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The potential of Malaise traps as an important tool in butterfly (Lepidoptera, Papilionoidea) inventories, based on studies conducted in Republic of Congo

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Abstract: Results of butterflies sampled as by-catch in Malaise traps deployed in indigenous forests at Parc National de Nouabalé-Ndoki, Republic of Congo are presented. Using traps deployed with cyanide as a killing agent, rather than the standard ethanol, 153 species of butterfly belonging to five families were sampled, which constituted nearly one-third of the butterfly species known from the Park, with numerous species not encountered during general collecting. The benefits and drawbacks of using this technique, as well as the potential for these traps to be used as part of future butterfly inventories are discussed. The species samples are presented in a tabulated form.

Résumé: Les résultats des papillons de jour échantillonnés comme prises accessoires dans les pièges Malaise déployés dans les forêts indigènes du Parc National de Nouabalé-Ndoki, République du Congo sont présentés. En utilisant des pièges déployés avec du cyanure comme agent letal, plutôt que de l'éthanol standard, 153 espèces de papillons de jour appartenant à cinq familles ont été échantillonnées, ce qui constituait près d'un tiers des espèces de papillons connues dans le parc, avec de nombreuses espèces non rencontrées lors de la collecte générale. Les avantages et les inconvénients de l'utilisation de cette technique, ainsi que la possibilité d'utiliser ces pièges dans le cadre de futurs inventaires de papillons, sont discutés. Les espèces échantillonées sont présentés sous forme de tableau.

Key words: Afrotropical Region, biodiversity, Central Africa, faunistics, Republic of Congo, Wildlife Conservation Society.
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INTRODUCTION

The Malaise trap was designed to collect insects that fly close to the ground and around obstacles, and is regularly used as part of biodiversity inventories. Since the Swedish entomologist René Malaise (1892–1978) developed the first trap (Malaise 1937), there have been numerous other designs, modifications and improvements, but the principle of the Malaise trap remains largely the same. Although used primarily for the sampling of Diptera and Hymenoptera (*e.g.*, Cambell & Hanula 2007; Karlsson *et al.* 2005), these traps have previously on occasion been utilised successfully to evaluate the butterfly fauna of a given site (Covell & Freytag 1979), and even as by-catch, have indicated the potential for sampling interesting or poorly-known species (Rosa *et al.* 2019). The collecting bottle (or bottles) attached to a Malaise trap is often charged with ethanol, which has both advantages and disadvantages for Lepidoptera research. Ethanol is suitable for storage of many insect orders and on a broader scale using high-throughput sequencing technologies, it has become possible to estimate species diversity and characterise biodiversity using these samples (*e.g.*, Steinke *et al.* 2022). Molecular studies of butterflies sampled in these traps have yielded excellent results (Morinière *et al.* 2016) and it has been shown that ethanol-preserved specimens from Malaise traps can be successfully prepared for morphological analyses (Schmidt *et al.* 2019). Lepidoptera specimens, however, stored in an ethanol solution with large quantities of other insects are often in poor condition for morphological analyses (Schmidt 2016) and it has been shown that the more delicate specimens cannot be set adequately, whilst certain colours are not retained once the specimen has been fixed in ethanol (Schmidt *et al.* 2019).

