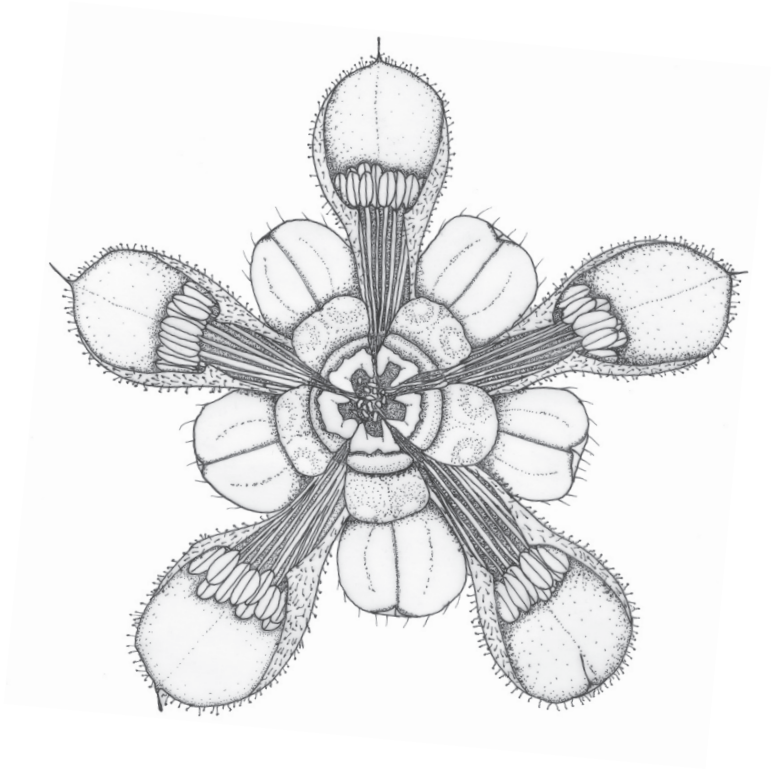
Systematics and floral ecology of *Nasa* (Loasaceae subfam. Loasoideae) and its allies



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The work here presented was carried out between 2007 and 2012 at the Institut für Biologie, Morphologie und Systematik der Phanerogamen, Freie Universität Berlin under the supervision of Prof. Dr. Maximilian Weigend, Nees-Institut für Biodiversität der Pflanzen, Rheinische Friedrich-Wilhelms-Universität, Bonn.

1st Reviewer: Prof. Dr. Maximilian Weigend

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We´re a virus with shoes!

Bill Hicks (†1994)

Cover: Flower of ***Nasa humboldtiana*** (Urb. & Gilg) Weigend subsp. ***subtrifoliata*** Henning & Weigend

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1. Introduction

1.1 Foreword

The author´s interest in Loasaceae and their fascinating pollination biology was initiated at an early stage of his biology studies in 2001 with a 3-month field trip to the Peruvian Andes. In the following years, additional field trips followed, yielding several new taxa and the first taxonomic study was published. Greenhouse experiments on the flower biology and pollination ecology and a constant interest in the taxonomy, systematics and morphology of Loasaceae in general and the genus *Nasa* Weigend* in particular accompanied the author´s biology study. Some minor aspects of these themes could be completed in the author´s diploma thesis. At the same time many questions regarding the α-taxonomy of *Nasa* and the flower morphology and biology of Loasaceae subfam. Loasoideae arose. An "Else-Neumann-Stipendium" (former NaFöG) offered the opportunity to address these issues in the framework of the author´s PhD-study, here presented. A DAAD grant allowed the comparison of the results of greenhouse experiments with data gained by a field study carried out in 2008 in north Peru.
The α-taxonomy of *Nasa* is now largely resolved and several aspects of the stunning pollination biology of Loasaceae have been extensively studied. The results of these two often closely linked topics have either been successfully published or are (or will be) submitted to international journals (see footnotes).

1.2 Loasaceae

Loasaceae are a medium sized plant family, established by de Jussieu (1804). The family is placed in the Cornales as sister to the Hydrangeaceae (Angiosperm Phylogeny Group, 2009), although this sister-relationship is only moderately supported by molecular data, the morphological similarities between some groups of Loasaceae and some of Hydrangeaceae are striking (Weigend, 2004a). Initially, the family consisted of the two genera *Loasa* Adans. and *Mentzelia* L. (de Jussieu, 1804) and was later on constantly expanded (e.g. *Gronovia* L., Bartling, 1825, *Blumenbachia* Schrad., Schrader, 1825). Reichenbach (1837) divided Loasaceae into the three groups Gronovieae, Mentzelieae and Blumenbachieae. The subfamilies, circumscribed by Gilg (1895, 1925) and especially Urban and Gilg (1900), largely correspond to Reichenbach´s (1837) classification. Their comprehensive systematic treatment of Loasaceae led to the recognition of the subfamilies Gronovioideae, Mentzelioideae and Loasoideae (incl. Blumenbachieae). This classification has since been largely followed and has (on the subfamiliar level) only been extended by the Petalonychioideae (Weigend, 1997b), a small, monogeneric subfamiliy of five species of (sub-)shrubs from SW USA and Mexico with a divergent inflorescence morphology.
Urban and Gilg´s (1900) treatment also provides revisions of several genera. Their generic circumscriptions and new subgeneric groups have since been debated by several authors

* Author citations are provided at first mention only (Chapterwise, except titel and abstract), if not otherwise indicated.

