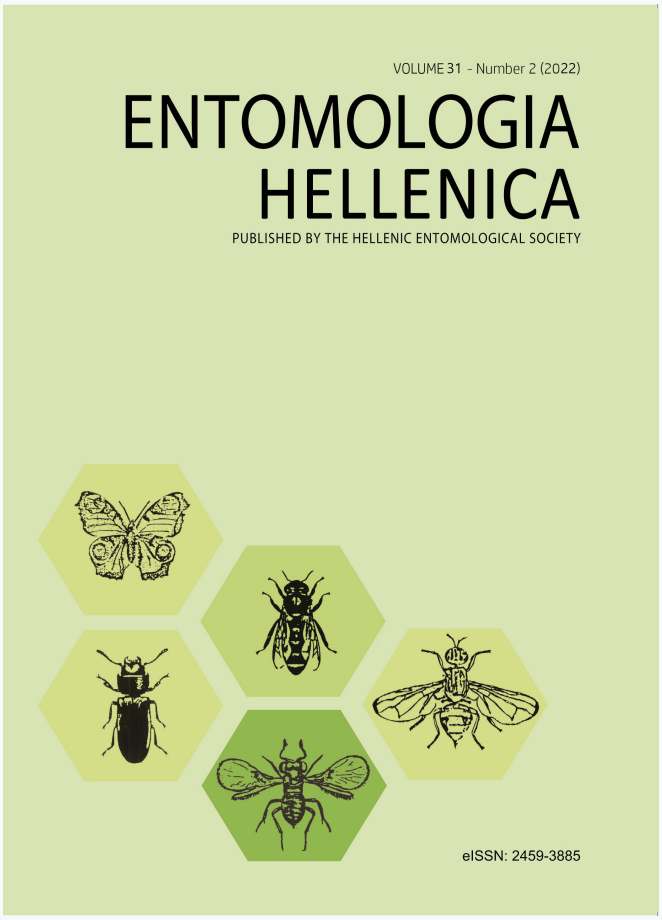
ENTOMOLOGIA HELLENICA

Vol 31, No 2 (2022)

Entomologia Hellenica 31(2)



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doi: 10.12681/eh.30648

To cite this article:

Galanos, C. (2022). Ecobiology of Danaus chrysippus (Linnaeus, 1758) (Lepidoptera: Nymphalidae, Danainae) throughout the autumn – early winter breeding period on Rodos Island, Greece. *ENTOMOLOGIA HELLENICA*, *31*(2), 71–80. https://doi.org/10.12681/eh.30648

ENTOMOLOGIA HELLENICA **31** (2022): 71–80

Received 26 April 2022 Accepted 26 August 2022 Available online November 2022

Ecobiology of *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae, Danainae) throughout the autumn – early winter breeding period on Rodos Island, Greece

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ABSTRACT

The present study is focused on the biology and ecology of *Danaus chrysippus* on Rodos Island, Greece, contributing on the knowledge of its life cycle in nature throughout the autumn – early winter breeding period, which is described and illustrated here for the first time for the country. Distribution range, habitat requirements and larval hostplant preferences, which are closely related with specific microclimatic conditions and the availability of milkweed resources in the region, are discussed.

KEY WORDS: Lepidoptera; Danainae; life cycle; *Cynanchum;* Greece; Aegean; Rodos.

Introduction

Danaus chrysippus (Linnaeus 1758) is a wide-ranging migrant species belonging to Danainae, a subfamily of Nymphalidae. It is a polyvoltine, polyphagous species, the larvae of which feed on plants containing cardenolides, especially species of the families Asclepiadaceae, Apocynaceae and Moraceae. The species prefers bushy, rocky and coastal places, usually near gardens and cultivated areas (Pisciotta et al. 2008). According to its status in the most recent IUCN Red List, it is classified as of Least Concern (LC) (Westrip 2021). It is widespread in the North African coastal regions (western Morocco, northern Algeria and Tunisia), from where it has colonized the coastal areas of the Canary Islands, southern Spain (where the first European population was found in 1980), southern France, Corsica, Sardinia, Sicily, Italy, Malta, Serbia, Montenegro, Croatia, Albania, Turkey, Cyprus and Greece (Perković 2006; Baytas 2007; Pisciotta et al. 2008; Pamperis 2009; John et al. 2019). In Greece, it has been reported mainly from the western coastal regions (Ionian Islands, Epirus, Sterea Hellas, Peloponnese, Attiki), while regarding the Aegean Sea region, it has been reported from Chios, Samos, Iraklia and Kriti Islands (Pamperis 2009; Gavalas 2013). On the islands of the Dodecanese complex, it has so far been reported from Astipalea, Kalymnos, Kos, Nisiros, Tilos, Rodos and Simi (Albrecht & Kissling 2013; Cuvelier & Mølgaard 2014; Galanos 2016; Mølgaard 2002; Nemec 2016; Pamperis 2009). Moreover, new sightings have also been reported to date both from continental Greece – coastal areas in Macedonia are also included – as well as from other Greek islands (Pamperis 2022).