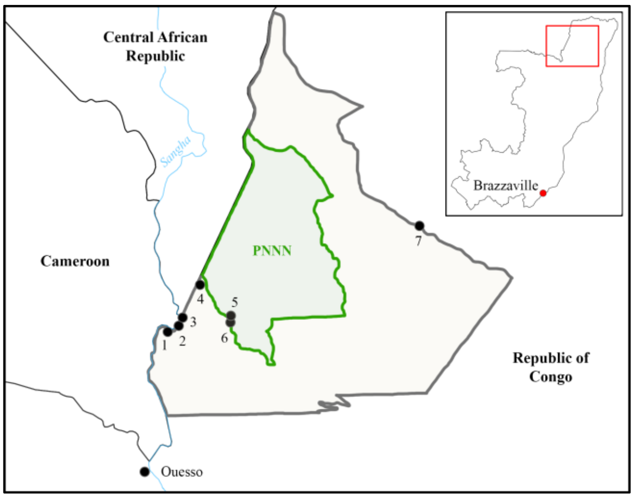

Figure 1 – Parc National d'Nouabalé-Ndoki (PNNN) and the surrounding Unité Forestière d'Aménagement. Sampling localities: 1. Mombongo Camp; 2. Bomassa Forest; 3. Wali Forest; 4. Mondika Camp; 5. Mbeli Camp; 6. Ndoki Formation; 7. Makao Forest.

During two recent entomological surveys undertaken by the African Natural History Research Trust (ANHRT) in Parc National de Nouabalé-Ndoki (PNNN), Republic of

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Congo, Malaise traps of two different types were deployed as part of Diptera-orientated sampling regimes (Fig. 1; Table 1), with all specimens collected dry, rather than into ethanol. This dry preservation has allowed the first thorough review of the effectiveness of such traps in the sampling of butterflies in a tropical African forest.

In this paper, the results of the butterfly by-catch are summarised and the very real potential for using Malaise traps as part of butterfly inventories is discussed.

Table 1 – Sampling conducted using Malaise traps in indigenous forest in Parc National de Nouabalé-Ndoki, Republic of Congo in September-October 2022 (CG-02) and February-March 2023 (CG-03).

Expedition No.	Locality	Coordinates	Dates (duration)
CG-02	Bomassa Forest	02°11'58.1"N 16°11'16.9"E	17–21.ix.2022 (5 days)
CG-02	Makao Forest	02°36'02.5"N 17°09'23.8"E	23–28.ix.2022 (6 days)
CG-02	Ndoki Formation	02°12'47.7"N 16°23'45.8"E	29.ix-01.x.2022 (2 days)
CG-02	Mbeli Camp	02°14'23.8"N 16°23'52.1"E	02–10.x.2022 (9 days)
CG-02	Wali Forest	02°13'56.8"N 16°12'13.9"E	11–16.x.2022 (6 days)
CG-03	Mombongo Camp	02°10'30.7"N 16°08'37.7"E	02–06.ii.2023 (4 days)
CG-03	Mondika Camp	02°21'50.6"N 16°16'25.8"E	07–14.ii.2023 (8 days)
CG-03	Mbeli Camp	02°14'23.8"N 16°23'52.1"E	15–19.ii.2023 (5 days)

METHODS AND MATERIALS

The two expeditions, upon which this study is based, took place between September/October 2022 (CG-02) and February/March 2023 (CG-03). Four expeditions were conducted in Parc National de Nouabalé-Ndoki of which butterflies were sampled from Malaise traps in the last three. The results of the paper only deal with data from the second (CG-02) and third (CG-03) expeditions and some of the species only collected in these traps were net-collected on the fourth expedition (CG-04). Additional unique species were also collected in the Malaise traps on the fourth expedition.

The methods used in the deployment of Malaise traps was outlined in detail by Kirk-Spriggs (2017). Two designs of Malaise traps were deployed in indigenous forest in Parc National de Nouabalé-Ndoki, viz. the 6 metre Gressitt & Gressitt-style Malaise trap (Gressitt & Gressitt 1962) (Figs. 2, 4, 5) and smaller Townes-style Malaise trap (Fig. 3). The larger traps are better suited to large flight paths in forested habitats, especially streambeds, forest clearings, disused roads and wider paths, whereas the smaller traps are suitable for narrow flight paths, such as forest paths and narrow streambeds.

