

# Integrative Taxonomy, a New Approach to Answer the Questions in the Biosystematics: A Case Study of the Genus *Gnopharmia* (Geometridae)

Integrative Taxonomie, ein neuer Ansatz Fragen der Biosystematik zu beantworten: Eine Fallstudie an der Gattung *Gnopharmia* (Geometridae)

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**Summary:** Looking back to the history of taxonomy reveals that thinking of the classification of organisms is an old engagement of human being. However, accumulation of knowledge, invention of new technologies and discovery of many biological facts and new conceptual definitions open a new era of taxonomy. Currently, the classical taxonomy undergoes a transformation into an integrative taxonomy, which is a multidisciplinary approach for species delimitation. Thus, integrative taxonomy will be more trustworthy and, if worldwide established, faster in recognizing species and biodiversity. Using the genus *Gnopharmia* as an example, a geometrid moth that is distributed in the Middle East, Central Asia and Levant, the present survey shows the wealth of information (e.g. taxonomy, species boundaries, zoogeographic distribution and biology) which can be generated with an integrative approach.

**Key words:** Integrative taxonomy, morphology, geometric morphometry, phylogeography, DNA-barcoding, life history

**Zusammenfassung:** Die Geschichte der Taxonomie zeigt, dass Überlegungen zur Klassifikation von Organismen ein altes Anliegen der Menschen sind. Allerdings eröffnen die enorme Anhäufung von Wissen, die Entwicklung neuer Technologien und die Entdeckung neuer biologischer Phänomene sowie neue konzeptionelle Definitionen eine neue Ära der Taxonomie. Zurzeit wird aus der klassischen Taxonomie eine integrative Taxonomie, die als ein multidisziplinärer Ansatz, u. a. zur Artabgrenzung, zu verstehen ist. Daher wird die integrative Taxonomie eindeutiger Ergebnisse liefern und, wenn erst einmal etabliert, auch schneller Arten und generell Biodiversität erfassen können. Am Beispiel der Gattung *Gnopharmia* (Geometridae), deren Arten ausschließlich in Mittelost, Zentralasien und der Levante verbreitet sind, wird in der vorliegenden Übersicht aufgezeigt, welche Fülle von Informationen (z. B. Taxonomie, Artgrenzen, zoogeographische Verbreitungen und Biologie) mit Hilfe dieses Ansatzes zu erhalten ist.

**Schlüsselwörter:** Integrative Taxonomie, Morphologie, geometrische Morphometrie, Phylogeographie, DNA-Barcoding, Biologie

## 1. Historical background

### 1.1. From artificial to natural classification

As introduction into the present survey only some hints will be given to characterize the de-

velopment of the classification of organisms (for details see JAHNS 2000). ARISTOTELES (384–322 B.C.), known as one of the most effective philosophers in ancient Greek, was one of the first pioneers who thought on taxonomy. He formulated his thoughts on taxonomy and biodiversity in the book

“*Historia Animalium*” (Greek: Περὶ Τῶ Ζῴων Ἱστορίαι). ARISTOTELES had a hierarchical view on the living organisms, which positioned the low complex organisms in the lower level and those more complex ones in higher levels of the ladder of life (*scala naturae*). Furthermore, he was an essentialist believing that species are fixed without any changes in time. In this sense, he saw no intraspecific variation. So he tried to classify the organisms based on their similarities (e.g. on their type of movement; he divided the animals into three groups: walkers, swimmers and flyers). ARISTOTELES used a descriptive binomial name for the organisms (e.g. he called human as featherless bipod). This attitude was subsisted until the 17th century, when several taxonomists started new efforts.

In 1735, the Swedish naturalist CAROLUS LINNAEUS (1707–1778) published his significant book “*Systema Naturae*” establishing the new taxonomy. Introduction of the binomial nomenclature and using a hierarchical classification system were two big steps in upgrading the taxonomy. LINNAEUS and the taxonomists, who followed him, also belonged to the essentialism’s school using morphological similarities for their classification. However, LINNAEUS couldn’t explain why the members of one taxonomic group (or taxon) show more similarity to each other than to the members of the other taxa.

This very fundamental question was answered over one century later by CHARLES DARWIN (1809–1882) in “*The Origin of Species*” (1859). DARWIN argued that the similarities of the members of one taxonomic group originate from their “common ancestor”. The idea of a common ancestor led to the development of a “natural” classification system (vs. artificial system), in which the members of a taxon share characters with common ancestors (phylogenetic signals). Today we have many algorithms, software and statistical methods for reconstructing the phylogenetic relationship, but for DARWIN

finding the phylogenetic relationships was a big dream and question. The question that needs around 100 years to be answered.