(Rydberg, 1903; Davidson, 1916; Davis & Thompson, 1967; Thompson & Ernst, 1967; Poston & Thompson, 1977; Hempel, 1995; Hempel & Jansen, 1996; Weigend, 1997a, b; Grau, 1997; Moody & Hufford, 2000; Weber & Wittmann, 2001) and/or were complemented by new genera (*Schismocarpus* S.F.Blake, Blake, 1918; *Plakothira* J.Florence, Florence, 1985; *Xylopodia* Weigend, Weigend, 1997b).
The mainly North American subfamilies Gronovioideae (with *Cevallia* Lag., *Fuertesia* Urb., *Gronovia* L.), Mentzelioideae (*Eucnide* Zucch., *Mentzelia* L., *Schismocarpus* Blake) and Petalonychioideae (*Petalonyx* A. Gray) have been in the focus of several phylogenetic and taxonomic studies within the last decades (Moody *et al.,* 2001; Hufford *et al.*, 2003; Weigend, 2007). Loasaceae subfam. Loasoideae have received particular attention only within the last few years, although it is by far the largest (ca. 2/3 = >200 spp. of all taxa recognized within Loasaceae) and most diverse subfamily in terms of morphology and ecology (Weigend *et al.*, 2004; Hufford *et al.*, 2005). Urban and Gilg (1900) recognized seven genera in three tribes: Kissenieae (*Kissenia* Endl., 2 spp.), Klaprothieae (*Klaprothia* Kunth, *Sclerothrix* C.Presl, 2 spp.) and Loaseae (*Blumenbachia* Schrad., *Caiophora* C.Presl, *Loasa* Adans. and *Scyphanthus* D.Don., ca. 150 spp.). Only minor generic changes have been undertaken since Urban and Gilg´s (1900) treatment, leading to the reduction of *Sclerothrix* under synonymy of *Klaprothia* (Poston & Nowicke, 1990) and the re-separation of *Huidobria* Gay from *Loasa* (Grau, 1997).
That the subfamily has long been scientifically neglected is clearly an artefact of its mainly South American distribution and the resulting undercollection within the 20th century. Extensive field work during the last decades, mainly by M. Weigend and colleagues, has added considerably to the knowledge of the subfamily. Repeated collection trips have yielded a great number of new taxa and led to the segregation of new genera. The most important rearrangement is the division of *Loasa* sensu Urban and Gilg (1900) into the four genera *Nasa* Weigend, *Aosa* Weigend, *Presliophytum* (Urb. & Gilg) Weigend and *Chichicaste* Weigend.

1.3 The genus *Nasa* Weigend

Nasa (short for "North Andean Loasas") now comprises four Series of *Loasa*, namely ser. *Grandiflorae* (Urb. & Gilg) Weigend, *Saccatae* (Urb. & Gilg) Weigend, *Carunculatae* (Urb. & Gilg) Weigend and *Alatae* (Urb. & Gilg) Weigend originally proposed by Urban and Gilg (Gilg, 1893, Urban and Gilg, 1900, Fig. 1.1). Its monophyly, claimed by Weigend (1997) on the basis of morphological and karyological data, has since been repeatedly confirmed by molecular studies (Hufford *et al.*, 2003; Hufford *et al.*, 2005; Weigend *et al.*, 2004, see Fig. 9.2). Until recently, less than half of the species were known to science (Fig. 9.1). Due to extensive collecting effort during the last two decades, many new taxa were discovered and formally described (Dostert & Weigend, 1999; Weigend, 1996a, b, c, 1997, 1999, 2000 a, b,2001a, 2002a, 2004b; Weigend & Rodríguez, 2001, 2002, 2003; Weigend *et al.*, 1998, 2003, 2006; Henning & Weigend, 2009b, Henning *et al.*, 2009, 2011; Chapters 2–4). *Nasa* is now by far the largest genus of Loasaceae and comprises ca. 100 species and a large set of subspecies (c. 40). The infrageneric systematics are actually not satisfyingly understood. The subdivision into the series established by Urban and Gilg (Gilg, 1893; Urban & Gilg,

1900) on the basis of morphological data appears doubtful in the light of recent molecular studies (Weigend *et al.*, 2004; Weigend & Gottschling, 2006, Fig. 1.1). Apart from *Nasa* ser. *Grandiflorae* (sensu Urban & Gilg, 1900), the other series are evidently paraphyletic (ser. *Saccatae,* ser. *Alatae*) or remain largely unresolved (ser. *Carunculatae*). The small ser. *Carunculatae* (5 spp.) is a morphologically well circumscribed species group of (sub-) shrubs from dry inner Andean valleys with a typical leaf morphology (Weigend *et al.*, 2003; Henning *et al.*, 2009). It lacks, however, a reliable placement in the molecular trees published so far, due to a insufficient taxon sampling. Nevertheless, some species-rich infrageneric groups clearly represent natural entities, identified by both, morphological and molecular data. These groups, namely the *Nasa triphylla* Juss. Weigend and *N. poissoniana* (Urb. & Gilg) Weigend group (both ser. *Saccatae*) together with *Nasa* ser. *Grandiflorae,* contain c. 40% of all species of *Nasa* and the vast majority of the subspecies known so far.

