence, each female carried an average of 573 eggs, and of these, 202 were mature; this was about 35% of the total.

Reproductive attributes: A summary of reproductive attributes is shown in Table 4. Females laid an average of 479 eggs (range = 306–676) over their lifetime, with a hatching rate of 55%. Females can deposit their highest number of eggs on the

Table 4. Reproductive attributes and longevity for ten paired adults of *Darna pallivitta*.

Parameter	Mean ± SEM	Range	Unit
Pre-oviposition period	1.1 ± 0.3	1–2	Days
Oviposition period	6.1 ± 1.0	4–8	Days
Post-oviposition period	2.6 ± 1.5	0–5	Days
Age of first oviposition	2.1 ± 0.3	2–3	Days
Age of peak oviposition	2.1 ± 0.3	2–3	Days
Peak oviposition number	229.3 ± 72.8	124–339	Highest no. eggs laid on one day
Daily oviposition	80.4 ± 20.0	38.2–101	No. eggs laid during oviposition pd.
Total oviposition	479.3 ± 113.4	306–676	No. eggs laid during oviposition pd.
% egg hatch	55.5 ± 18.0	30.4–78.7	No. eggs hatching
Adult ♂♂ longevity	9.7 ± 1.1	8–12	Days
Adult ♀♀ longevity	11.0 ± 1.3	9–12	Days
Potential fecundity (ovarian eggs from ten 1-day old females)			
Mature eggs	201.5 ± 53.5	66–257	No.
Immature eggs	372.0 ± 146.6	110–569	No.
Total eggs	573.5 ± 184.1	176–796	No.

second day after emergence, averaging 229 (range = 124–339). From then on, the number of eggs declined over the six-day oviposition period, while the postoviposition period was 2.6 days. Female and male longevity averaged 9.7 and 11.0 days, respectively.

Discussion

Darna pallivitta has the potential to be a major pest in Hawaii from medical problems resulting from caterpillar stings and from its polyphagous larval feeding habit. The latter is of concern because this species has a high reproductive capacity and invasive status from a limited natural-enemy complex. The average of 479 eggs laid per female is several times higher than other reported pest species of Limacodidae, such as *Latoia viridissima* Holland in Africa (Igbinosa 1992). New generations of caterpillars of *D. pallivitta* are produced rapidly because the female ecloses with 35% of its eggs mature, not needing to feed to lay eggs, while depositing its highest number of eggs on the day after mating. Furthermore, it has continuous generations because there is no diapause (discussed further below). Limacodid caterpillars tend to be generalists on plants with glabrous leaves rather than hostplant specialists (Dyar 1899; Epstein 1996; Wagner 2005; Lill et al. 2006); this enables species such as *D. pallivitta* to feed on a broad array of plants. Although this general strategy as well as larval polyphagy are normal for Limacodidae, *D. pallivitta* appears to have the survival advantages of being the first species of Limacodidae to become established in Hawaii; these include being without the specialized parasitic Hymenoptera and Diptera that normally attack this moth family (see Cock et al. 1987 for information on these parasitoids).

We may now compare the number of instars and duration of larval and pupal stages of *D. pallivitta* with other species of Limacodidae, many of which are considered to be pests. The eleven instars found for some caterpillars of *D. pallivitta* matches the number found for *Parasa* (as *Latoia*) *lepida* (Cramer) (Chayopas 1982), the maximum number known for a species of Limacodidae. Nine instars have been reported for *Phobetron pithecium* (Cramer) (Dyar 1896) and *Acharia hyperoche* (Dognin) (as *Sibine megasomoides* Walker) (Mexzón et al. 1996). Although Dyar (1896) stated that he did not investigate the variability of instars of *P. pithecium*, many other species he reared had seven or eight instars (Dyar & Morton 1896; Dyar 1896; Dyar 1897). While the length of the pupal stage is not known for many limacodids, temperate species appear to remain as prepupae throughout the winter, only pupating a short period before eclosion the following summer (e.g., *Prolimacodes badia* (Hübner))(Wagner 2005; Epstein unpublished). Similarly, several Neotropical species examined by using the cocoon window remained as prepupae for lengthy periods, presumably in diapause to avoid adverse conditions during the dry season (e.g., *Talima postica* Walker)(Epstein unpublished). It is presumed that what is often referred to as "pupal duration" in the limacodid literature is in fact "cocoon duration," since there was no direct observation of the insect inside the cocoon. Therefore, only comparisons of the latter kind can be made between *D. pallivitta* and other limacodid species. The African species *L. viridissima* has a cocoon period of 32.9 days (Igbinosa 1985), suggesting that prepupal diapause did not take place, but this is a longer cocoon period than was found in *D. pallivitta* by nearly two weeks. *Parasa lepida* has a similar cocoon period of 22 days (Desmier de Chenon 1982) to that of *D. pallivitta*. The summer generation of the southern Arizona species of the

