Taxonomy of spiny eels (Synbranchiformes: Mastacembelidae) in Thailand

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The present taxonomic study is an updated revision of spiny eels (Synbranchiformes: Mastacembelidae) found in Thailand. It is based on specimens deposited in local reference collections and new specimens collected throughout Thailand from June 2006 to September 2007. It represents an attempt to alleviate difficulties with species identification of individual specimens by providing a treatment of the nomenclature and taxonomy of all available local members. Two genera and twelve species, *Macrognathus aculeatus*, *M. circumcinctus*, *M. maculatus*, *M. meklongensis*, *M. semiocellatus*, *M. siamensis*, *M. zebrinus*, *Mastacembelus alboguttatus*, *M. armatus*, *M. erythrotaenia*, *M. favus* and *M. tinwini*, were recognized. All of them are freshwater fish. However, *M. erythrotaenia* can be found in both fresh and brackish waters. From this study, *M. tinwini* is a newly recorded species.

Species identification within the family Mastacembelidae is based largely on configurations and the presence or absence of colour markings on the body and the different numbers of spines, fin rays and vertebrae. Dichotomous keys to genera and to species are provided. Information about scientific names, synonyms and citations, common names, local names, examined materials, diagnoses, descriptions, distributions, habitats, frequency distributions of spines, fins and vertebrae and photographs are available. Morphometric and meristic dendograms using Hierarchical cluster analysis were constructed.

Demography of an invasive alien sailfin molly, *Poecilia velifera* (Regan, 1914) (Cyprinodontiformes: Poeciliidae), in Thale Sap Songkhla and Haad-kaew Lagoon, south Thailand

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Established populations of the sailfin molly, *Poecilia velifera* (Regan, 1914), have been recorded for the first time in Thale Sap Songkhla sub-basin (the outermost part of Thale Sap Songkhla) and Haad-kaew Lagoon, south Thailand. From a coast survey along the coast, we know that they are currently established along the entire coastline of Thale Sap Songkhla sub-basin and Haad-kaew Lagoon (adjacent to the mouth of Thale Sap Songkhla). Biological data shows that their male : female ratio is 1.0:1.8. First maturation occurs at 16.8 mm SL in males, and 17.1 mm SL in females, whereas their maximum standard length is 69.8 mm. They can reproduce several times a year with 3-252 offspring each female. Their preferred habitat is vegetated coastal areas and they tend to spread rapidly throughout the region and probably have an adverse impact on many indigenous species as a result of predation and competition for food and space. On the other hand, populations of *P. velifera* may provide a food source for predatory fishes in their environment.

The ecology of fish communities in rivers in eastern Thailand: Co-existence strategies

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Diversity of freshwater fishes in Thailand is among the highest in the world but information on their abundances and co-existence strategies in rivers is almost non-existent. Concern for conservation of biological diversity requires the development of practical tools for quantitatively sampling small and medium-sized rivers to determine numbers of species and their spatial distribution which is a project currently under study in south eastern and central Thailand using multipass electrofishing techniques. Research has also been initiated to examine strategies employed among species for co-existence including feeding dynamics, morphology and reproduction. Our studies on reproduction strategies include fecundity, oocyte growth rate and gonadosomatic indices. Studies on feeding dynamics are currently focusing on feeding periodicity among fishes as well as precise descriptions of diets and potential food resources including macrobenthos, periphyton and stream drift. Feeding periodicity is important as we have clearly demonstrated distinct species-specific patterns. For example, *Shistura* sp. and *Amblyceps foratum* feed most actively in early morning while *Balitora* sp. and *Channa guacha* are nocturnal feeders. Finally we are investigating ways employed by fishes that allow them to live where they are found. Information has been collected on specific gravity or density, body and fin shapes, sizes and locations to understand how their morphologies define specific hydrodynamic characteristics. This information, along with other morphological adaptations and selected physical and biological characteristics of their habitat will be synthesized to provide some understanding of why they live where they are found.