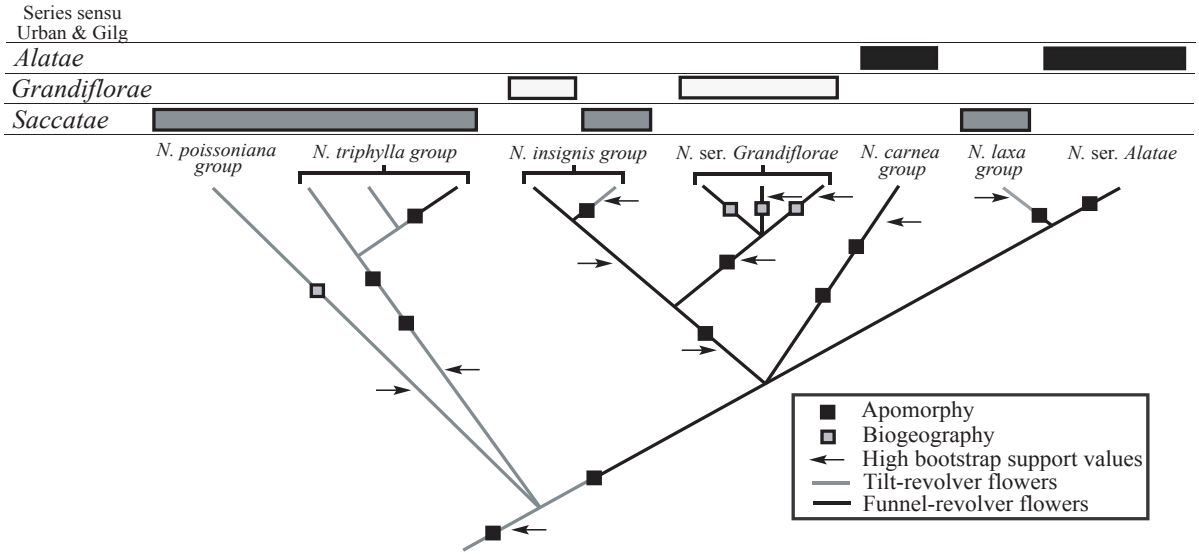

Figure 1.1: Annotated cladogram of *Nasa*, modified from Weigend & Gottschling (2006). Infrageneric relationships are based on molecular findings (ITS1 & trnL-ITS1, Weigend & Gottschling, 2006) and a conservative, parsimonious interpretation of the evolution of both flower types. The presence of tilt-revolver flowers in the *N. insignis* Weigend & E.Rodr. and *N. laxa* (J.F.Macbr.) Weigend groups are most likely reversions. Funnel-revolver flowers accordingly developed two times independently in the *N. venezuelensis* (Steyerm.) Weigend species group [nested within the *N. triphylla* (Juss.) Weigend group] and on the stem lineage to *N.* ser. "*Grandiflorae*" and *N.* ser. "*Alatae*" with two independent reversals in the *N. insignis* and *N. laxa* clades. Putative monophyletic *Nasa* ser. *Carunculatae* is not included here, since its placement is uncertain and not supported by molecular data so far.

1.3.1 Biogeography

The distribution of species of *Nasa* extends mainly from Bolivia to Colombia with only a few outliers in Central America (Weigend, 2001b, 2002a) and the vast majority of taxa are native to Peru. It is predominantly an Andean genus and most of the taxa can be found between 2000–4000m a.s.l., only few species occur at lower elevations [e.g. *Nasa urens* (Jacq.) Weigend, a typical floristic element of the so-called Lomas vegetation of the coastal deserts of Peru and Chile, Henning & Weigend, 2009b; Fig. 1.2 A, Chapter 2)]. Conversely, members of *Nasa* ser. *Grandiflorae* (Henning *et al.*, 2011, Chapter 4) have adapted to High Andean habitats (Figs. 1.2 G, H) and can be found at elevations well above 3000m to nearly 5000m.

Figure 1.2: Typical habitats of Loasaceae in Andean South America. A: Coastal Lomas in Sama Grande, Peru, with large stands of *Nasa urens* (Photograph taken by H. Förther), B: Inner-Andean valley near Contumaza in North Peru with species-rich forest remnants; C: Roadside in a former cloudforest area, habitat of *N. macrothyrsa* above Contumaza; D: Quebrada below Guzmango (Prov. Contumaza), habitat of narrowly endemic *N. contumazensis*; E: Remnants of a humid montane forest (Bosque de Monteseco) near La Florida (Depto. Cajamarca); F: Relict forest „Bosque de San Mateo" near Contumaza (Depto. Cajamarca); G: High Andean landscape of the Cordillera Negra in north Peru; H: Puna vegetation close to the Laguna Negra Huacanan, habitat of narrowly endemic *Nasa ranunculifolia* subsp. *macrorrhiza*.

Two main centers of diversity can be recognized, both in the Peruvian Andes. The first, the so called Amotape-Huancabamba Zone (Young & Reynel, 1997; Weigend, 2002b, 2004c), is a species-rich region in north Peru with an exceptional percentage of endemism, not only for Loasaceae but also for other Andean plant groups (see Chapters 2-4). Almost all groups of *Nasa* are represented here with many, often extremely narrowly endemic species (for example *Nasa* ser. *Carunculatae*, Weigend *et al.*, 2003; Henning *et al.*, 2009; *Nasa stuebeliana* (Urb. & Gilg) Weigend group, Weigend & Rodríguez, 2003; *Nasa triphylla* group, Dostert & Weigend, 1999; *Nasa ranunculifolia* group, Henning *et al.*, 2011 – Chapter 4). It is the primary center of diversity of *Nasa* and various ecological diversifications originated here (Weigend *et al.,* 2004). For example, the colonization of high altitudinal habitats by *Nasa* ser. *Grandiflorae* accompanied by profound changes in morphology and pollination biology has taken place in this region (Weigend, 2002b).
The second center of diversity, although less important, are the inner Andean valleys of central and southern Peru (esp. Departments Cuzco and Apurimac), which are home to the species of the *Nasa poissoniana* group (*Nasa* ser. *Saccatae*, Henning & Weigend, 2009b, 2011; Chapters 2 & 3). It has to be understood as a secondary center of diversity, resulting from a rapid diversification of ancestral taxa that likely migrated from the Amotape-Huancabamba Zone in north Peru and adjacent Ecuador.
Species of *Nasa* have occupied various habitats throughout their distributional range. Herbaceous taxa of "ser. *Saccatae*" are typically found in the undergrowth of shrub communities that provide rather moist conditions in the overall drier inner Andean valleys. Most collections are from forest remnants (Fig. 1.2 E, F), quebradas (Fig. 1.2 D) and roadsides in former cloudforest areas (Fig. 1.2 C). Annual species are often found in disturbed habitats like field margins, new road cuttings and landslide sites. Many members of *N.* ser. *Grandiflorae* are found in the High Andean Puna and Jalca habitats (Fig. 1.2 D, H), where they typically prefer sheltered spots at the base of rocks and boulders. Annual *N. urens* is the only exception by being restricted to the fog-oases (Lomas vegetation) in the coastal deserts of Peru and Chile.