Figures 2–5 – Malaise traps deployed in various localities and habitat types in Parc National de Nouabalé-Ndoki, Republic of Congo. **2** – Bomassa Forest (Gressitt & Gressitt-type trap over streambed). **3** – Makao Forest (Townes-type trap over streambed). **4** – Mondika Camp (Gressitt & Gressitt-type trap across forest path). **5** – Mombongo Camp (Gressitt & Gressitt-type trap across disused forest road). Photographs: Violette Dérozier.

Two Gressitt & Gressitt-style traps and three Townes-style traps were deployed at each sampling site, which were serviced twice daily, once in the early morning and once in the late afternoon. The collecting bottles were charged with cyanide and the specimens collected dry into tissue paper (see Kirk-Spriggs 2017), for later sorting and packeting.

Five sites were sampled during the CG02 expedition (Bomassa Forest, Makao Forest, Ndoki Formation, Mbeli Camp and Wali Forest) and three sites during the CG-003 expedition (Mombongo Camp, Mondika Camp and Mbeli Camp). Details of these sites and trapping durations are provided in Table 1.

Specimens were stored conventionally in glassine envelopes and dried using silica gel. All reference material is deposited in the collections of the ANHRT.

RESULTS

A total of 153 species of butterfly belonging to 69 genera and five families (Table 2 & Appendix) were sampled in Malaise traps over the course of two expeditions, which constituted 29% of the total number of species sampled (529 species). Of these species, 19 were solely caught in Malaise traps and were not encountered during general collecting.

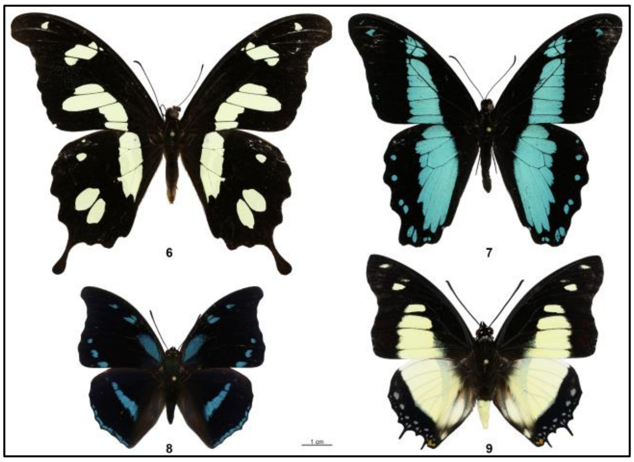
All the families sampled at PNNN were represented in the Malaise trap catch, and aside from Papilionidae, there was at least one species unique to the trap sample. Over half of the Pieridae species (54%), a little over one-quarter of the Nymphalidae (28%) and one-quarter of the Lycaenidae (25%) were sampled in Malaise traps. The Hesperiidae in the traps accounted for over one-third of the species (37%), with nine out of 67 species (13%) unique to the trap catch. The most abundant taxa in the Malaise trap samples were: *Mylothris* Hübner, [1819] (Pieridae), *Lachnoptera* Doubleday, [1847], *Neptis* Fabricius, 1807 (Nymphalidae), *Neurellipes* Bethune-Baker, 1910, *Triclema* Karsch, 1893 (Lycaenidae) and *Coeliades* Hübner, 1818 (Hesperiidae). For certain genera of Hesperiidae such as *Gretna* Evans, 1937 and *Pteroteinon* Watson, 1893, the Malaise trap sampled far more individuals than were netted.

Table 2 – Butterflies collected in Malaise traps deployed in Parc National de Nouabalé-Ndoki, Republic of Congo (PNNN), listed by family.

	Number of species			
Family	PNNN total	Malaise traps	Percent-age	Unique to trap (% of total)
Papilionidae	22	4	18%	0
Pieridae	24	13	54%	1 (4%)
Lycaenidae	178	45	25%	4 (2%)
Nymphalidae	238	66	28%	5 (2%)
Hesperiidae	67	25	37%	9 (13%)
Total	**529**	**153**	**29%**	**19 (4%)**

DISCUSSION

The deployment of Malaise traps in a Central African forest yielded a large diversity of butterfly species, some of which were not sampled through general collecting. The number of species trapped represented nearly one-third of the total number of butterfly species sampled during two periods of fieldwork. Aside from the work of Owen (1971) in Sierra Leone and Uganda, this is the only study to concentrate solely on the butterflies from Malaise traps deployed in the Afrotropics and the first to tabulate the species sampled.