Finally, the German entomologist WILLI HENNIG (1913–1976) overcame the problem and developed a practical method for calculation of phylogenetic relationship based on coded morphological data (see HENNIG 1950). HENNIG’s publication has transformed the traditional classification into “phylogenetic systematics” (see JAHNS 2000).

## 1.2. Species concept define the approaches of species delimitation

It seems that the process of species delimitation directly depends on the species concept, which the taxonomist are working with. The typologists used the morphological similarities to classify the species, but after DARWIN the evolutionists described species as lineages that evolve separately as own units (SIMPSON 1951). With his cladistic concept HENNIG (1950) understands species as unbranched lineages in a cladogram. Later, ERNST MAYR (1904–2005) summarized his biological concept of species, which based on reproductive isolation (MAYR 1970). Although the biological concept of species encouraged the taxonomists to check the reproductive isolation as a definite criterium for many species, the ecological concepts of species (VAN VALEN 1976) draw attention to the environmental and ecological conditions of a zone, which is occupied by a lineage. Usage of these or other species concepts directly defines the methodological approach of taxonomy. Because each of these concepts has its own limitations, advantages and disadvantages, an ultimate, standard procedure to answer all taxonomic questions does not exist.

## 1.3. Accelerating the taxonomy

Up to now, around two million species are described and it has been estimated that

between ten and 100 million eukaryote species have not yet been discovered. Although the number of taxonomists strongly decreased, in the last 50 years the number of new described species was stable between 14,000 and 25,000 species per year (PADIAL et al. 2010). Taking a minimum quantity of undescribed eukaryote species (ca. ten million), we need more than 400 years to describe nearly all biodiversity of the earth! This is an important argument for thinking about acceleration of taxonomy and finding novel methods for automation of species identification.

Following this dream, development and implementation of DNA barcoding was a revolutionary achievement in taxonomy. The idea of DNA barcoding seems to be simple: finding a species-specific piece of DNA (barcode); generating a library of this piece of DNA for all known species; comparing the barcode of an unknown species with the known specimens and identifying the species.

As an optimal candidate, cytochrome oxidase I (*COI*) was realized as barcode (HEBERT et al. 2003) for many organisms, and during the last decade many taxonomists were convinced to use this method. However, several new problems arose directly affecting the quality and accuracy of DNA barcoding (e.g. introgression, co-amplification of nuclear copies of mitochondrial DNA, heteroplasmy and contamination with different endosymbionts). Facing with these biological processes in a DNA barcoding survey may lead to a completely different interpretation of the results and to a wrong taxonomic decision.

#### 1.4. Acceleration or accuracy?

There are many species misidentifications in the taxonomic literature. Some of these misidentifications cost much more than one may imagine. The outbreak of Cassava mealybug (*Phenacoccus manihoti*) on agriculture

fields of Manihot (*Manihot esculenta*) in Africa and its misidentification is just one of many examples (SMITH et al. 2011).

Outbreak of the pest in the 1970s strongly decreased the yield of Manihot (as one of most important agriculture crops in Africa). Besides using pesticides, a hymenopteran parasitoid was imported from South America to control this pest. However, none of these methods could effectively reduce the population of the mealybug. In the 1980s over 80 % of crop loss was reported. In 1990 taxonomists realized that the pest species and therefore its related parasitoid were not correctly identified. In the next step the right parasitoid (*Anagyrus lopezi*) was identified and its introduction to the fields successfully controlled the pest. The economic loss due to a single taxonomic error was approx. over 20 million dollar (SMITH et al. 2011). Such examples (which are not seldom) warn us not to sacrifice the accuracy of determination to the profit of an acceleration in taxonomy.

#### 1.5. Integrative taxonomy: an alternative

Many taxonomists have recently acclaimed the “integrative taxonomy” as a multidisciplinary approach for species delimitation and for answering taxonomic questions (PADIAL et al. 2010; SCHLICK-STEINER et al. 2010). As highly complex systems, species have multidimensional relationships with their environment and other species. To understand these systems we are forced not only to check their appearance (morphological characters) or genetic data, but also their natural history, phylogenetic relationships, developmental stages, ecological demands, behavior and biology etc., in an objective sense. In other words, when a complex historical background has influenced a population of organisms to have formed a unity (e.g. species), we may see this background in its genome, morph, behavior, niche

preference, etc. Looking at the species in an integrative context gives us the opportunity to compare the validity of different concepts and regard the species valid, when different methods confirm its unity. This approach was used in many new taxonomic projects during recent years (including the genus *Gnopharmia* Staudinger, 1892).