1.3.2 Morphology

The genus is particularly rich in morphological characters due to its rapid diversification, its extensive Andean distribution and the high number of narrowly endemic taxa. The species of *Nasa* are annual to perennial, rarely biennial herbs (most species of ser. *Saccatae,* Fig. 1.3 A, some of ser. *Alatae* and ser. *Grandiflorae*) or (sub-)shrubs (*Nasa* ser. *Carunculatae*, ser. *Grandiflorae*, ser. *Alatae,* Fig. 1.3 B-E). Dwarf specimens of annual, herbaceous taxa (e.g. *Nasa picta* (Hook.) Weigend) barely reach 5cm in height, whereas biennial species of *Nasa* ser. *Grandiflorae* can form tall, profusely flowering shrubs that reach a size of more than 3m (Fig. 1.3 D). Most of the species have an erect stem that branches only above the ground. Additionally, rosulate herbs and (sub-)shrubs, often with extensive rhizomes, horizontal shoots and underground runners (e.g. *Nasa rugosa* (Killip) Weigend subsp. *llaqtacochaensis* T.Henning, E.Rodr. & Weigend, Fig. 1.3 F), as well as climbing species occur.
The leaves are opposite or alternate and almost all lamina shapes conceivable can be found, from entire to variously divided to peltate and reniform leaves (Fig. 1.3 G-K). The same is true for the leaf margins, which can be entire, denticulate, serrate or variously lobed.

Leaf types and shapes can vary extremely, even between closely allied taxa (Fig. 1.3 G, H), but can also be the characterizing feature of some infrageneric entities (e.g. *Nasa triphylla* group, Dostert & Weigend, 1999).
As typical for Loasaceae, species of *Nasa* are usually covered with different types of hairs on almost all parts of the plant surface, although few, more or less glabrous taxa occur in different infrageneric groups. Three basic types, stinging hairs (setae), uniseriate gland-tipped trichomes and scabrid-glochidiate trichomes, can be distinguished and their occurrence and/or density often provides valuable information for taxonomic delimitation (e.g. *Nasa humboldtiana* (Urb. & Gilg) Weigend ssp., Henning & Weigend, 2009a).
By far the most interesting feature in terms of both, morphology and function, are the flowers of Loasaceae in general and those of Loasaceae subfam. Loasasoideae, and hence *Nasa*, in particular. Throughout the last two centuries the flowers of Loasaceae have repeatedly been in the focus of several studies regarding their morphology and ontogeny, often within a taxonomic framework (Payer, 1857; Gilg, 1893, 1925; Urban, 1886, 1892; Urban & Gilg, 1900; Brown & Kaul, 1981; Leins & Winhard, 1973; Hufford, 1990, 2003; Weigend, 1997b, 2004b). Only recently, different authors provided some functional approaches either with a focus on the behaviour of the flower visitor (Harter *et al.*, 1995; Schlindwein & Wittmann, 1992, 1997b; Wittmann & Schlindwein, 1995) or the different adaptations of floral morphology and function by the plants (Ackermann & Weigend, 2006; Weigend & Gottschling, 2006; Weigend *et al.*, 2010).

1.3.3 Flower morphology, floral function and pollination

The flowers of Loasoideae have a particular complex morphology with a unique combination of structural and functional adaptations. The bisexual flowers are pentamerous, choripetalous, poly- and proterandric. Apart from the inferior ovary (with three parietal placentae and numerous ovules, developing into a predominantly many seeded capsule with persisting, usually simple calyx-lobes), all other flower organs show more or less spectacular modifications that are functionally closely linked. Two floral types can be distinguished: tilt- and funnel-revolver flowers (Weigend, 2004b; Weigend & Gottschling, 2006). Both types are conceptual derivations of the term "revolver-flowers", coined by Endress (1994, p. 116) for flowers with more than one access point to the nectar-bearing flower part. Tilt-revolver flowers are the plesiomorphic flower type for Loasaceae subfam. Loasoideae (Weigend & Gottschling, 2006, Fig. 1.3 L, M) and are widespread in the genera *Aosa*, *Blumenbachia*, *Caiophora, Loasa*, *Nasa*, *Scyphanthus*, *Plakothira* and *Xylopodia* (Weigend, 2004a). Conversely, funnel-revolver flowers are the derived floral type limited to some younger lineages within *Nasa* (*N. venezuelensis* group, ser. *Alatae* and *Grandiflorae,* cf. Fig. 1.1, Fig. 1.3 N). Tilt-revolver flowers have spreading to reflexed, hooded to boatshaped petals (Fig. 1.3 L, M). The numerous stamens are arranged in antepetalous fascicles of c. 10–40 each and initially hidden in the petals. The stamens mature successively and perform a movement into the center of the flower, one by one, presenting their pollen (cf. Fig. 5.1, 6.1). Between the stamen fascicles, antesepalous staminodial complexes can be found. These complexes consist of three outer, fused staminodes that form a “floral scale” and two free inner staminodes that lean against the scales from the center of the flower. Nectar is secreted at the inner base of the nectar scales and can only be harvested through