Figures 6–9 – Examples showing the condition of larger butterflies sampled in Malaise traps in Parc National de Nouabalé-Ndoki, Republic of Congo. **6** – *Papilio (Princeps) hesperus hesperus* Westwood. **7** – *Papilio (Princeps) chrapkowskoides nurettini* Koçak. **8** – *Laodice mycerina nausicaa* (Staudinger). **9** – *Charaxes nobilis nobilis* Druce.

Despite the entrances of the collecting bottles of the larger Gressitt & Gressitt-type Malaise traps being relatively narrow, albeit markedly wider than that of the Townes-style Malaise trap, even the largest butterflies (*e.g.*, *Papilio (Princeps) hesperus hesperus* Westwood, [1843]) were able to enter the collecting bottles undamaged. Cyanide gas has a fast knock down rate, thus maintaining the condition of the specimens, with the majority, including even the most powerful fliers (*e.g.*, *Charaxes* Ochsenheimer, 1816), recovered in excellent condition (*e.g.*, Figs 6–9, for a selection of specimens sampled in Malaise traps). A surprising number of specimens from both expeditions, especially the Hesperiidae, were extremely fresh, suggesting that many of these individuals were recently eclosed and were perhaps intercepted by the traps on their initial flight. Cyanide as a killing agent has been reported to be suboptimal for DNA preservation in insects (Knyshov *et al.* 2019), but butterflies from the Malaise traps barcoded as part of on-going taxonomic studies (H. Takano, in prep.) sequenced successfully and it is here suggested that post-mortem conditions, especially the speed at which the specimens are dried are of greater importance than the killing agent itself.

It has been demonstrated that different types of Malaise trap sample different groups of insects (*e.g.*, Uhler *et al.* 2022), but it is not yet clear which type is most effective for Lepidoptera. The majority of Malaise traps are manufactured using either black or grey netting material, sometimes with a contrasting white roof on predominantly black traps, which being positively phototrophic, presumably encourages insects to fly upwards towards the light upon encountering the barrier sheet. In general, black traps are more effective as compared to those constructed of grey netting, probably because these are less visible to flying insects in shaded forests (A.H. Kirk-Spriggs, pers. obs.). Although samples from each separate Malaise trap deployed in PNNN were not segregated, it was apparent that the Gressitt & Gressitt-type Malaise traps (Figs 2, 4, 5) with their greater surface area for interception, yielded more individuals and species than did the smaller Townes-style Malaise traps (Fig. 3). At least half of the Malaise traps were set over small streams (*e.g.*, Figs 2 & 3), which may account for the large number of species known to “mud-puddle”, but conversely, numerous species of butterflies that feed from extra-floral nectaries (*e.g.*, Lycaenidae: Liptenini), or from fermenting fruit (*e.g.*, *Bicyclus* Kirby, 1871), and species from both vertical components of diversity (understory and canopy), as defined by Molleman *et al.* (2006), were sampled in these traps.

The Malaise traps were particularly useful and productive in collecting crepuscular species, especially in the Hesperiidae, which in areas where African forest elephant and African forest buffalo are abundant, such as at PNNN, is a safer, if not only alternative; many of the research sites were several kilometres distance from camps and walking after dark was forbidden in the Park, meaning the sites had to be left in good time to return to camp. Moreover, encountering and netting fast-flying Hesperiidae in low-light conditions is always difficult, and the Malaise traps were particularly effective at sampling these butterflies.