## 2. The taxonomic problems of the genus *Gnopharmia* (Geometridae) resolved by integrative taxonomy

The genus *Gnopharmia* Staudinger, 1892 is a member of the family Geometridae (Geometer moths), which is distributed only in the Middle East, Central Asia and Levant. Before the revision (RAJAEI et al. 2012), 19 taxa (all species and subspecies) of this genus were described by different authors (SCOBLE & HAUSMANN 2007). High intraspecific variation in both genitalia and wing pattern led to over 130 years misinterpretation of the species delimitations in this genus. Additionally, biology and food plants of *Gnopharmia* spp. were poorly known. Therefore, species identification and definition of the geographic distribution proved to be extremely difficult. To solve this problem, an integrative approach using a wide spectrum of methods was used to appraise the taxonomy, distribution, phylogeography and biology of this genus.

### 2.1. Methodological aspects

To realize the integrative approach, over 2,000 specimens including the type series of all species were studied. Specimens were collected or loaned from 14 museums and many private collections in Iran and various European countries. A variety of methods was used:

**Morphological studies:** Light microscopy and scanning electron microscopes were used to study the external and internal anatomy of specimens (including over 900 genitalized species; see RAJAEI et al. 2012).

**Geometric morphometry (GM):** Landmark-based geometric morphometry was applied to the aedeagus of 298 specimens of *Gnopharmia* spp. to compare the results of GM on species-specific characters with those of traditional morphology (see RAJAEI et al. 2013a for more details).

**Life history studies:** Two *Gnopharmia* species were studied in their natural habitats (RAJAEI 2010).

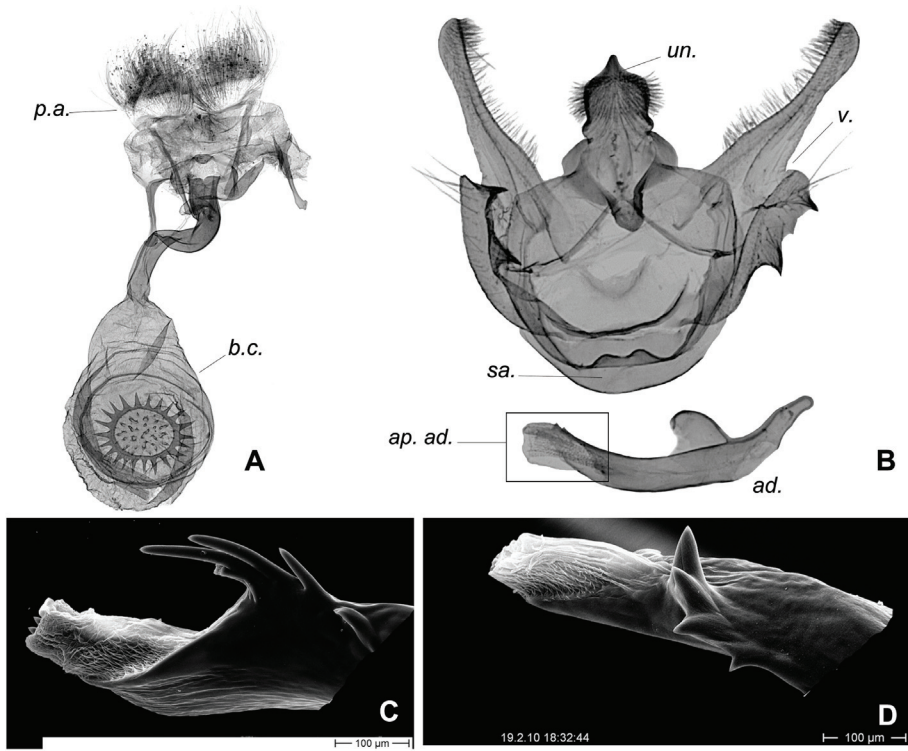
**Molecular taxonomy:** Three nuclear sequence regions (28S, ITS1 and ITS2) and fragments of one mitochondrial gene (CO1) were examined for their utility for phylogenetic inference and species delimitation of *Gnopharmia* spp. (see RAJAEI et al. 2013a).

**Phylogeography:** The genetic population indices of the *Gnopharmia* spp. were studied to explore a reasonable explanation for current population distribution (see RAJAEI et al. 2013a).

**Ecological survey:** To estimate the current and past potential distribution dynamics of *Gnopharmia* spp. and their host-plants and to study the existence of any refugium in Iran during the last glacial maximum (LGM) the species distribution modeling was used (see RAJAEI et al. 2013b for details).