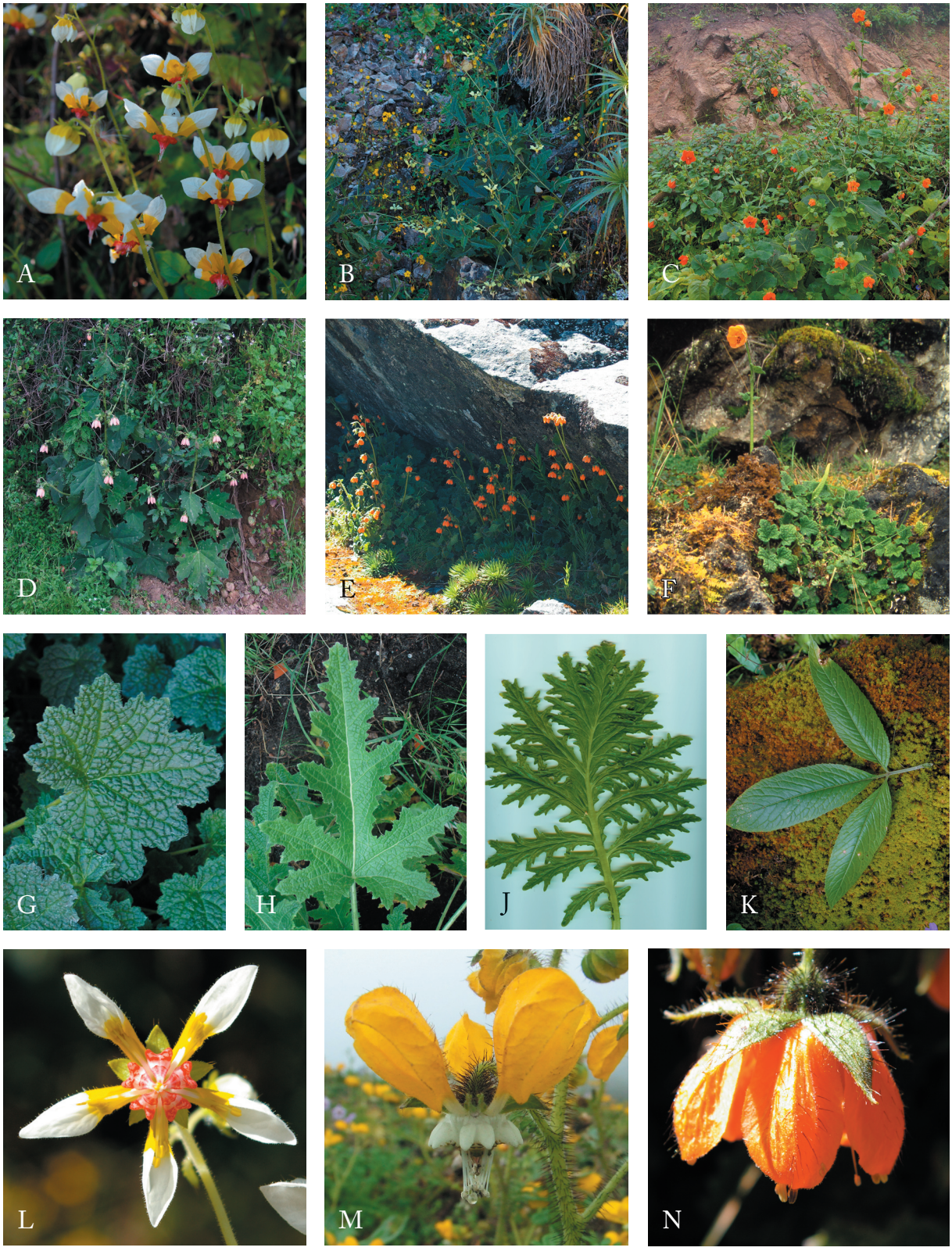

Figure 1.3: Morphological characters and variation in *Nasa*. A: Flowers of *Nasa picta* ssp. *pamparomasii* (*N.* ser. *Saccatae*, Henning 08/05); B: Shrubby *Nasa macrothyrsa* near Contumaza (*Nasa* ser. *Carunculatae*, Henning 08/14); C: *Nasa olmosiana* in relict forest remnants in north Peru (*N.* ser. *Alatae*, Henning 08/23); D: *Nasa magnifica* from Ancash (*N.* ser. *Grandiflorae*, Weigend 8476); E: *Nasa ranunculifolia* subsp. *macrorrhiza* close to the Laguna Negra Huacanan at ca. 5000m (*N.* ser. *Grandiflorae*, Henning 08/08); F: *Nasa rugosa* subsp. *llaqtacochaensis* from the Laguna de los Condores area (Depto. Amazonas, *N.* ser. *Grandiflorae*, Henning & Schneider 303); G-K: Leaf shapes in *Nasa*; G: Rounded leaves of *Nasa ranunculifolia* ssp. *macrorrhiza*; H: Elongated leaves of closely allied *Nasa ranunculifolia* subsp. *pamparomasii*; J: Bipinnatifid leaves of *Nasa urens*; K: Trifoliolate leaves of *Nasa humboldtiana* ssp.; L-N: Flowers of *Nasa picta* (L), *Nasa urens* (M) and *Nasa ranunculifolia* subsp. *macrorrhiza* (N)

the apical opening formed by the scale neck and the two free inner staminodes. This opening is constructed in a way that the principle pollinator has to get over a mechanical resistance by bending the hinged scale outwards.

Funnel-revolver flowers (Weigend, 2004b) share the same overall flower morphology but have a more or less campanulate corolla with less hooded petals. The stamen bundles are accordingly only slightly bended backwards and only shallowly covered by the petals. The much larger floral scales in these flowers are stiffly erect with a notably wider apical opening. The nectar harvest is only restricted to specialized pollinators by the longer distance to the scale base, where the nectar is held.

Overall flower morphology of Loasaceae has long been subject of research and has been studied already by Payer (1857) and Urban and Gilg (1893, 1925). A stamen movement was first described by Urban (1886, 1892) and later on by Brown and Kaul (1981). A functional connection between the stamen movement and the behaviour of nectar harvesting flower visitors was discovered only quite recently by Schlindwein and Wittmann (1992). By inserting their proboscis into the apical opening of the nectar scales in order to take up nectar, the flower visitor is forced to bend the scale outwards. This mechanical stimulus triggers a stamen movement. Schlindwein and Wittmann described this thigmonastic stamen movement for three species of *Caiophora* [*C. arechavaletae* (Urb.) Urb., *C. clavata* Urb. & Gilg, *C. eichlerii* (Urb.) Urb] and *Blumenbachia insignis* Schrad. in the context of a complex foraging strategy of specialized bees (Wittmann & Schlindwein, 1995; Schlindwein & Wittmann, 1997b).