2008 year of the frog

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At present, global amphibian decline is regarded as one of the critical threats to biodiversity around the world. Several potential causes have been linked to this problem including deforestation, acid precipitation, fungal infestation, and pollution. To understand this problem, Amphibian Ark, a branch of the IUCN Amphibian Specialist Group, has created a campaign "2008 Year of the Frog" to generate public awareness and understanding of the amphibian extinction crisis. Some of the specific aims of this project include: 1) to create partnerships among Zoos, Aquaria, Botanical Gardens, and private and public institutions (universities, etc) around the world to ensure the global survival of amphibians; 2) to highlight ways in which the public can make positive contributions to conservation through activities in their daily lives; 3) to stimulate a sustained and long-term interest in amphibian conservation and related interactions within the wider environment; and 4) to raise increased awareness of the protection of biodiversity through the conservation of amphibians.

Cloning antimicrobial peptide genes from *Rana nigrovittata*

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Frog genes encoding for antimicrobial peptides were cloned and their sequences were analyzed. Mucous from the skin of *Rana nigrovittata* was obtained using mild electrical shock. The secretion contains mRNA which was used as template for reverse transcription reactions (RT-PCR). To analyze the sequence of cDNA, the cDNA was cloned into either pCR4 or pCR8 plasmids using a TOPO cloning method. Next, the ligated products were transformed into TOP10 *E. coli* cells. The bacteria were then grown overnight and plasmids were extracted. Sequencing reactions were done using universal primers. After that the sequences were analyzed, conceptually translated into amino acid sequences and classified into groups based on sequence similarity. According to the classification, they were divided into eight groups. Six groups of peptides have the typical properties of antimicrobial peptides found in other *Rana* frogs, that is, positive net charge, and two Cysteines which were separated from each other by about seven amino acids. The peptides in the other two groups have negative net charge and their sizes were too small. Therefore, these two groups may not be antimicrobial peptides. Finally, the peptide sequences were used for alignment against known peptide sequences in a public database using BLAST. All of the BLAST results showed % similarities between 30-70%.

The genetic diversity of rice field frogs *Hoplobatrachus rugulosus* (Wiegmann, 1835) inferred from cytochrome *b* gene sequences

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The Rice Field Frog (*Hoplobatrachus rugulosus*) is an economically important species in Thailand. It belongs to the family Ranidae, genus *Hoplobatrachus*, and can be found from East Asia and throughout the Thai-Malay peninsula. Recently, populations of this frog in its natural habitat have been decreasing. The main reasons are habitat destruction, pollution, pesticide use, climate change and hunting. In a study of genetic diversity, we sampled rice field frogs from 4 regions (10 populations) in Thailand and extracted DNA from the liver or toe clips of each species. A segment of the mitochondrial cytochrome *b* (Cyt-*b*) gene was amplified and the nucleotide sequences of this gene were analyzed. Comparisons of sequence divergence of the Cyt-*b* gene among populations suggested that the populations form two major clades within this species, divergent from each other by high values of 12.94-14.54%. The first clade included populations from the Northeastern montane region, Southeastern Asian lowlands and the Tenasserim-Malay peninsula in Thailand, and the other clade included a population from the Thai-Lao dry plateau and some populations from the Southeast Asian lowlands in Thailand. The highest value of sequence divergence occurred between Chonburi and Nakhon Ratchasima populations (14.54%).

Effects of atrazine on the early development and gonad development of the rice field frog, *Hoplobatrachus rugulosus* (Wiegmann, 1835)

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Amphibians have declined dramatically around the world. Agrochemical contamination is considered one of the disastrous causes of amphibian declines. A triazine-herbicide, "atrazine", is the most commonly used herbicide in Thailand and probably in the world. Atrazine has been found to be an endocrine disruptor pollutant capable of feminizing male amphibians. However, there is no report on the effects of atrazine at environmentally relevant concentrations on amphibians in Thailand. The aim of this study is to determine the effects of atrazine on the development of the rice field frog, *Hoplobatrachus rugulosus* (Weigman, 1835), a common frog species in Thailand. To study the effects of atrazine on embryonic and early larval development, a modified FETAX procedure (ASTM E 1439-98, 2004) was used. Nominal concentrations of atrazine used were: 0.01, 0.1, 1, 10, 100 and 1000 ppb. The aim of the other experiment was to study the effect of atrazine on gonad development. The embryos of *H. rugulosus* were treated with the nominal concentration of atrazine until the end of metamorphosis. The gonads of froglets will be investigated morphologically and histologically. The expected results can be used to explain the effects of atrazine on embryonic and gonadal development of the rice field frog, from which its role in the amphibian decline situation can be inferred.