### 2.2. Results

As a result of the morphological surveys, a series of new morphological diagnostic characters were discovered, e.g. tibial spines, octavals of the male pregenitalia and arrangement of subapical spines on the aedeagus (see Fig. 1). Comparisons of all morphological characters revealed that many synonymies could be recognized and the total number of valid taxa in this genus was reduced from 21 to ten taxa (seven species and three subspecies). Two taxa were transferred to the closely related genus *Neognopharmia* Wehrli, 1953. One taxon (*G. colchidaria cocandaria*) is revived as valid species. One species (*G. sinesefida*) was downgraded to a subspecies of *G. colchidaria*



**Fig. 1:** *Gnopharmia rubraria*. **A** Female genitalia. **B** Male genitalia. Scanning electron microscopy shows clearly the differences of the arrangements the spines on the subapical part of the aedeagus of **(C)** *Gnopharmia keasrunensis*, and **(D)** *Gnopharmia colchidaria*. Abbreviations: ad. = aedeagus, ap.ad. = apical part of aedeagus, b.c. = bursa copulatrix, p.a. = papilla analis, sa. = saccus, un. = uncus, v. = valva (see RAJAEI et al. 2012).

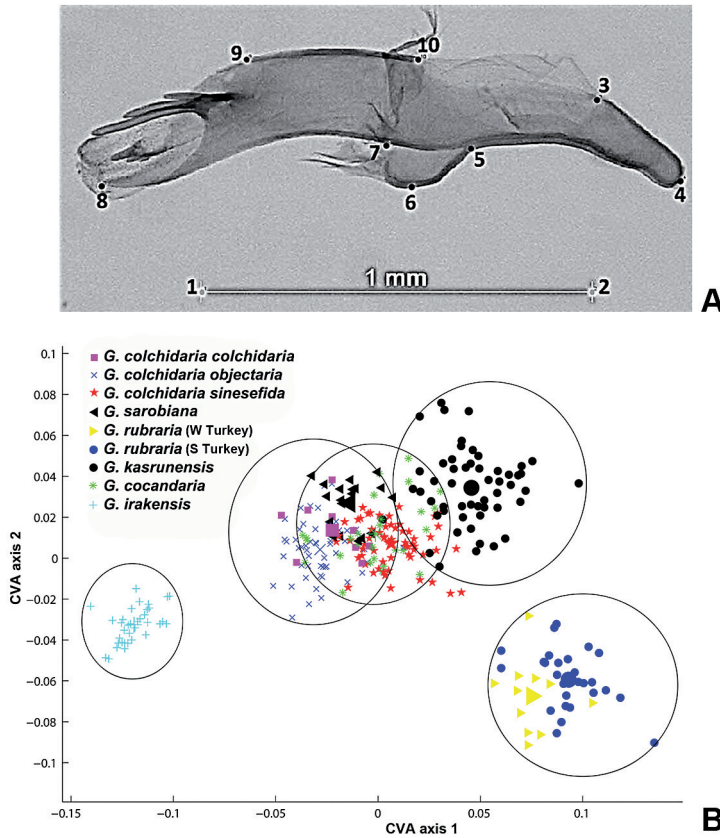
**Abb. 1:** *Gnopharmia rubraria*. **A** Genitalpräparat eines Weibchens. **B** Genitalpräparat eines Männchens. Die rasterelektronenmikroskopischen Aufnahmen zeigen den deutlichen Unterschied in der Anordnung der Dornen auf dem subapicalen Aedeagus von **(C)** *Gnopharmia keasrunensis* und **(D)** *Gnopharmia colchidaria*. Abkürzungen: ad. = Aedeagus, ap.ad. = apicaler Teil des Aedeagus, b.c. = Bursa copulatrix, p.a. = Papilla analis, sa. = Saccus, un. = Uncus, v. = Valva (s. RAJAEI et al. 2012).

Lederer, 1870. Additionally, five new records of the valid taxa are reported for the fauna of different countries in the Middle East (see RAJAEI et al. 2012 for details).

Using only the data of descriptive morphology didn't help us in some cases to postulate a reasonable result. For example, populations of *Gnopharmia rubraria* from Southwest Turkey show clearly darker color than those from southeastern Turkey and Levant. In this case, fine results of geometric morphometry show that these populations form a cluster (Fig. 2). Also DNA taxonomy

shows a very low genetic distance between them (Fig. 3). Therefore, it was concluded that these populations belong to the same species and that the dark colored specimens from Southwest Turkey are a melanistic form of *G. rubraria*. Based on these new results, the geographic pattern of all valid species was depicted. Using the identification key and referring to the distribution maps, the identification of all *Gnopharmia* specimens is easily possible.