The occurrence of tilt-revolver flowers is strongly correlated to entomophily (Weigend & Gottschling, 2006). A wide range of insect pollinators have been reported for this plesiomorphic flower type (Schlindwein, 2000; Ackermann & Weigend, 2006; Weigend & Gottschling, 2006). The vast majority of taxa is visited by bees (e.g. Colletidae, Apidae, Megachilidae, Halictidae), but flies (*Plakothira*) and butterflies (*Presliophytum*) also act as pollinators in single lineages. Funnel-revolver flowers are the derived flower type of Loasoideae and are restricted to some infrageneric groups of *Nasa*. This floral type evolved two or three times independently (*Nasa venezuelensis* (Steyerm.) Weigend group, *N.* ser. *Grandiflorae*, *N.* ser. *Alatae* - Weigend & Gottschling, 2006). Generally, the driving force of the transformation from tilt- to funnel-revolver flowers was the colonization of High Andean habitats (except the *N. venezuelensis* group which has an Andean but not High Andean distribution, Weigend, 2004a; Noguera-Savelli & Ruíz-Zapata, 2006). At elevations above 3000m, insects fail to be reliable pollinators due to the climatic conditions. Hummingbirds are very diverse in these heights and are the most important pollinator guild. The High Andean lineages within Loasoideae (e.g. *Nasa* ser. *Grandiflorae*, *N.* ser. *Alatae*) have repeatedly switched to ornithophily. The corresponding transformations in the floral morphology are similar and their paraphyletic origin has only been revealed by molecular data (Weigend & Gottschling, 2006).

Flower morphology and floral function are closely linked. This is particularly true for highly derived flowers as is the case in Loasoideae. The modifications between tilt- and funnel-revolver flowers are obviously linked to different pollinator taxa and their respective behaviour. Floral function in Loasoideae was initially analyzed in detail in species with tilt-revolver flowers (Schlindwein & Wittmann, 1992, 1997b, 2000; Wittmann &

Schlindwein, 1995). As mentioned above, they discovered the thigmonastic nature of the stamen movement and finally described it for a few melittophilous species of *Caiophora*, *Blumenbachia* and *Loasa* from southern South America (Brazil, Argentina and Chile). Later on, they reported the loss of a thigmonastic stamen movement in hummingbird pollinated *Caiophora hibiscifolia* (Griseb.) Urb. & Gilg (Harter *et al.*, 1995). The putative loss of thigmonasty by a shift from entomophily to a different pollination syndrome was later on underlined by Cocucci and Sércic (1998), who reported it for rodent pollinated *Caiophora coronata* (Arn.) Hook. & Arn. Ornithophilous lineages of *Nasa* with funnel-revolver flowers have so far not been analyzed in this respect. Thigmonasty was hence thought to be intimately and exclusively correlated with entomophily. The first data on species with a divergent pollination syndrome indicated the immediate loss of thigmonasty. In these cases, this loss was not correlated with profound changes in flower morphology, both species have tilt-revolver flowers that show only minor modifications in corolla shape, colour and orientation. The stamen movement in these taxa was thought to be reduced to a spontaneous movement that follows a daily rhythm (Cocucci & Sércic, 1998).

1.4 Pollen presentation

Male function plays a crucial role in outbreeding success and is thus substantial for the fitness of a plant species. In animal pollinated plants, male function is based on flower-pollinator-interaction (pollen release and dispersal) and this has traditionally mainly been considered as adaptations in flower morphology, such as corolla shape, color or orientation of particular sexual organs. A zoophilic flower is predominantly visited several times by a potential pollinator. Thus, the chance to successively produce offspring improves with additional visits, making the timing (of pollen release) an important adaptive trait, too. Lloyd and Yates (1982) and Lloyd (1984) concluded that the major selective force guiding floral evolution is to increase the proficiency of pollen donation. They emphasize that this in particular means to exert influence on the number of visits and the timing of pollen pickup by pollinators. In the following 25 years, some seminal works emphasized the importance of floral traits that determine the pollen pickup by pollinators (Harder & Thomson, 1989; Harder & Wilson, 1994, 1998; LeBuhn & Holsinger, 1998; Thomson, 2003). Especially Thomson and colleagues provided detailed insight in the process of pollen transfer by modelling plant-pollinator interaction. "Pollen presentation theory" (PPT) (Percival, 1955) was introduced by Thomson *et al.* (2000) to circumscribe all models and theories that embrace the fact that plants can increase male fitness by adjusting pollen presentation to the quality and quantity of the pollinator.

The timing of pollen presentation shows a broad variety, from presenting all pollen simultaneously within minutes to few hours (e.g. *Drosera tracyi* (Diels) Macfarl., Wilson, 1995; *Mentzelia* L. Loasaceae subfam. Mentzelioideae - Weigend, 2008) to a decelerated pollen presentation over several hours or days (*Erythronium* L. Harder & Thomson, 1989; *Penstemon* Mitch. Castellanos *et al.*, 2006; for other examples see Percival, 1955). For the latter, two principle floral mechanisms that restrict pollen removal can be considered: *packaging* and *dispensing*. Packaging means that pollen is presented sequentially at some organizational level. The per-plant pollen production can be divided into inflorescences, individual flowers, stamens or even anther sacs that open consecutively. Dispensing of pollen

might be less obvious, but shows a greater diversity of mechanisms employed. Here, anthers slowly present either only parts of the pollen contained at a time (poricidal anthers or gradual opening, e.g. *Dodecatheon* L. - Primulaceae, Harder & Barclay, 1994), or pollen is presented via secondary pollen presentation as common in the large families Asteraceae, Campanulaceae and Fabaceae (Lavin & Delgado, 1990; Etcheverry, 2001). Finally, active pollen-dusting-mechanisms, such as the stamens of *Kalmia* L. (Ericaceae - Real & Rathcke, 1993) and *Berberis* L. (Berberidaceae - LeBuhn & Anderson, 1994) that spring inwards upon tripping by an insect, fall into that category.