Vertical distribution and diets of median-striped bullfrogs *Kaloula mediolineata* (SMITH, 1917) in Sam Ngao District, Tak Province

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The vertical distribution and diet composition of the median-striped bullfrog, *Kaloula mediolineata* (Smith, 1917), were studied in Sam Ngao District, Tak Province, Thailand, from July 2006 to June 2007. The results showed that the average depth of frog burrows in dry months (December 2006 to March 2007, average rainfall = 56.60 mm, N = 75) was significantly deeper than in wet months (July to November, 2005, April to June, 2006, average rainfall = 31.59 mm, N = 140). Significant negative correlations were observed when vertical distribution was compared with the following physical factors: soil surface moisture (R = -0.298; p = 0.000), relative humidity (R = -0.249; p = 0.000) and air temperature (R = -0213; p = 0.002).

Moreover, diet composition was analyzed. The results showed that only empty stomachs were observed during the dry months, whereas during the wet months, empty stomachs were observed in 42.9% of specimens. The main food items were ants (Order Hymenoptera, Family Formicidae), termites (Order Isoptera) and beetles (Order Coleoptera). The stomach contents were similar in both female and male frogs (Simple Similarity Index between 0.91-0.99). Furthermore, a relationship between diet and prey availability was observed (tau = 0.469, p = 0.046). In conclusion, the results suggest that the median-striped bullfrog is a generalist predator which is active in wet months, and ants, termites and beetles are the main food-sources of this frog.

Phylogenetic relationships among Thai newts assessed using mitochondrial DNA sequences

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The genetic variation of the Himalayan newt (*Tylototriton verrucosus*) in Thailand was examined through field surveys, which were carried out at 14 locations in 7 provinces of Thailand, from December 2001 to September 2006. It was observed that, in Thailand, this species could be divided into two morphotypes based upon body coloration (orange to yellow and dull colorations), and female size (the orange to yellow type was noticeably bigger than the dull type). The two morphotypes, as described, also coincided with their current distribution ranges (northern and northeastern mountain ranges). The current local distributions within Thailand of both of the two types of *T. verrucosus* were determined, with new localities discovered, and are reported.

Among 7 populations, the genetic variation among 21 individuals was examined using DNA sequence analysis of two mitochondrial DNA gene fragments: 16S ribosomal RNA (498-500 base pairs) and D-loop (729-730 base pairs). Phylogenetic relationships were established using distance and maximum likelihood methods. The clear findings revealed the existence of two distinct genetic lineages, which are related to their geographic distributions and color patterns. These data showed that there are two morphotypes of *T. verrucosus* in Thailand based on distribution patterns and molecular characters.

Recent occurrence of the endangered big-headed turtle *Platysternon megacephalum* Gray, 1831 in Thailand

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The big–headed turtle, *Platysternon megacephalum*, is a species of concern to conservationists. It is listed as endangered in the IUCN Red List of Threatened Species, 2007, and is given in Appendix II of CITES. In addition, it is a protected species under the Preservation and Protection of Wild Animals Act (No. 2), B.E. 2546. Areas where this species has been reported are in Chiang Mai, Mae Hong Son, Phrae, Tak, Kanchanaburi, Loei, Phetchabun and Chaiyaphum Provinces (Nabhitabhata and Chan-ard, 2005). The purpose of this study is to explore the present distribution of this turtle in Thailand, and to provide data required for its conservation. The data were collected by mailing questionnaires to authorities and field surveys were conducted in the areas that had positive information. In addition, literature and museum records of this species in Thailand were compiled.

Preliminary results suggest that these turtles are widely distributed in northern, northeastern, central and western Thailand. Protected areas in Chiang Rai, Nan, Prayao, Uttaradit, Phitsanulok and Sukhothai Provinces are new localities for the big-headed turtle found in this study.