Despite there being numerous benefits of using Malaise traps for sampling butterflies highlighted above, there is one clear drawback which is the time it takes to erect and service the traps. Finding a suitable location and setting up a small Malaise trap is time-consuming, but setting up the larger traps will take considerably longer. Moreover, unlike ethanol, cyanide is not a preserving agent and so the collecting bottles need to be emptied at least once a day, preferably twice in these tropical conditions. With five traps spread out at each site in PNNN, albeit for the purpose of sampling Diptera, it took one team member an entire morning and an afternoon to empty the collecting bottles before sorting and storing the specimens.

CONCLUSIONS

The results of the butterflies sampled as Malaise trap by-catch at PNNN have demonstrated that there is great potential for utilising these traps as part of butterfly inventories. This technique sampled numerous species, including some which were not observed in the field, resulting in specimens suitable for molecular studies, which more often than not, were recovered in excellent condition, thus enabling accurate identification. Deploying these traps is, however, time-consuming and the practicality of utilising these in rapid assessments would depend upon a number of factors, such as the time available at a particular site, and the number of personnel available.

It is not advised that these traps be used alone, or as a replacement for general net-collecting; a great proportion of the species would otherwise be missed. Although baited aerial traps are regularly used as part of such faunistic surveys, other collecting techniques, such as Malaise traps and light traps, often yield species that are otherwise rarely encountered. For example, light traps at PNNN attracted numerous taxa that were not encountered during general net collecting, such as the genera *Aslauga* Kirby, 1890, *Iridana* Aurivillius, 1921 and an undescribed species of *Anthene* Doubleday, 1847 (H. Takano, in prep.). Moreover, data from these traps could be used within a statistical framework to analyse relative abundance and estimate population sizes, whilst direct comparisons could be made between traps set across Africa.

The positioning of the traps is an important aspect of how successful the catch is, and it is worth noting that the use of Malaise traps in open habitats yields far fewer butterflies in general (A.H. Kirk-Spriggs, pers. obs.). Much like in afforested environments, however, deploying a trap across insect flight corridors in xeric environments (*e.g.*, dry river beds), will increase the likelihood of collecting the greatest diversity.

Further experiments are planned by the authors to investigate the stratification of butterflies by deploying specially-designed Malaise traps in the canopy to understand their vertical distribution and diversity. Butterflies only made up a small proportion of the Lepidoptera sampled as by-catch in the Malaise traps and the observed diversity of the moth fauna was high, especially of families such as Crambidae and Pyralidae as well as crepuscular Geometridae and uncommon female Sphingidae specimens. It is believed many species not encountered at light were sampled and a full inventory and comparison would make an interesting study.

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APPENDIX: List of species collected in Malaise traps deployed at Parc National de Nouabalé-Ndoki, Republic of Congo.

(*) indicates species only sampled in traps.
(+) indicates species not encountered through general net collecting (but were attracted to light traps).