Before this study, we had no knowledge on the biology and on host plants of *Gnophar-*



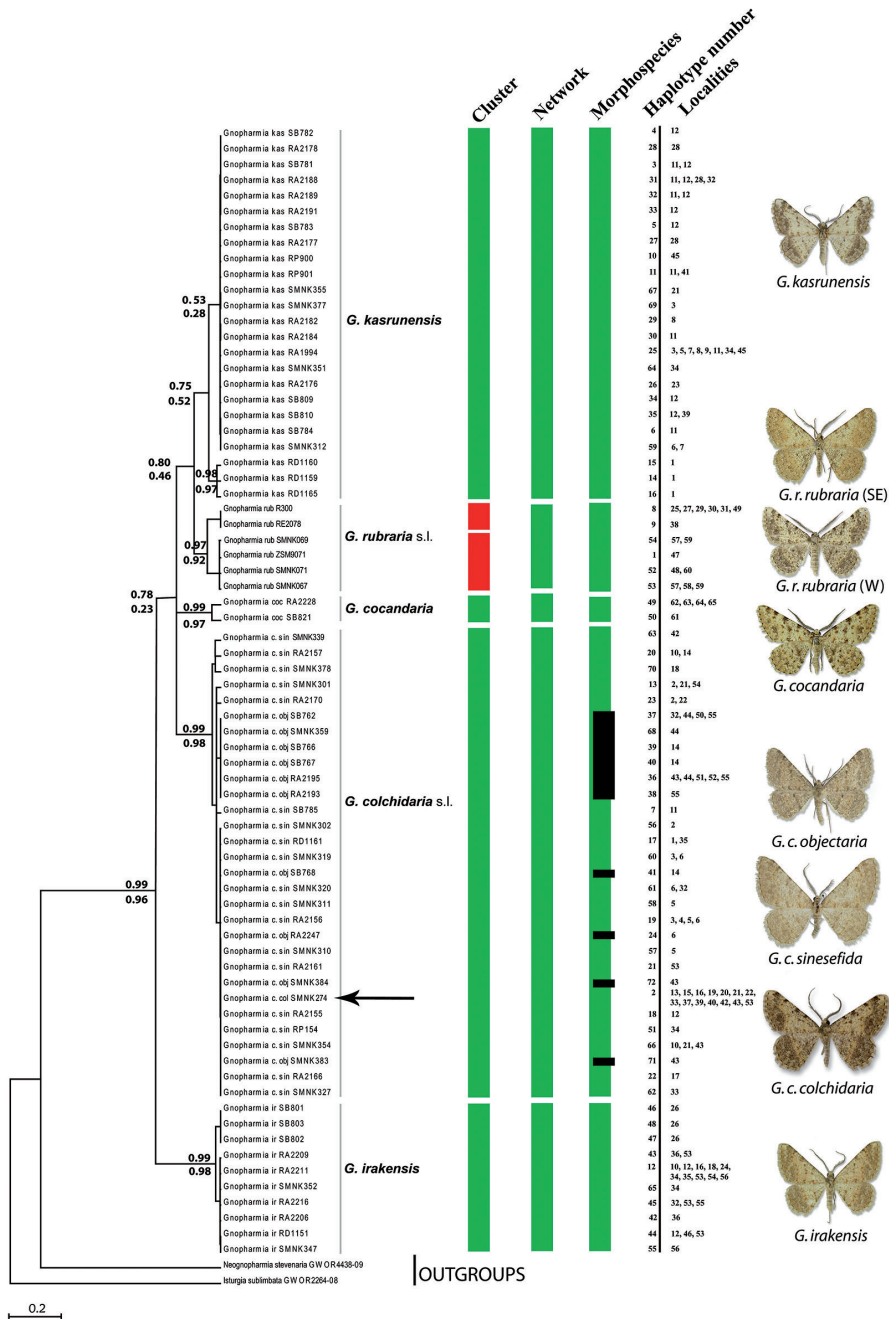
**Fig. 2: A** Position of selected landmarks for geometric morphometry on the aedeagus. **B** Canonical variates analysis (CVA) confirms the results of morphological and molecular studies on species delimitation within the genus *Gnopharmia* (after RAJAEI et al. 2013a).

**Abb. 2: A** Lage der für die geometrische Morphometrie ausgewählten Landmarken auf dem Aedeagus. **B** Die kanonische Merkmalsanalyse (CVA) bestätigt die Ergebnisse der morphologischen und molekularen Untersuchungen zur Artabgrenzung innerhalb der Gattung *Gnopharmia* (nach RAJAEI et al. 2013a).