Appraisal of the evolution of testudinoid turtle diversity from the Late Palaeogene and Neogene of Thailand

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The evolution of testudinoid turtles of Thailand was assessed using both fossil records and recent turtle data. Two localities were selected for studying fossil turtles, Nong Ya Plong (Oligocene), Petchaburi Province, and Tha Chang Sandpits (Neogene), Nakhon Ratchasima Province. The turtles from the first locality were described and the specimens are considered to be a new species of *Mauremys* and a new genus closely related to *Malayemys*. The turtle from the second locality is reminiscent of a giant testudinid turtle. Turtles living before the late Neogene have not been found in this study.

The geographical distribution of the superfamily Testudinoidea was studied using clustering methods for separating five biogeographical provinces (Sino-Indian Province, Taiwanese/Eastern Chinese Province, Philippines Province, Indochinese Province, and Indonesian Province) based on the similarity of taxa. These provinces were then integrated with a published phylogeny in order to study endemic and migration patterns. The results showed that the *Heosemys* group is an endemic taxon to the Indochinese Province. In addition, the turtles in Thailand are more related to the Indonesian Province than to turtles in India and China. However, evidence of fossil *Heosemys* has not been found before the Pliocene or Pleistocene. It means that the biogeographical identity of living turtles in Southeast Asia began recently.

Species diversity of amphibians at different elevations in Num San Noi Stream at Phuluang Wildlife Sanctuary

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Species composition and abundance of frogs at Nam San Noi stream, Phu Luang Wildlife Sanctuary, northeastern Thailand were determined at three elevations, 800, 950, and 1250 m, by visual encounter surveys during May 2006 to May 2007. Three 100 m-stream transects at each elevation were surveyed each month during 19:00-21:00 hrs. A total of 22 species was found. The species diversity of frogs at 800 m (n=17, Shannon-Wiener's index=1.54) was highest, followed by 950 m (n=15, Shannon-Wiener's index=1.38), and 1250 m (n=10, Shannon-Wiener's index=0.71). The average abundances of all stream-dwelling frogs, except for *Limnonectes gyldenstolpei*, showed significant differences across elevations. Compared with other species, *Limnonectes kuhlii* had the highest abundance at 1250 m, whereas *Sylvirana nigrovittata* was the most common at both 950 m and 800 m. *Xenophrys major* was found only at one elevation, 950 m. It was concluded that the diversity and abundances of frogs in Nam San Noi stream are largely determined by elevation.

Reproductive cycle of the rainbow water snake, *Enhydris enhydris*, in Prachinburi Province, Thailand

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From March 2006 to April 2007, two hundred and thirteen *Enhydris enhydris* females at Ban Borthong, Kabinburi District, Prachinburi Province were collected for reproductive cycle examination. Thirty-nine gravid females, at carrying stages 30-37, were found during the nine month period from February to October, of which the number of gravid females was highest in August. Mean snout to vent length of gravid females was 560.65 ± 6.53 mm. Mean clutch size was 13.12 ± 5.41 embryos, ranging from 1 to 27. The full-term stage of embryos, stage 37, was found in May, June and October. The complete reproductive cycle of the rainbow water snake is demonstrated.

Genetic diversity of the Thai roundleaf bat (*Hipposideros halophyllus*)

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The Thai roundleaf bat (*Hipposideros halophyllus*), a mammal endemic to Thailand, has been categorized in the low risk subcategory: Near Threatened (LR/nt) on the IUCN Red List. There are currently three free living populations of the roundleaf bat in Thailand which are at least 130 km apart from each other and this may prevent gene flow between these populations. This study aimed to evaluate the genetic diversity of the Thai roundleaf bat using microsatellite markers that were developed from different bat species. A total of 41 microsatellite primer pairs were developed and 8 primer pairs showed polymorphic patterns within the samples. The results showed that the bats from Ta-Pa and Fa-Tho Caves had the closest genetic relationship. This may be due to the two caves being located in close proximity and as such the bats are likely to have an opportunity to exchange individuals between populations. In contrast, the bat population of Tai-Din Cave was genetically divergent from the bat populations of the other caves, and this may be due to the geographical distance. Interestingly, the bats from Tai-Din Cave had lower heterozygosity than that found in the other populations suggesting that the smaller size of the bat population at Tai-Din Cave results in inbreeding of the species. Due to the crisis of a sharp decline in the numbers of the Thai Roundleaf bat, our data will be useful for establishing an effective conservation plan for this species.

