LIFE HISTORY AND KARYOLOGY OF *PARALASA NEPALICA* (LEPIDOPTERA: NYMPHALIDAE, SATYRINAE)

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Abstract – Partial life history and dividing chromosomes are described and illustrated for a rare highland satyrine from Nepal, *Paralasa nepalica* Paulus. These characters, heretofore unknown for the genus *Paralasa,* are discussed in the context of the modern perception of the phylogenetic position of the genus.

Key words: chromosome, egg, *Erebia*, Erebiina, evolution, karyology, larva, morphology, Nepal, Nymphalidae, *Paralasa*, systematics, Satyrinae, *Ypthima*, Ypthimina, Scanning Electron Microscopy.

The basic chromosome number for Satyrinae, according to numerous studies, seems to fall within narrow limits of n=30, with n=29 being the predominant number (e. g., Robbinson, Kudrna, ed., 1990, Brown et al., 2007). However, some genera show great variation in this character, which variation in the past has been successfully applied to systematics (e.g., Lorkovic, 1972; Lukhtanov, 1987). The genus *Erebia*, for instance, exhibits remarkable variation in chromosome numbers even within a single species group. Thus, in the *E. tyndarus* group, the chromosome number varies from n=8 (*E. calcarius* Lork.) to n=51 (*E. iranica* Gr. Gr.) (Lorkovic, 1961). This species group has become a textbook example of speciation in butterflies (e. g., Descimon and Mallet, 2008). Many *Erebia* species, however, have 28-29 chromosomes and, probably due to the fusion of several chromosomes, numbers in the lower 20s are also frequently observed.

The genus *Paralasa* Moore was separated from *Erebia* in 1883, and Warren (1936) distinguished these two genera by male genitalia characters. Though Miller (1968) shows unequivocally that *Paralasa* belongs to the *Callerebia* series of Ypthimina, many authors continue to refer to it as a subgenus of *Erebia* (e.g. Schetkin and Schetkin, 1991), probably mostly as a result of similar ecology and distribution, rather than in consideration of morphology of *Paralasa*. The recent molecular phylogeny of Satyrinae (Peña et al., 2006) supports Miller's placement of *Paralasa* within Ypthimina as a sister of the Old World genus *Ypthima*.

Here, we provide illustrations of the partial life history and karyotype of one of the rare, recently described (Paulus, 1983) *Paralasa* species, *P. nepalica*, from the Himalayas of northwestern Nepal. As far as we know, this is the first information of this kind for the genus.

MATERIALS AND METHODS

The first author collected the specimens of *P. nepalica* in June 1997 above Phoksumdo village, Dolpa Province, Nepal, at the elevation of 3,500 m. Females were kept alive and fed daily with sugar solution. They laid eggs on the dry stems of grass supplied to them randomly. Eggs and first instar larvae were preserved in alcohol. The larvae refused to feed on the grasses randomly offered to them, thus we can conclude that they are oligophagous in nature. Preserved material was subjected to critical point drying and examined using a Hitachi Scanning Electron Microscope. Testes of live, wild-caught males were removed with fine dissecting forceps and fixed in vials containing 3:1 alcohol: acetic acid (Emmel 1969). Upon returning from the field the vials containing the testes were stored at -20°F until processing in the Gainesville laboratory. For microscopic observation, testes were stained with lacto-aceto-orcein, and squashed under a press until the testicular material was approximately one cell layer thick. Preparations were examined with a Carl Zeiss Research Microscope Standard WL fitted with 25x, 40x, and 100x planapochromatic flatfield objectives and automatic camera (refer to Emmel 1969 for further details). All uniformly spread metaphase plates of each individual were counted in order to verify the count and determine any variation within a particular specimen.