related family Dalceridae, *Dalcerides ingenita* (Hy. Edwards), has a similarly short prepupal period to *D. pallivitta* of 3 days (Epstein 1997).

The absence of plant feeding in the 1st instar, as found for *D. pallivitta*, appears to be typical of limacodid species that have caterpillars with spines on scoli or verrucae (e.g., *Euclea* spp., *Acharia* spp., *Parasa* spp., *Natada* spp.) (Dyar & Morton 1896; Dyar 1897). Limacodid caterpillars that become smooth beyond the 1st instar (= gelatine) (e.g., *Apoda*) or retain fleshy tubercles that become hairy and deciduous (e.g., *Phobetron*) begin feeding soon after hatching from the egg (Dyar 1896). Curiously, the first report of nonfeeding 1st instar limacodids was erroneous for *Apoda y-inversa*, a smooth limacodid caterpillar (Dyar & Morton 1895); this, however, was later corrected by Dyar (1898). Dyar's observations on feeding versus nonfeeding 1st instar limacodids have been verified over the last two decades by Epstein (unpublished) and J. Lill (personal communication).

MEE considers the development of nonfeeding 1st instars in spiny limacodids to be related to the tendency for species with spiny caterpillars to lay eggs in overlapping clusters and developmental factors (as discussed further on). This absence of feeding allows these caterpillars to avoid feeding on unhatched embryos in the same egg clusters. Conversely, limacodid caterpillars that feed as 1st instars and become smooth or retain tubercles in later instars hatch from eggs spaced further apart or on different leaves (Dyar & Morton 1895; Dyar 1896). This presumably reduces the probability of unhatched embryos being consumed. Nonfeeding 1st instars can also be viewed to be the result of developmental constraints between spiny caterpillars and their eggs. Unhatched limacodid caterpillars have inverted tubercles or scoli presumably to fit inside the flat egg (Epstein 1996). This is illustrated by the observation that once hatching occurs, the tubercles (Fig. 9) or scoli with 3 to 5 spines evert and the larva greatly expands in height. The shorter time spent as a 1st instar versus the other instars in *D. pallivitta* (2 d vs 5 d or longer) and in other limacodids with spiny caterpillars such as *Parasa lepida* (1 d vs 3.6 d) (Chayopas 1982) suggest that the 2nd instar is nearly developed when the egg hatches. In fact, the short nonfeeding period of 1st instar spiny limacodids can be viewed much like the nonfeeding days that occur prior to molting in the other instars. Furthermore, the 1st instar cuticle before hatching is in essence a covering that protects the flat, ultrathin chorion found in limacodid eggs (Epstein 1996) from the spines of the 2nd instar beneath. The related family Megalopygidae, in contrast, has a feeding spiny 1st instar with a dorsoventrally broad egg roughly the same height and shape of the larva inside, and has a thick chorion (Epstein 1996). This protects the egg from the spiny verrucae beneath and the caterpillar does not need to inflate after hatching.