A review of bat research in Thailand with eight new species records for the country

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A review of the literature relating to the history of bat research in Thailand (1821–2006) is included together with lists of the 119 bat species currently recorded from the country and the 16 that are omitted for lack of supporting data. The geographical distribution within Thailand of some of the more significant bat field studies (1896–2004) is mapped and briefly discussed. Based on field work conducted in peninsular Thailand in 1993 and 2003–2004, eight bat species (*Hipposideros ridleyi*, *Myotis hermani*, *Pipistrellus stenopterus*, *Hesperoptenus tomesi*, *Murina suilla*, *Murina aenea*, *Kerivoula pellucida*, and *Mops mops*) were recorded from the country for the first time; information is provided on their taxonomy, distribution, and ecology. Recommendations are made for further bat studies in Thailand, with emphasis placed on selecting less well known species groups, such as forest bats, in under-researched habitats in neglected geographical areas (for example, the deciduous dipterocarp forests of eastern Thailand and the semi-evergreen forests of peninsular Thailand). A need to develop in-country skills in bat acoustics and taxonomy is also highlighted.

A taxonomic review of *Rhinolophus pusillus* and *Rhinolophus lepidus* (Chiroptera: Rhinolophidae) in Thailand

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Rhinolophus pusillus and *R. lepidus* are horseshoe bats which have essentially similar external morphology and cranio-dental measurements. Previous taxonomic studies suggest that their taxonomy is confused as there are number of synonyms and a smaller number of subspecies. In the past taxonomic studies of the two species have been of the classical kind with a qualitative and quantitative review of external and cranio-dental morphology. Today, morphometric analysis techniques are more sophisticated and better able to discriminate between taxa. Species identification can also be supported with recordings and analysis of echolocation calls and it has been shown elsewhere that the calls of many bat species are a useful aid to identification at genus and species levels. Current information suggests that the constant frequency of the echolocation call for *Rhinolophus lepidus* is 95-105 kHz in Thailand and 100 kHz in Malaysia. The frequency for *Rhinolophus pusillus* is 105-110 kHz. From recent studies, there is a third taxon that is morphologically similar to *R. lepidus* and *R. pusillus* but larger and with a lower frequency of about 85.2-91.6 kHz. It is known from Pho Soun Sai National Park, Loei province, in Northeast Thailand. There is also another taxon which is smaller than *R. pusillus* with a higher frequency of about 126.3 kHz, known from Khao Samohkhon, Lopburi province, in Central Thailand. From the current study, it is shown that the *R. pusillus* taxon comprises a species complex and that there are more than two taxa of this group in Thailand.

Asian origin of anthropoid primates and the evolution of Miocene apes

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New paleontological results have firmly established Asia as the ancestral homeland for the anthropoids. Asian anthropoids are not only the oldest recorded but also the most primitive known. During the Early to Middle Eocene, the Eosimidae, *Eosimias* from China (45 Ma) and *Bahinia* from Myanmar (37 Ma), are recognized as the earliest undoubted Anthropoidea. During the late Eocene, the Amphipithecidae, *Myanmarpithecus* and *Pondaungia* from Myanmar (37 Ma) and *Siamopithecus* from Thailand (35 Ma) radiated. This group which is widely distributed from Pakistan to Thailand, has developed the same characters as those of crown anthropoids, such as a short face, massive jaw, low crowned molar with flat occlusal surface, orbit frontation, a.s.o. This morphological evolution is interpreted as the result of an adaptation to a strongly seasonal climate and to a hard-food dominated diet. The evolutionary scenarios relative to early anthropoid evolution imply therefore one or several dispersal events between Southeast Asia and Africa.

Thai Miocene hominoids, *Khoratpithecus chiangmuanensis* from northern (13.5-10 Ma) and *Khoratpithecus piriyai* from northeastern Thailand (9-7 Ma), share one unique derived character with orang-utans and are therefore considered as their closest fossil relatives. Both Thai fossils evolved in a humid tropical forest. Climatic fluctuations recorded from the marine realm suggest that these hominoids did not migrate “Out of Africa” around 12.5 Ma, as commonly stated, but were already present in Southeast Asia and extended their distribution area further north during the warm climatic events. In conclusion, an “Out of Asia” scenario for early anthropoids and for African late Miocene hominid primates is more strongly established than the classical “Out of Africa” scenario. This emphasizes the importance of climatic changes and of paleogeographic constraints on their evolution.