In Rodos, specifically, *D. chrysippus* was first discovered and collected by Ghigi in August 1926 (Olivier 1993). Since then,

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however, it should be noted that no thorough fieldwork has been done so far on the species' life cycle throughout the year on the island. Thus, following recent studies of the butterfly fauna in the Dodecanese Islands conducted by Galanos (2014, 2016, 2017, 2020), further investigations were carried out to define the number of species' generations throughout the year, as well as to obtain more accurate data by monitoring its seasonal stages and to evaluate the bionomic information. In addition, notes on the butterfly's hostplant range, larval polymorphism and duration of immature stages depending on weather conditions, were also examined, and are herein illustrated.

Materials and Methods

Photographs of living butterflies and oviposition on the larval hostplant were taken in situ. Early stages of *D. chrysippus* were measured recording larval body length, as well as the pupal length and width (at its broadest point). The moulted female larval head capsules of each instar, from 1 to 5, were collected and their widths were measured comparatively under laboratory conditions to determine accurately the number of moults and consequently of species' instars that have occurred. Moreover, we were able to track the different larval stages by measuring larval frass particles in accordance with Bean (1959) and Southwood and Henderson (2000). All measurements were taken using a digital calliper (Total TMT321501). No adult specimen was collected due to conservation considerations, considering that it was not necessary since the species could not be confused with any other. Taxonomy and nomenclature follow Wiemers et al. (2018) for the butterfly fauna, Hassler (2004-2022) and Kleinsteuber et al. (2016) for the flora. All photographs were captured by Christos Galanos.

Results and Discussion

During 2020 and 2021, a series of field surveys was carried out at selected locations, which had previously been confirmed as breeding habitats of *D. chrysippus*, to in-depth study the species' metamorphosis from the early life stages, i.e. egg, larva and pupa, to the adult, with the aim of ascertaining the number of generations over the year. In particular, butterflies of *D. chrysippus* begin to arrive along the coastal areas of the island, where they remain for breeding, subject to suitable climatic and habitat conditions, i.e. absence of winds, storms and freezing temperatures, exposure to dappled sunlight, prevalence of high humidity and availability of water and food (Pelton et al. 2016). More specifically, it was found that migrants gradually appear on the island from early September to October, although butterflies have occasionally been recorded flying earlier, i.e. in May (Rebel, 1936), 1st of June (Mølgaard, 2002), as well as in August 1926 and 2012 by Ghigi (Olivier 1993) and the author, respectively.

Life cycle

Three broods are produced over autumn and early winter, specifically from September to early January, each one succeeding in the completion of its life cycle. In particular, the developmental interval from egg to adult of the first, second and third autumn – early winter generations lasts for 26, 28 – 30 and 40 days, respectively (Fig. 1).

In order to accurately study the life cycle of *D. chrysippus*, mature hostplants of *Cynanchum acutum* ssp. *acutum* (Apocynaceae) (Fig. 2A), where females deposited their eggs, were examined in situ during the ovum, larval and pupal stage. Additionally, shoots of plants, where females deposited eggs, were transferred to the laboratory to allow further study. Fresh

shoots with leaves were supplied on the morning of each day until pupation.

The female individuals were observed to lay eggs singly on the upper and the underside of the leaves, as well as on the stems of *C. acutum* plants (Fig. 2B). During oviposition, the forewings were seen to flutter continuously. It is worth noting that the presence of *D. chrysippus* was confirmed in every location where populations of *C. acutum* were recorded through the entire island, whereas the opposite did not occur. Thus, the specific plant species is here documented for the first time as its larval hostplant in Rodos, on which the butterfly has successfully developed through its full biological cycle.