RESULTS AND DISCUSSION

The chromosome count, based on two *P. nepalica* individuals, shows that haploid chromosome number in this species is n=10 (Fig. 1). This number is also not unusual for *Erebia* (e. g. *E. cassioides*), and, probably, represents a derived character state. Among Satyrinae the chromosome number n=10 is known in *Auca nycteropus* (while *Auca coctei* has n=20), in *Pampasatyrus ocelloides* (while *Pampasatyrus nilesi* has n=41), in *Erebia cassioides* (while other species of *Erebia* have from n=8 to n=52) (Brown et al., 2007; Robinson, 1971). It is clear that this chromosome number (n=10) evolved independently at least three times in Satyrinae, and thus, being homoplesious, as a character has limited use for phylogenetic reconstruction. Chromosome

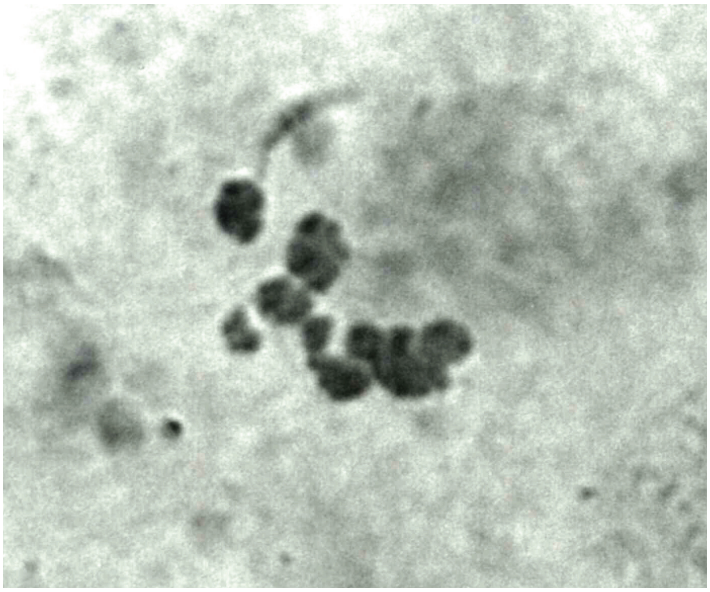

Fig. 1 Dividing meiotic chromosomes of *Paralasa nepalica*, n=10.

numbers known for *Ypthima* are n=24, 27, and 29, representing a common, perhaps ancestral satyrine number.

Brown et al. (2007) state that "*a chromosome number change is very unlikely to become fixed in a large, outbreeding population. The novel karyotype must somehow land in a small population, with an effective size of a few individuals. This small population must remain reproductively isolated from other members of the species for a long enough period, at least for two generations. The novel karyotype can then become fixed in individuals homozygous for it through inbreeding*." If this is correct, the genus *Paralasa* represents an ideal model for chromosomal change. Isolated in high mountain valleys, these butterflies are weak fliers and probably disperse through founding females, whose offspring remain in isolation for prolonged periods of time.

The immature stages illustrated here support an affinity of *Paralasa* with the genus *Erebia*, rather than with *Ypthima*. *Paralasa nepalica* has the egg sculptured with vertical ribs (Fig. 2), which is typical of *Erebia*. The egg is cream colored with pink spots. The head of the first instar larva is reticulate, with short setae, not unlike those found in *Erebia* (Fig. 3). On the contrary, in *Ypthima*, the first instar larvae have a smooth head with very long setae and light reticulation. Their eggs are like those of Pronophilina and some Maniolina: thin-walled spheres that are supported by irregularly shaped or hexagonal sculpturing (Sourakov and Emmel, 1996). The ribbed, robust structure of the *Paralasa* egg is probably an ancestral characteristic, as it is found in many subfamilies of Nymphalidae and in different tribes of Satyrinae (e.g. *Cercyonis* of Maniolina (Sourakov, 1995), *Chazara* of Satyrina, *Erebia* of Erebiina (e. g., Hasselbarth et al., 1995)).

To conclude, while novel characteristics for the genus *Paralasa* that are described here are similar to these of *Erebia*, we found no new evidence supporting its affinity with Ypthimina.