Limacodid caterpillars are known to be heteromorphic, that is undergoing a change in form from first to later instars (Epstein 1996). *Darna pallivitta* is the first known example of a limacodid caterpillar known to convert elongate 1st instar tubercles into spiny scoli in the 2nd instar. This transformation may be more common than presently known because early instars have been described from only a few Old World taxa such as the spiny *Monema flavescens* Walker (Dyar 1909) and the smooth *Belippa horrida* Walker (Epstein 1996), both from Asia. The elongate 1st instar tubercles of *D. pallivitta* are similar to those in the Holarctic *Apoda* and New World *Phobetron* generic complexes (sensu Epstein 1996). The *Apoda* complex usually has a smooth dorsum after the 1st instar, while the *Phobetron* complex

retains tubercles throughout the larval stage, although they become detachable and hairy. In structure, the tubercles found on *D. pallivitta* are more similar to those of the *Apoda* complex in having a forked apex, whereas in number they match those of *Phobetron* complex in lacking an SD tubercle on the first abdominal segment.

Dyar (1899) referred to limacodid caterpillars with elongate 1st instar tubercles as a "primitive first stage" because they had the most complete setal representation and therefore most primitive known at the time. Dyar's (1899) genealogical tree of limacodid (= eucleid) species occurring in New York State, which is similar to a modern cladogram, has the primitive first stage at the base of the tree, while it is present with some fusion in both the "Palaearctic smooth Eucleids" (= *Apoda* complex) and "Tropic hairy Eucleids" (= *Phobetron* complex), and absent at branch points for the spiny genera termed the "Tropic spined Eucleids" (= *Parasa* and *Natada* complexes) and for the "Tropic smooth Eucleids" (= *Prolimacodes* complex)(all names of generic complexes *sensu* Epstein 1996). First instars of spiny caterpillars of *Parasa* and *Natada* generic complexes typically have three and up to seven spines (in the latter complex) on protuberances that are referred to as scoli rather than tubercles (Epstein 1996). Epstein (1996) followed Dyar's assessment of the 1st instar scoli in the two spiny complexes as being derivative of more simple tubercles; however, he considered those in the *Prolimacodes* complex to be a more primitive type. The setal arrangements found in two African genera, *Pantoctenia* Felder and *Crothaema* Butler, and Asian *Belippa* Walker are now considered to be the most complete and primitive based on homologies of relatively unfused tubercles found in the sister group Dalceridae (Epstein 1996).

Darna pallivitta is also the first known example of a limacodid species with a spiny caterpillar having SD2 verrucae or setal derivative. The only previous report of an unfused SD2 derivative were warts found in 1st instars of smooth caterpillars of African *Crothaema* and *Pantoctenia* (Epstein 1996). At present we do not know whether the SD2 verrucae (Figs. 15–17) occur in other species of *Darna* because examination of images in the literature are inconclusive (e.g., Holloway et al. 1987); the lateral position of the SD1 scoli obscures the spiracles and possible verrucae associated with them. While it is not known whether the phenotypic expression of the SD2 verrucae in later instars of *D. pallivitta* is an anomaly, other examples of developmental shifts of larval characters such as crochets previously reported in Dalceridae (Stehr & McFarland 1985; Epstein 1996) and Limacodidae (Epstein 1996) suggest otherwise for *Darna* and perhaps elsewhere in Limacodidae.

The first attempt to determine the relationship of *Darna* to other limacodid genera was made by Holloway (1986) and Holloway et al. (1987), who placed *Darna* in his "split-back" group. This was the only one of the four assemblages of genera defined by forewing venation rather than by a combination of the type of signum of the female genitalia and the general form of the caterpillar. Future investigation of the caterpillars in the group, both early and late instars, as well as other evidence will be needed to determine whether this grouping is monophyletic. For example, the late instars of two of the "split-back" genera, *Trichogyia* Walker and *Olona* Snellen, have a completely different shape and texture compared to those of *Darna* species (Holloway et al. 1987). However, a close look at the figure of *Olona gateri* West in Holloway et al. (1987) reveals that it has at least three tubercles on each side of a segment, similar to the condition we report here for *D. pallivitta*, except that the tubercles lack spines and are deciduous. Since the early instars of *Olona* are

undescribed, it is not known whether a SD2 row is added beyond the early instars, as occurs in *D. pallivitta*, or is present during the 1st instar as occurs in primitive *Crothaema* and *Pantoctenia* (Epstein 1996).

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