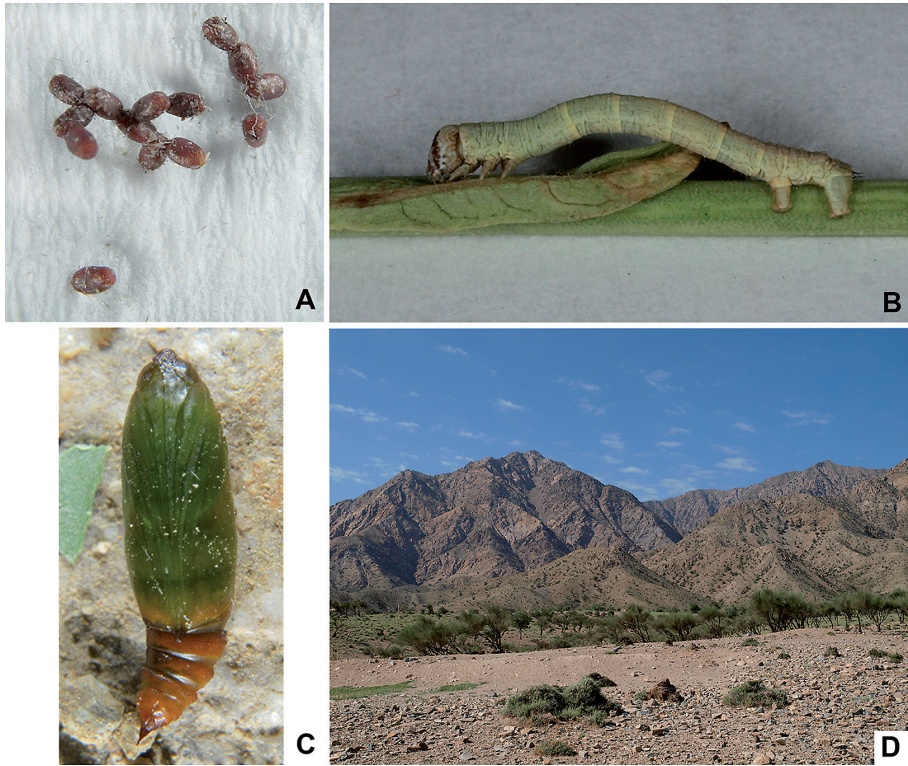
*mia* species. During collecting trips in Iran, the life history of two sympatric *Gnopharmia* species (*G. kasrunensis* and *G. colchidaria*) was successfully studied. Eggs, larvae, pupae, adults and habitats of both species were illustrated and the larval host plant (*Prunus*

(*Amygdalus scoparia*) was confirmed (first time for the genus). This biological data congruently confirm the species differentiation of these two sympatric species (see Fig. 4). The effects of the past climate oscillations on the populations are reflected in the pres-

**Fig. 3:** Ultrametric maximum likelihood (ML) tree of haplotypes with bootstrap support (upper numbers for ML analysis, lower for *Neighbor-Joining* analysis (NJ)); 1000 replicates were used). Green shows the congruence and red shows incongruence between molecular analyses and morphological analyses (see RAJAEI et al. 2013a for details). In general, these analyses are fully in agreement with the results of the geometric morphometry.



**Abb. 3:** Ultrametrischer Stammbaum (Maximum likelihood, ML) der Haplotypen mit Unterstützung durch Bootstrap (Zahlen oben für die ML-Analyse, Zahlen unten für die *Neighbor-Joining*-Analyse (NJ); 1000 Replicas). Kongruenz (grün) und Inkongruenz (rot) zwischen molekularer und morphologischer Analyse (s. RAJAEI et al. 2013a). Generell decken sich die Ergebnisse dieser Analysen mit den Ergebnissen der geometrischen Morphometrie.



**Fig. 4:** Developmental stages and habitat of *Gnopharmia kasrumensis*. **A** Eggs. **B** Larva. **C** Pupa. **D** Ras-Kuh, Fars province, Iran (after RAJAEI 2010).

**Abb. 4:** Entwicklungsstadien und Habitat von *Gnopharmia kasrumensis*. **A** Eier. **B** Larve. **C** Puppe. **D** Ras-Kuh, Provinz Fars, Iran (nach RAJAEI 2010).

ent population variation and speciation. These effects could be traced by comparison of the population genetics. In a part of our study the population structures of *G. colchidaria* and *G. kasrumensis* were compared. Different genetic indices (e.g. haplotype and genetic diversity) and haplotype relationships of these two *Gnopharmia* species indicate the existence of at least three Quaternary refugia in Iran and its neighboring countries. As these refugial areas match with the current distribution of the subspecies of *Gnopharmia colchidaria*, these results confirm also our taxonomic hypothesis about this species (Fig. 5).