Conflict and commensalisms between long-tailed macaques and humans

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Long-tailed macaques (*Macaca fascicularis*) are the most frequently encountered primate in Thailand. They are currently considered at low risk of extinction on the IUCN Red List 2007, However, they are threatened by genetic pollution (hybrids and translocation), and by habitat fragmentation, loss and transformation. Their habitats have been greatly changed from natural forests to recreation parks or Buddhist temples. Living in temples, besides provisioning by monks and pilgrims, monkeys are safe without fear of hunters or predators. Thus, the populations of long-tailed macaques are currently in a state of over-population in many locations. There are always pros and cons regarding the maintenance of macaque populations in Thailand, and people fall into one of two groups. The group with a positive attitude, such as banana venders, hotel owners and shop keepers, mostly benefit from monkeys. They have established a foundation for monkey food and they feed the macaques. The group with a negative attitude, such as people living near the sites, are disturbed by the monkeys. The monkeys raid their houses for food or damage their crops during the dry season when natural foods are scarce. Up to now, there have been no concrete management plans to overcome the problems of over-population of Thai long-tailed macaques and of conflict with humans. This is a delicate matter. To solve the problem, we need mutual understanding among people and require cooperation from various groups, including primatologists, conservationists, governmental agencies, and NGO's.

Morphological and genetic characters of stump-tailed macaques in the Indochinese and Sundaic sub-regions of Thailand

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The stump-tailed macaque (*Macaca arctoides*) is one of five species of macaque monkeys in Thailand. They are categorized as a vulnerable species by IUCN 2007. Although they were reported to be distributed throughout Thailand, information on this macaque presently is scarce. Based on geological and palaeontological evidence, marine transgressions occurred over the Thai-Malay Peninsula and caused the separation of land, especially at the Isthmus of Kra, into the Indochinese and Sundaic sub-regions. There have been reports that show that fauna and flora have restricted distributions in different areas of the Indochinese and Sundaic sub-regions. Thus, this project aims to 1) research the distribution of stump-tailed macaques in Thailand, and 2) investigate differences in morphological and genetic characters of stump-tailed macaques inhabiting the Indochinese and Sundaic sub-regions of Thailand. Methods used will include a census of stump-tailed macaques based on previous reports, replies to questionnaires which were sent to heads of subdistricts throughout Thailand (7,410 questionnaires), assessment of demography, taking photos and measuring body size and determining pelage color from these photographs, and analyzing genetic characters and phylogenetic relationships.

Managing human-elephant conflict based on elephant and human behavior: a case study at Thong Pha Phum National Park, Kanchanaburi Province, Thailand

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Most mitigating methods for Human-Elephant Conflict (HEC) tend to solve immediate problems without considering long-term and accumulated changes in elephant and human behaviors. Adaptive management based on elephant and human behavior principles have been explored. Field experiments on elephant responses to different repellent methods were conducted on 3 farms. Farm I used a single sound repellent (firecracker), farm II used a single light repellent (spotlight) and farm III used double and simultaneous repellents (firecracker and spotlight). Lone male and family herd elephants showed hiding patterns and stayed longer on the farm in response to a single sound or a single light repellent method compared with a shorter stay for the double and simultaneous repellents method. The light repellent could elicit a flee response more than the sound repellent in family herds. This information was used on other farms to select the most effective repellent technique. For elephant welfare, human behavior was assessed by direct observation and categorized as verbal aggression (insult elephant) and physical aggression (intend to harm elephant with a gun) during elephant raiding. Farm III selected a win-win solution by applying ecotourism in addition to the existing repellent technique for a long-term solution. However, Farms I and II decided not to include ecotourism. Results showed that villagers in Farm III expressed verbal aggression and physical aggression less than Farms I and II after ecotourism implementation, and physical aggression disappeared after Farm III set rules not to harm elephants. Adaptive management based on elephant and human behavior may help to provide the long-term solution for HEC.