C. acutum is an infrequent native perennial climbing shrub, which can be found along the coastline and prefers moist soils, rivers with patches of cane and reed beds. Such plants produce toxic cardenolides, which the larvae have adapted to remain unaffected and retain these toxins in their own bodies, which consequently pass on to the pupa and adults, protecting them against vertebrate predation. As a result, the majority of predators (birds, mammals, etc.) avoid consumption on account of their toxicity (Gil 2006; Malcom & Brower 1989).

Based on field and laboratory investigations it is clearly shown that the species' larvae develop through five instars, with developmental time dependent on temperature and availability of food sources. In accordance with the findings of comparative studies (Alam et al. 2019; Cockrell et al. 1993; Golestaneh et al. 2009; Smith et al. 1988; Talavera & Vila 2017; Zalucki 1982) and taking into account species' monitoring data collected by the author since 2012, the following may be stated: the first generation takes place in September and the duration from egg to adult lasts for about 26 days at an average maximum monthly temperature of 29°C ±2°C. The second generation takes place in October with an average maximum monthly temperature of 26°C ± 2°C and the development time from egg to adult lasts for 28 - 30 days. The third generation takes place in November and lasts up to December with average maximum monthly temperatures of 21°C ± 2°C and 18°C ± 2°C, respectively. The development time from egg to adult extends to about 40 days. Females were detected flying and laying eggs throughout December (6th, 12th and 22nd).

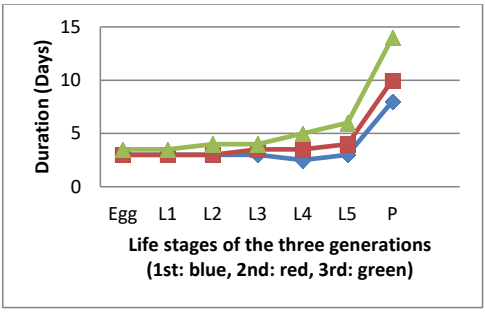


FIG. 1. Duration of early life stages of *D. chrysippus* over the three generations (L1, L2, L3, L4, L5 =1st, 2nd, 3rd, 4th and 5th instar, P=Pupa).

It is worth mentioning that during autumn (October, November, and early December), adults, eggs and larvae of all instars were also present at the same habitat, showing that there is an overlap of migrants gradually arriving in the region with the first-generation natives (male and female adults) (Fig. 2C). Final ovipositing was recorded on the 2nd of January, along with very few larvae of the first and the second instars, which fed on the last fresh leaves of the larval hostplant. At the end of January, with an average higher monthly temperature at 14°C ± 2°C, no traces of immatures were found, while the whole population of *C. acutum* plants had already withered, consequently larvae obviously died due to lack of food. It is pointed out that diapause was not confirmed at any biological stage. As perceived in the field,

the seasonal absence of the larval hostplant lasts until April when new plants begin to sprout. However, it is noted that an extensive plant-to-plant research of the total population was carried out, from April to August, but neither adults nor traces of the species' immature stages were found.

Considering all the above, we conclude that the most important factor that affects species' overwintering population dynamic is the absence of its principal hostplant, *C. acutum*, for a limited time.

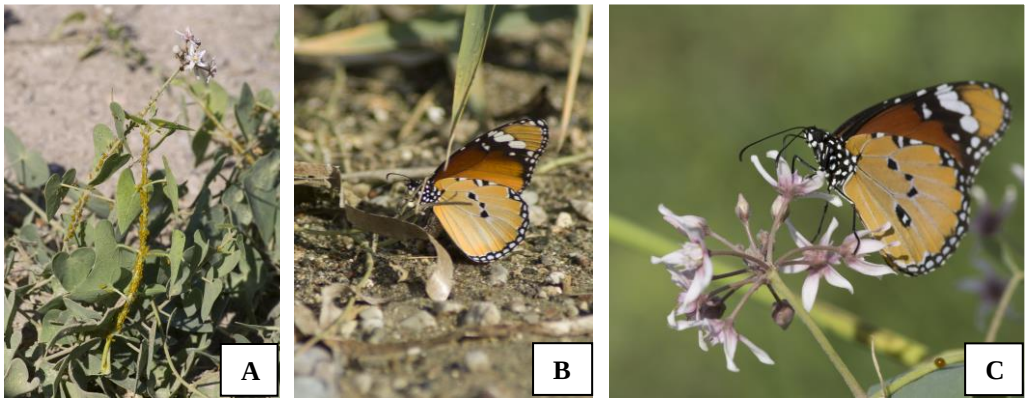


FIG. 2. (A) *C. acutum*, the larval hostplant of *D. chrysippus* in Rodos. Rodos, 12 Oct 2020; (B) Female of *D. chrysippus*, ovipositing on the leaves of its larval hostplant, *C. acutum*. Rodos, 19 Dec and 13 Nov 2020, respectively; (C) Male adult recently emerged, showing excreted meconium. Rodos, 25 Oct 2020. (Photos by Christos Galanos).