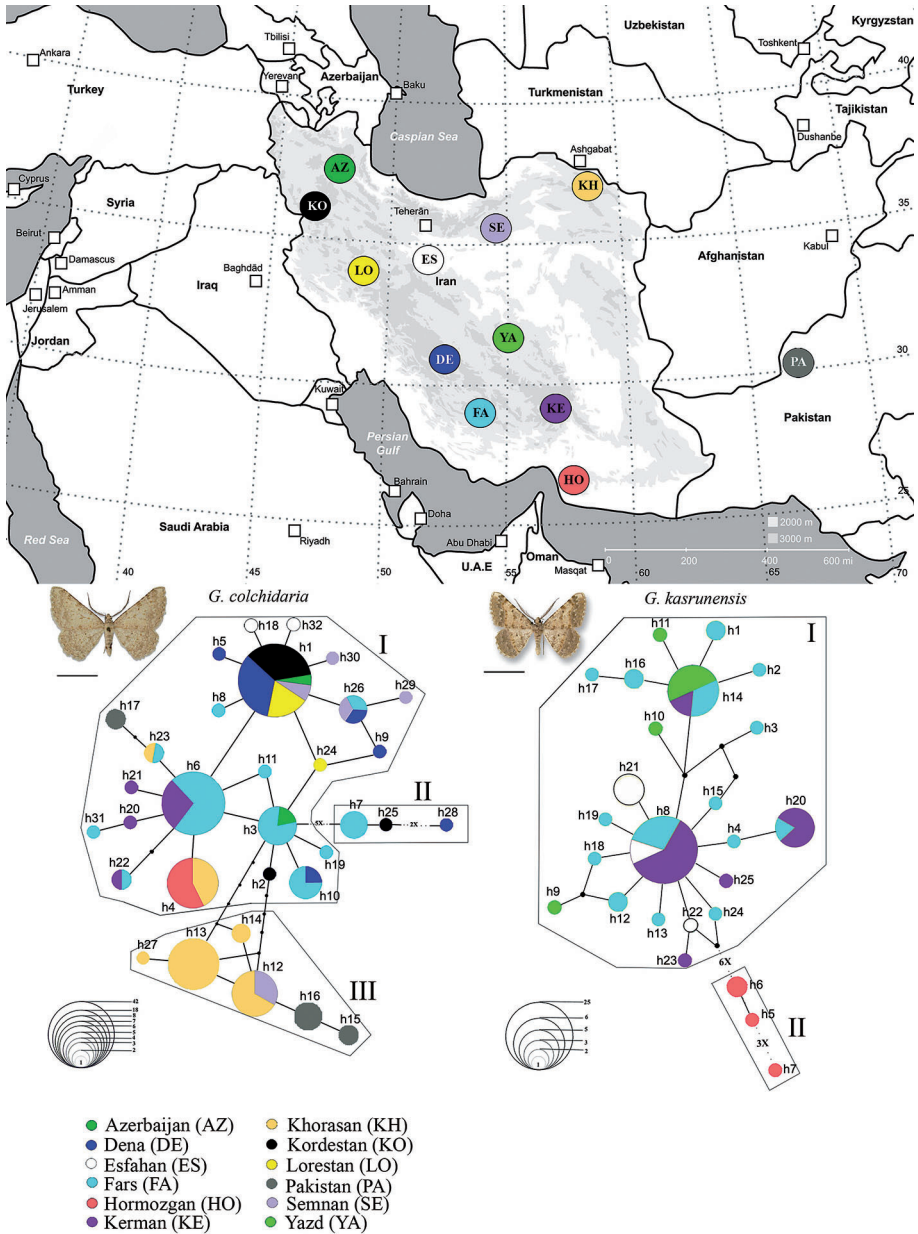
As an independent line of data, the results of species distribution modeling of *Gnopharmia colchidaria* were compared with the

genetic data of this species. The results of these two datasets fully allow to see their population distributions in the past (Fig. 6). Based on these results we were able to postulate a likely scenario for postglacial distribution of these species (Fig. 6).