Papilionidae
Papilio (Princeps) chrapkowskoides nurettini Koçak, 1984
Papilio (*Princeps*) *hesperus hesperus* Westwood, [1843]
Papilio (*Princeps*) *lormieri lormieri* Distant, 1874
Graphium (*Arisbe*) *policenes policenes* (Cramer, [1775])
Pieridae
Nepheronia argia argia (Fabricius, 1775)
Nepheronia pharis pharis (Boisduval, 1836)
Belenois theora ratheo (Suffert, 1904)
Belenois calypso dentigera Butler, 1888
Belenois theuszi (Dewitz, 1889)
Appias (*Glutophrissa*) *sabina sabina* (Felder & Felder, [1865])
Appias (*Glutophrissa*) *sylvia sylvia* (Fabricius, 1775)
Mylothris maxima maxima Berger, 1981
Mylothris zaireensis zaireeeptinsis Berger, 1981
Mylothris asphodelus asphodelus Butler, 1898
Mylothris sulphurea basalis Aurivillius, 1906
Mylothris chloris chloris (Fabricius, 1775)*
Mylothris rhodope (Fabricius, 1775)
Lycaenidae
Lachnocnema exiguus Holland, 1890
Spalgis lemolea lemolea Druce, 1890
Pentila tachyroides tachyroides Dewitz, 1879
Ptelina carnuta (Hewitson, 1873)
Telipna cameroonensis Jackson, 1969
Ornipholidotos amieti amieti Libert, 2005
Ornipholidotos gemina fournierae Libert, 2005
Citrinophila tenera (Kirby, 1887)
Liptena fatima fatima (Kirby, 1890)
Liptena xanthostola xanthostola (Holland, 1890)
Falcuna margarita (Suffert, 1904)
Falcuna cf. *kasai* Stempffer & Bennett, 1963*
Tetrarhanis ilma ilma (Hewitson, [1873])
Epitolina dispar (Kirby, 1887)
Oxylides gloveri Hawker-Smith, 1929
Aphnaeus argyrocyclus Holland, 1890
Iolaus (*Epamera*) *farquharsoni* (Bethune-Baker, 1922)*
Hypolycaena antifaunus antifaunus (Westwood, [1851])
Hypolycaena lebona lebona (Hewitson, [1865])
Hypolycaena dubia Aurivillius, 1895
Paradeudorix ituri ituri (Bethune-Baker, 1908)
Paradeudorix cobaltina (Stempffer, 1964)
Anthene rubricinctus rubricinctus (Holland, 1891)
Anthene sylvanus (Drury, 1773)
Anthene afra afra (Bethune-Baker, 1910)
Anthene princeps (Butler, 1876)
Anthene larydas (Cramer, [1780])
Anthene irumu (Stempffer, 1948)*
Neurellipes lachares toroensis (Stempffer, 1947)
Neurellipes leptines extensa Libert, 2010
Neurellipes ngoko ngoko (Stempffer, 1962)
Neurellipes sp. n.
Neurellipes ducarmei occidentalis Libert, 2010
Neurellipes makala (Bethune-Baker, 1910)
Neurellipes pyroptera (Aurivillius, 1895)
Neurellipes zenkeri zenkeri (Karsch, 1895)
Triclema fasciatus subnitens (Bethune-Baker, 1903)
Triclema lutzi Holland, 1920
Triclema phoenicis (Karsch, 1893)
Triclema rufoplagata ituriensis Joicey & Talbot, 1921*
Pseudonacaduba aethiops (Mabille, 1877)
Uranothauma cyara cyara (Hewitson, [1876])
Leptotes pirithous pirithous (Fabricius, 1767)
Azanus mirza (Plötz, 1880)
Azanus isis (Drury, 1773)
Nymphalidae
Elymnias bammakoo bammakoo (Westwood, [1851])
Bicylcus xeneoides Condamin, 1961
Bicyclus medontias (Hewitson, 1873)
Bicyclus sebetus (Hewitson, [1877])
Bicyclus sandace (Hewitson, [1877])
Bicyclus moyses Condamin & Fox, 1964
Bicyclus dorothea dorothea (Cramer, [1779])
Bicyclus auricruda fulgida Fox, 1963
Hallelesis asochis congoensis (Joicey & Talbot, 1921)
Charaxes lucretius intermedius van Someren, 1971
Charaxes brutus angustus Rothschild, 1900
Charaxes nobilis nobilis Druce, 1873
Eriboea etesipe etesipe (Godart, [1824])
Eriboea hildebrandti hildebrandti (Dewitz, 1879)
Eriboea ochracea (van Someren & Jackson, 1957)
Eriboea cedreatis (Hewitson, 1874)
Eriboea virilis virilis (van Someren & Jackson, 1952)
Viridixes eupale latimargo (Joicey & Talbot, 1921)
Laodice lycurgus (Fabricius, 1793)
Laodice mycerina nausicaa (Staudinger, 1891)
Polyura kahldeni (Homeyer & Dewitz, 1882)
Polyura pleione congoensis (Plantrou, 1989)
Polyura paphianus paphianus (Ward, 1871)