Immature stages

Egg: The eggs are white in colour when laid, turning creamy grey on the last day before hatching with an average size of 0.88 mm in diameter and 1.53 mm in height, barrel-shaped with a rounded top, flattened at base, longitudinally ribbed with ridges between the ribs (Fig. 3A, B).

Larva: The larvae passed through five instars, molted four times and the length of their fully grown bodies was measured (Fig. 4). Their frass pellets (Fig. 3L) and exoskeleton heads were collected and measured (Fig. 3M). Polymorphism in larval color was observed in the same studied areas, however it is not being considered as common (Fig. 6A, B, C).

1st Instar: Immediately after hatching, the young larva consumes its eggshell. Body is cream to yellowish with lateral hairs and shiny black head with a pair of black horns. Larvae measured from 2.88 mm up to 3.16 mm in length (Fig. 3C, D) and were observed to consume the soft leaves of the hostplant. At this stage the larvae grow up to 4.30 mm. The exoskeleton head measured 0.67 mm in width.

2nd Instar: At this stage the head capsule has a triangular white mark and a prominent white arch (Fig. 3E). Larvae grow up to 9.30 mm, while the exoskeleton head measured 1 mm in width.

3rd Instar: An extra white arch becomes apparent at the rear periphery of the head capsule (Fig. 3F). At the same time, frass pellets became clearly bigger than in the previous instar and larvae grow up to 19.86 mm. Exoskeleton head measured 1.81 mm in width.

4th Instar: Larvae were observed immediately after ecdysis to consume their discarded larval skins (Fig. 3G). At this stage frass pellets were found to be distinctly larger than in the previous instar,

while larvae grow up to 25 mm. Exoskeleton head measured 2.02 mm in width.

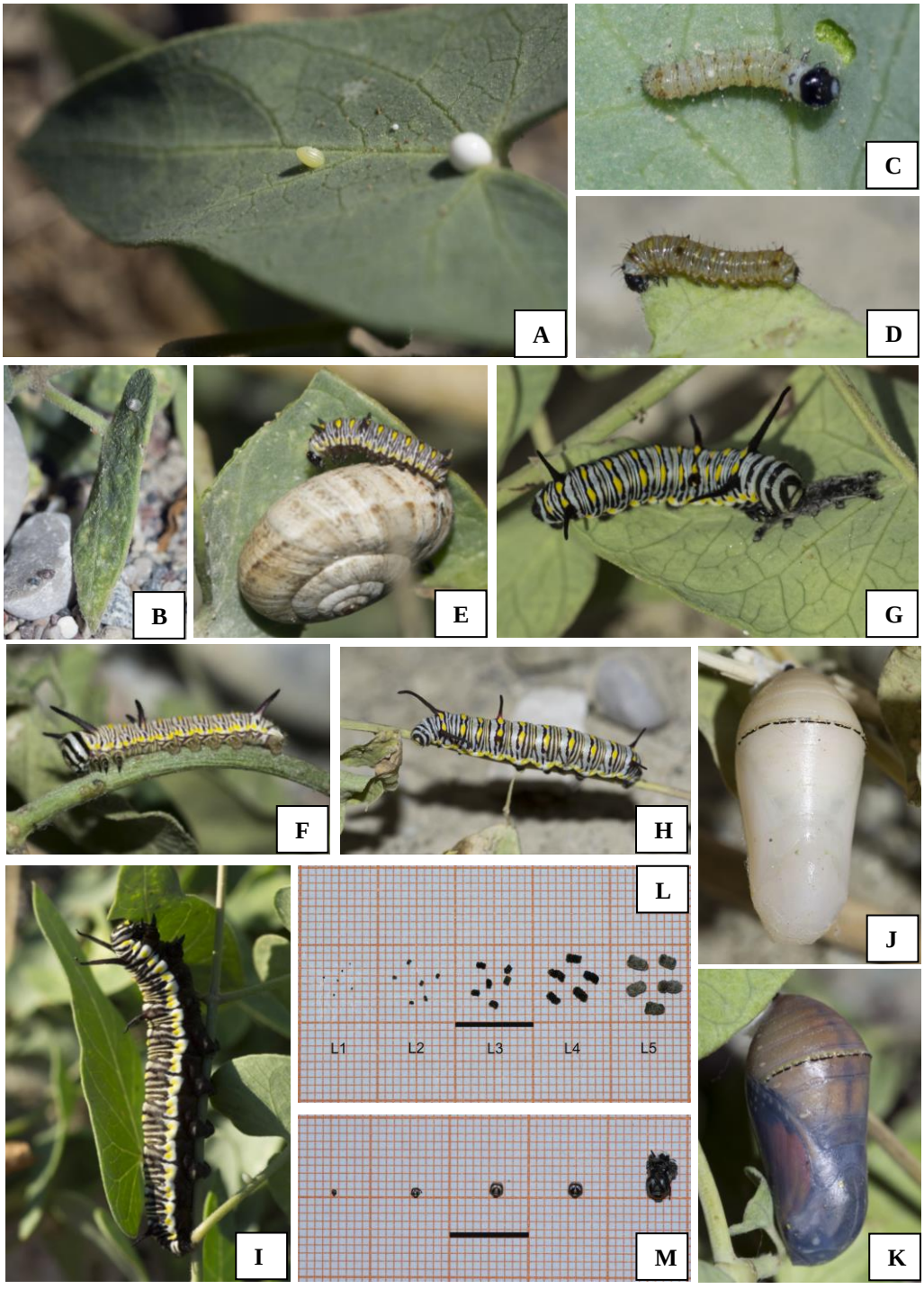
5th Instar: At this stage larvae grow up to 34.60 - 40 mm (Fig. 3H, I). Exoskeleton head measured 3.11 mm in width. Frass was collected and measured comparatively. The comparison revealed each instar to be noticeably distinct from the foregoing. It should be noted that the frass pellets were collected from a limited area where the larvae fed exclusively on the particular hostplant, *C. acutum*, both localised frass and larval specialisation being regarded as significant parameters in such an experiment (Southwood and Henderson 2000).