The occurrence and extent of gradual pollen presentation reflects the overall pollination syndrome found in a species. For *Penstemon* Castellanos *et al.* (2003, 2006) could show that there is an intimate correlation between the degree of deceleration in pollen presentation and the type of pollinator observed. Their results roughly revealed that hymenopteran-adapted species present pollen more gradually than their hummingbird-adapted relatives. Hummingbirds, on the one hand, are known not to preen so often and lack special grooming devices. Hence, pollen loads on a hummingbird forehead can stay for a long time. On the other hand, Hymenopterans, especially bees, are likely to groom pollen grains off and tend to intensify grooming after heavy dusting with pollen (Harder & Thomson, 1989; Harder, 1990a, b), which leads to a big pollen loss. Conversely, hummingbirds are unreliable in terms of flower constancy and revisits, since they are known to fly long distances during a foraging bout. In turn, Hymenopterans (esp. bees) often are short distance trapliners that are more or less flower constant and shuttle frequently between populations of the same species, making repeated visits likely and a gradual pollen presentation useful. The optimal mode of pollen presentation is thus closely connected to the quality and quantity of the pollinator and its behaviour. A mechanism of pollen presentation that is perfectly adapted to a pollinator taxon should upgrade the outbreeding success. In the case of pollination by traplining Hymenopterans, the pollen presentation should be extended to make use of several revisits. LeBuhn and Holsinger (1998) concluded that: "A plant should allocate pollen such that all pollinators that visit remove pollen". Hence, by packaging and dispensing a plant can ideally control the amount of pollen received by each pollinator visit. This system would require either a very constant frequency of revisits or a flexible mode of pollen presentation. Harder and Wilson (1994) called this the "unlikely case in which the number of visits to be received is higly predictable and the individual plant possess the ability to adjust pollen-dispensing schedules accordingly" by which "plant fitness may increase substantially".

Pollinator behaviour and especially visitation rates may, however, vary throughout the time a flower is presenting pollen. Visitation frequency may change depending on weather, daytime or competition among pollinators. Nevertheless, revisits of specialized trapliners will still be predictable if they are reliable (and do not exceed a specific time frame) and if the pollen presentation mode is connected with the previous visit.

This "unlikely case" was first described by Schlindwein & Wittmann (1992, 1997) for *Caiophora arechavaletae* Urb. (Loasaceae) and *Perditomorpha pampeana* (Colletidae) from southern Brazil. This was the first description of pollen dispersal in several small packages (anthers) via a thigmonastic stamen movement in Loasaceae subfam. Loasoideae.

1.5 Stamen movement

Movements of floral organs are frequently found in several plant families. Kölreuter (1761) mentioned the stamen movement initiated by flower visitors in *Berberis*, Smith (1788) reported pollen-throwing-stamens for *Parietaria* L. (Urticaceae) and Sprengel (1793) considered the stamen movement of *Nigella arvensis* L. (Ranunculaceae) as a special mechanism to support pollination. Later on, reports of floral movements tended to be restricted to spectacular movements that deposit pollen directly on the pollinators body, e.g. *Catasetum* Rich. ex Kunth (Orchidaceae - Romero & Nelson, 1986) or *Cornus canadensis* L. (Cornaceae - Edwards *et al.*, 2005). Other reports mainly concentrate on active pollen release in anemophilous plants, e.g. *Ricinus* L. (Euphorbiaceae - Bianchini & Pacini, 1996) or *Trophis* P.Browne (Moraceae - Bawa & Crisp, 1980; Simons, 1992). These very rapid movements are usually driven by a release of stored energy and are thus passive mechanisms. Stamen movement in terms of pollen packaging or dispensing (instead of unique pollen deposition as in *Catasetum*) usually requires a slower movement or a mechanism that can be triggered repeatedly (e.g. *Berberis* - LeBuhn & Anderson, 1994). Often these stamen movements can be tripped by a flower visitor. These thigmonastic stamen movements are reported for several plant families: Aizoaceae, Berberidaceae, Cactaceae, Cistaceae, Malvaceae, Portulacaceae and Tiliaceae (Kabsch, 1861; Unger, 1863; Juel, 1906; Bünning, 1959; Guttenberg, 1971; Jaffe *et al.*, 1977), but are often restricted to a genus or even a single species.

For Loasaceae, a stamen movement was first reported by Urban (1886, 1892). Brown and Kaul (1981) later on described it for *Loasa*, *Blumenbachia* and *Caiophora*. But its thigmonastic nature was discovered only very recently by Schlindwein & Wittmann (1992) who reported it for four species, namely *Caiophora arechavaletae*, *C. clavata*, *C. eichleri* and *Blumenbachia insignis* Schrad. (Wittmann & Schlindwein, 1995; Schlindwein & Wittmann, 1997), as part of a complex mutualistic pollination system.

Only recently (Weigend *et al.*, 2010, Chapter 5), thigmonasty in Loasaceae has been analyzed in detail for the first time. A series of upcoming publications will provide further insights into this complex floral "behaviour" (Chapters 6-8).

1.6 Aims and scope

1.6.1 Hypotheses

The unique floral morphology and function of Loasaceae subfam. Loasoideae was the main driving force for the process and rate of their evolution and diversification. The resulting (ongoing) rapid radiation in *Nasa* led to many taxon rich, infrageneric groups that successfully occupied various habitats by making optimal “use” of the available pollinators. If so, (i) a key invention regarding the complex floral morphology and function possibly separates the species-poor, basally placed genera from the diverse, species-rich derived lineages that underwent a rapid adaptive radiation. (ii) The detailed patterns of the pollination biology of the different lineages will differ notably between genera of Loasoideae as well as infrageneric groups within *Nasa*. These differences will be closely linked to taxon-specific adaptations to the respective habitat and pollinator. (iii) The

revision of large monophyletic infrageneric groups will reveal numerous new taxa at different taxonomic levels. This underlines the recent radiation and an ongoing diversification of *Nasa*, especially by the recognition of closely allied, but geographically clearly disjunct taxa. (iv) The complex flower morphology and a thigmonastic stamen movement is advantageous, irrespective of the type of habitat occupied and pollinator recruited. A complete secondary loss of thigmonasty, as indicated by Harter *et al.* (1995), did not take place. This is (v) also true for High Andean taxa that successfully colonized fragmented habitats with extreme climatic conditions. Diversity and distributional ranges of these lineages will be comparable to those of groups from lower elevations.