### 3. Conclusions

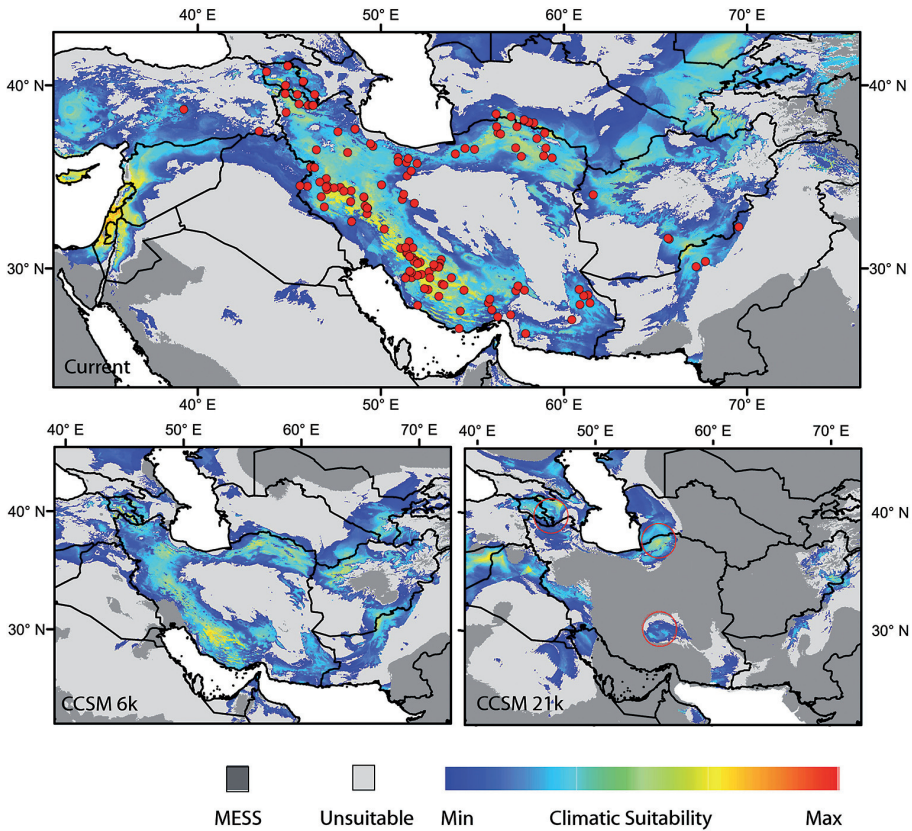
Complex taxonomic problems are like a multidimensional object, which in most cases need to multiple disciplines to be resolved. Looking to a multidimensional object only from one side will not help us to understand its whole concept. Today a wide range of standard tools (traditional and geometric morphometry, descriptive morphology, DNA barcoding, phylogeographical, eco-





**Fig. 5:** Haplotype networks of two *Gnopharmia* spp.: Localities are coded with colors and the diameter of the circles shows the haplotype frequency in the whole data set. High haplotype frequency of Fars and Khorasan localities confirms the existence of an old population from last ice age in these areas (see RAJAEI et al. 2013b for details).

**Abb. 5:** Netzwerk der Haplotypen von zwei *Gnopharmia* spp.: Fundorte sind farbig markiert; die Durchmesser der Kreise deuten die Häufigkeit der Haplotypen im gesamten Datensatz an. Die hohe Haplotyp-Frequenz bei Fars und Khorasan bestätigt für diese Gebiete die Existenz einer alten Population aus der letzten Eiszeit (s. RAJAEI et al. 2013b für Details).



**Fig. 6:** Species distribution models for *Gnopharmia colchidaria* based on the current distribution (map on the top). Projection of the current distribution to the past (6,000 and 21,000 years before present (BP) based on CCSM model system (below) confirms the existence of multiple refugia in 21,000 years BP (red circles) (see RAJAEI et al. 2013b for more details).

**Abb. 6:** Artverbreitungsmodelle für *Gnopharmia colchidaria* auf Grundlage der gegenwärtigen Verbreitung (Karte oben). Die Projektion der gegenwärtigen Verbreitung auf die Vergangenheit (vor 6.000 und 21.000 Jahren), basierend auf einem CCSM-Modellsystem (unten), bestätigt das Vorhandensein vieler Refugien vor 21.000 Jahren (rote Kreise) (s. RAJAEI et al. 2013b für Details).

logical and biogeographical methods) are developed and available to increase the quantity and quality of our arguments to support the best taxonomic hypothesis. In most cases, the disagreements between two methodological approaches originate from overlooked, unsolved (maybe new) problems, which need a new approach to be employed. This may open new windows for researchers. Furthermore, using a wide range of different methods and approaches will decrease the probability of failure and misunderstandings.

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