Pupa: At the pre-pupal stage the fully grown fifth-instar larvae become inactive, stop feeding and the last frass pellets are excreted. Subsequently, larvae become shorter and thicker before pupation and measured 21.10 mm in length. The pupa is translucent pale yellowish-green when first formed, eventually darkening while the wings and other parts of the body becoming visible through the pupal case. It measured 17.70 mm in length and 8.12 mm in width at its broadest point (Fig. 3J, K).

Conclusions

In conclusion, the present study aimed to assess the main reason for the species' temporary absence during winter, spring and summer, in particular, from late January to late August (Table 1). The results showed that the cessation of breeding is associated with the temporary absence of milkweed (*C. acutum*) from the end of January until April, which for the first time is documented here as the exclusive hostplant of the species on the island. No oviposition on other plant species of Apocynaceae or Convolvulaceae families, stated to be acceptable in other regions (Olivier 1993; Robinson et al. 2010) and which are present in Rodos, such as *Nerium oleander* L., *Ipomoea* sp. L. or *Gomphocarpus fruticosus* (L.) Ait. were observed. Despite the fact that suitable climatic conditions prevail in the area, specifically factors which favour the reproduction of the species throughout the year, i.e. protection from winds, absence of freezing temperatures, exposure to the right extent of sunlight, high humidity, as well as species' habitat requirements, such as the availability of water and nectar, the absence of the specific hostplant during the winter is decisive in terms of the lack of any form of life, up to arrival of the next wave of migration to the island at the end of the summer. Furthermore, this study contributes to the knowledge of the number of generations over the year, the identification of each larval instar of this species, the morphological traits and the development time of each life stage, as well as establishing life cycle duration depending on seasonal variations in ambient temperatures throughout the period of study.