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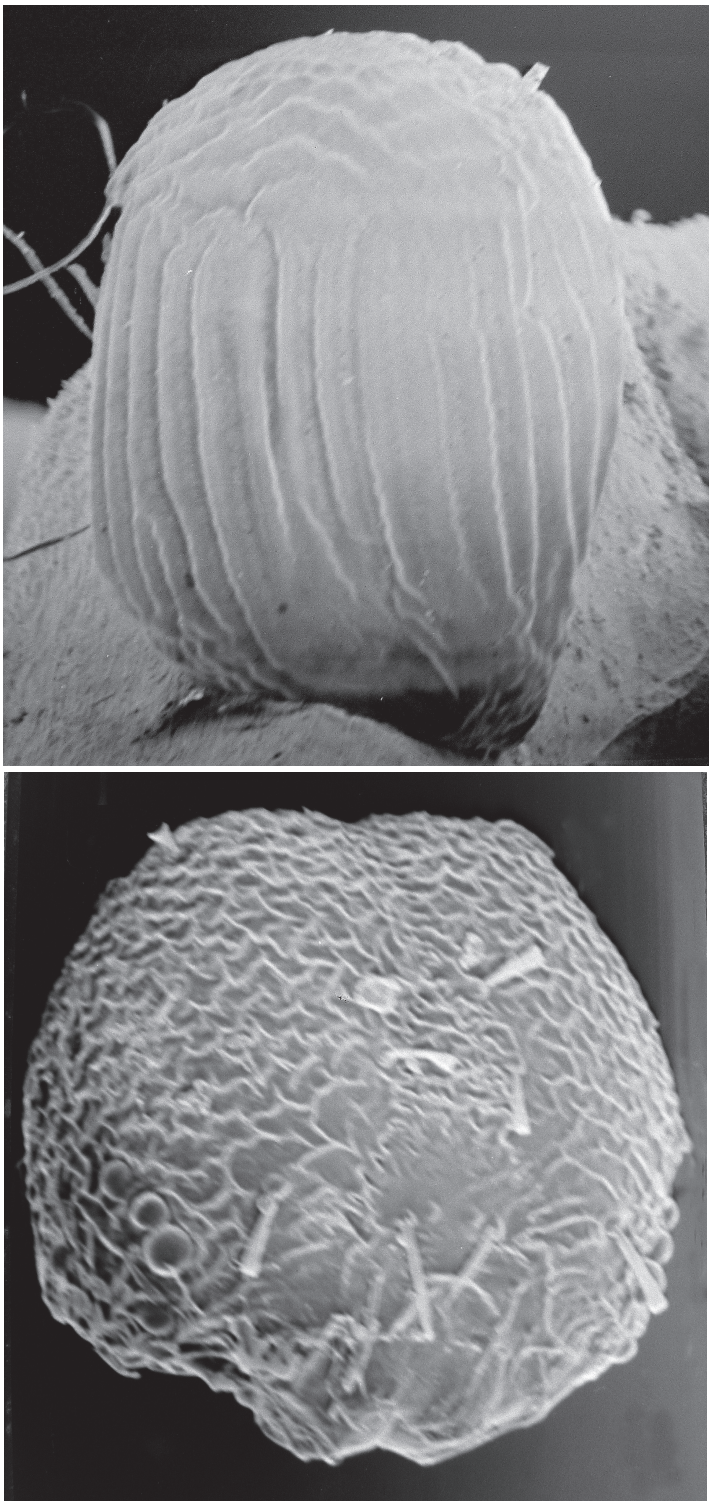

Fig. 2-3. Scanning electron micrograph of egg and first instar larval head of *Paralasa nepalica*.

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Fig. 4 (top). Adult male of *Paralasa nepalica.* The red coloration of the underside, normally hidden, is exposed by a torn hindwing.

Fig. 5 (bottom) Typical habitat of *Paralasa nepalica* is in the hills surrounding this village, Doh Tarap, located above 4,000 m elevation in Dolpa, northwestern Nepal.

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