Palla publius centralis van Someren, 1975
Apaturopsis cleochares cleochares (Hewitson, 1873)
Libythea labdaca Westwood, [1851]
Precis rauana silvicola Schultze, 1916
Hypolimnas anthedon anthedon (Doubleday, 1845)
Junonia sophia sophia (Fabricius, 1793)
Neptidopsis ophione ophione (Cramer, 1777)
Sevenia amulia amulia (Cramer, 1777)
Sevenia occidentalium occidentalium (Mabille, 1876)
Cymothoe haynae diphyia Karsch, 1894
Cymothoe hypatha hypatha (Hewitson, [1866])
Cymothoe confusa Aurivillius, 1887
Cymothoe indamora indamora (Hewitson, [1866])
Cymothoe caenis (Drury, 1773)
Cymothoe distincta distincta Overlaet, 1944
Cymothoe cf. *arcuata* Overlaet, 1944
Cymothoe sangaris sangaris (Godart, [1824])
Pseudacraea clarkii Butler & Rothschild, 1892
Pseudacraea kuenowii gottbergi Dewitz, 1884*
Pseudacraea lucretia protracta (Butler, 1874)
Neptis cf. *continuata* Holland, 1892
Neptis agouale Pierre-Balthus, 1978
Neptis nicoteles Hewitson, 1874*
Neptis nicomedes Hewitson, 1874
Neptis nicobule Holland, 1892
Neptis nigra Pierre-Balthus, 2007
Neptis stellata Pierre-Balthus, 2007
Neptis jamesoni Godman & Salvin, 1890
Neptis strigata strigata Aurivillius, 1894
Neptis metella metella (Doubleday, [1850])
Evena angustatum (Felder & Felder, [1867])
Aterica galene extensa Heron, 1909
Euriphene (*Euriphene*) *tessmanniana* (Bryk, 1915)*
Bebearia (*Apectinaria*) *zonara* (Butler, 1871)
Bebearia (*Apectinaria*) *micans* (Aurivillius, [1899])
Bebearia (*Apectinaria*) *amieti* Hecq, 1994*
Bebearia (*Bebearia*) *eliensis eliensis* (Hewitson, [1866])
Euphaedra (*Medoniana*) *medon viridinota* (Butler, 1871)
Telchinia parrhasia servona (Godart, [1819])*
Telchinia peneleos peneleos (Ward, 1871)
Telchinia bonasia (Fabricius, 1775)
Phalanta eurytis eurytis (Doubleday, [1847])
Lachnoptera anticlia (Hübner, [1819])

Hesperiidae

Coeliades forestan forestan (Stoll, [1782])
Coeliades libeon (Druce, 1875)
Apallaga illustris (Mabille, 1891)*
Procampta rara Holland, 1892
Abantis rubra Holland, 1920*
Gorgyra afikpo Druce, 1909*
Gorgyra minima Holland, 1896
Rhabdomantis galatia (Hewitson, [1868])
Osmodes adonides Miller, 1971
Osmodes thora (Plötz, 1884)
Semalea pulvina (Plötz, 1879)
Andronymus caesar caesar (Fabricius, 1793)
Gamia buchholzi (Plötz, 1879)*
Gretna carmen carmen Evans, 1937*
Gretna waga (Plötz, 1886)
Pteroteinon capronnieri (Plötz, 1879)
Pteroteinon caenira (Hewitson, [1867])
Pteroteinon concaenira Belcastro & Larsen, 1996*
Pteroteinon laufella (Hewitson, [1868])*
Leona meloui (Riley, 1926)
Caenides dacena (Hewitson, 1876)
Monza alberti (Holland, 1896)*
Melphinyet statirides (Holland, 1896)
Melphinyet unistriga (Holland, 1893)
Fresna carlo Evans, 1937*