FIG. 3. Metamorphosis of *D. chrysippus* during the early stages of its life cycle. A, B: *D. chrysippus* egg before and after hatching on fresh leaves of *C. acutum*; C, D: newly hatched 1st instar larva, dorsal and lateral view; E: 2nd instar larva moving on hostplant; F: 3rd instar larva, lateral view; G: newly moulted larva next to its exuvia after ecdysis from 3rd to 4th instar; H, I: fully grown 5th instar larva, lateral view; J, K: newly formed and fully grown pupa, dorsal and lateral view; L: comparison of collected frass pellets of fully grown larvae of the 1st, 2nd, 3rd, 4th and 5th instars. Scale bar: 1 cm; M: comparison of collected exoskeleton heads of all instars. Scale bar: 1 cm. Rodos 2020 - 2021. (Photos by Christos Galanos)



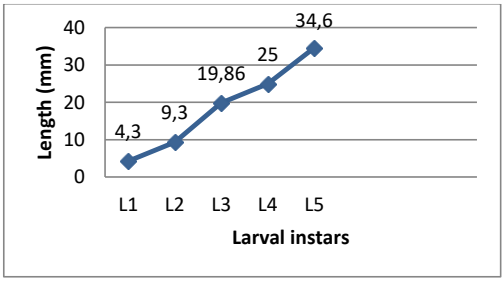


FIG. 4. Length of larval instars of *D. chrysippus.*

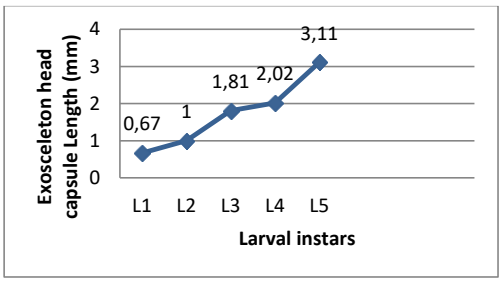


FIG. 5. Length of exoskeleton head capsules of different larval instars of *D. chrysippus.*

FIG. 6. Polymorphism in *D. chrysippus* larval color, in the same area of study. Rodos, 22-30 Oct 2020. (Photos by Christos Galanos).

Table 1: The life cycle of *D. chrysippus*, showing the annual emergence of adults, eggs, larvae and pupae in Rodos island. Each box corresponds to about 10 days.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Adult	■							■	■■■	■■■	■■■	■■■
Egg	■								■■	■■■	■■■	■■■
Larva	■								■■	■■■	■■■	■■■
Pupa									■	■ ■	■ ■	■

Acknowledgements

All research was conducted with the permission of the Greek Ministry of Environment, Energy and Climate Change (ref. numbers: 176960/2397, 1682/26-15.02.2021). The author gratefully acknowledges the General Directorate for the Protection and Development of Forests and the Rural Environment of the Greek Ministry of Environment and Energy, especially Mrs. Agapitou, for the permission to carry out surveys of both flora and fauna in areas protected by the European Network "Natura 2000", on the islands of the Dodecanese Complex. Special thanks go to the reviewers of the submitted manuscript, for their kind assistance, valuable comments, and excellent suggestions for improving this manuscript.

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Οικοβιολογικά χαρακτηριστικά της ημερόβιας πεταλούδας, *Danaus chrysippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae, Danainae) στη Ρόδο, Ελλάδα, καθ' όλη την περίοδο αναπαραγωγής του κατά τη διάρκεια του φθινοπώρου και, ειδικότερα, τον χειμώνα

ΧΡΗΣΤΟΣ ΓΑΛΑΝΟΣ

Ανεξάρτητος Ερευνητής επί της άγριας χλωρίδας και πανίδας λεπιδοπτέρων στο σύμπλεγμα των νήσων της Δωδεκανήσου, Πάροδος Φιλερήμου, 85101 Ιαλυσός, Ρόδος, Ελλάδα

ΠΕΡΙΛΗΨΗ

Η παρούσα έρευνα επικεντρώνεται στη βιολογία και την οικολογία της ημερόβιας πεταλούδας, *Danaus chrysippus*, στο νησί της Ρόδου, στην Ελλάδα, συνεισφέροντας στη γνώση του βιολογικού της κύκλου στη φύση, καθ' όλη την περίοδο αναπαραγωγής του είδους κατά τη διάρκεια του φθινοπώρου, και ιδιαίτερα κατά την περίοδο του χειμώνα, η οποία περιγράφεται και απεικονίζεται εδώ για πρώτη φορά για τη χώρα. Επίσης, το εύρος κατανομής, οι απαιτήσεις ενδιαιτήματος, οι οποίες σχετίζονται με τις μικροκλιματικές συνθήκες, που επικρατούν στις επιλεγμένες θέσεις αναπαραγωγής του είδους και η προτίμησή του προς το φυτικό είδος *Cynanchum acutum* ssp *acutum*, της οικογένειας Apocynaceae, ως μοναδικό ξενιστή των προνυμφών του στην περιοχή μελέτης για πρώτη φορά, συζητούνται.