1.6.2 Research questions

The hypotheses presented above raise the following questions, which will be in the focus of this work:

1. Is the interaction with a specialized pollinator in species of Loasaceae subfam. Loasoideae as tight as indicated by previous studies?

2. What are the regulatory mechanisms of both, the non-stimulated (autonomous) and the thigmonastic stamen movement?

3. Can be identified where a thigmonastic stamen movement arose in the phylogeny of Loasaceae subfam. Loasoideae?

4. Are there different levels of development and is there a gradual increase of complexity of the movement patterns, paralleling the phylogeny of Loasaceae subfam. Loasoideae?

5. Is the complex flower morphology and interaction with the main pollinator taxon an economical trade-off for the plants and is it advantageous under all circumstances?

6. Can a reversal and secondary loss of stamen movement, as described for two species of *Caiophora* pollinated by hummingbirds and non-flying mammals, be confirmed and is this a general trend in lineages with a derived pollination syndrome?

7. Is thigmonasty a key invention and has it influenced diversity and distribution in Loasoideae?

1.6.3 Specific goals

1. Completion of the taxonomic revision of the genus *Nasa*.

2. Studying the plant-pollinator interaction in detail in selected species of *Nasa*.

3. Performing analyses of the process of the (autonomous) stamen movement in absence of flower visitors.

4. Carrying out experimental analyses regarding the influence of the pollinator on the process of the individual stamen movement and the whole anthesis under different visitation regimes.

5. Providing quantitative and qualitative analyses of the stamen movement of as many taxa as possible from preferably all genera.

6. Analyzing the cost, effort and effect of the floral function compared to general trends in plants with comparable pollination syndromes.

7. Investigation of the presence and patterns of the stamen movement in Loasoideae with a derived pollination syndrome and/or a divergent altitudinal distribution.

1.6.4 Overview of the dissertation

This dissertation consists of seven manuscripts that are either published in, submitted to or in preparation to be submitted to international, peer-reviewed journals. Accordingly, the Chapters 2[a] to 8[g] are structured as journal articles, each containing a separate introduction as well as sections for materials and methods, results and discussion. For reasons of clarity and to avoid redundancies, the references cited are listed together following Chapter 9. Data, not necessarily needed for the understanding of the single chapters (e.g. lists of specimens examined, vouchers and supplementary data), are provided in the appendix at the end of this work.

The Chapters 2[a] to 4[c] are dedicated to the systematics of *Nasa* and its infrageneric groups. Two larger species groups are revised (Chapters 2[a] and 4[c]) and additionally, two new species are described as new to science (Chapter 3[b]). Chapters 2[a] to 4[c]are ordered chronologically, according to the date of their publication. The findings of these chapters are based on a comprehensive revision of all herbarium material available from herbaria worldwide, copious own collections (or by the coauthors) and on observations made on living plants either during field trips or on cultivars in the greenhouse. For each taxon, a formal description, including notes on the ecology and distribution, is given. Distribution maps are provided and each taxon is illustrated by a line drawing.

The *Nasa poissoniana* (Urb. & Gilg) Weigend group is revised in Chapter 2[a]. This group has been retrieved by earlier molecular findings and represents a monophyletic group of herbaceous, short lived species of *Nasa* with a distribution focussed on the south of Peru. One species, *Nasa raimondii* (Standl. & F.A.Barkley) Weigend, formerly placed in the *N. stuebeliana* (Urb. & Gilg) Weigend group (Weigend & Rodríguez, 2003), is transferred into the *N. poissoniana* group due to recent molecular findings (Weigend & Gottschling, 2006). A new subspecies of *N. poissoniana, N. poissoniana* subsp. *glandulifera* T.Henning & Weigend from North Peru, is defined based on consistent morphological differences and geographical vicariance.

Coincidentally, two collections of a new species, obviously belonging to the *N. poissoniana* group, came to our knowledge shortly after the revision (Chapter 2[a]) of the group has been published. This new species (*N. urubambensis* T.Henning & Weigend) is, together with the new *N. sanchezii* T.Henning & Weigend, taxonomically treated in Chapter 3[b]. The affinities of both species are discussed with a particular focus on the new extent of the *N. poissoniana* group.

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[a] Henning T, Weigend M. 2009. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical Journal of the Linnean Society* 161: 278-301.

[b] Henning T, Weigend M. 2011. Two new species of *Nasa* (Loasaceae) from Andean South America. *Phytotaxa* 26: 1-8.

The species rich and diverse *Nasa ranunculifolia* group is revised in Chapter 4[c]. Two species, *N. basilica* T.Henning, E.Rodr. & Weigend and *N. tulipadiaboli* T.Henning, E.Rodr. & Weigend, are described as new to science. The new circumscription of a variable and widely distributed taxon, formerly divided into four species [*N. ranunculifolia* (Kunth) Weigend, *N. macrantha* (Urb. & Gilg) Weigend, *N. cymbopetala* (Urb. & Gilg) Weigend and *N. macrorrhiza* (Urb. & Gilg) Weigend] became necessary in the light of the evaluation of all herbarium material assigned to either of these names and the observations made in the field. A subspecies concept has been adopted to take account of the rather gradual morphological differences and the geographical vicariance of this tribe. Finally, *N. ranunculifolia, N. macrantha, N. cymbopetala* and *N. macrorrhiza* are united as subspecies under (oldest) *N. ranunculifolia* and an additional set of five subspecies has been described (subspp. *pamparomasii* T.Henning, E.Rodr. & Weigend, *guzmangoensis* T.Henning, E.Rodr. & Weigend, *bolivarensis* T.Henning, E.Rodr. & Weigend, *patazensis* T.Henning, E.Rodr. & Weigend and *huanucoensis* T.Henning, E.Rodr. & Weigend). A second, closely allied species, *Nasa rugosa* (Killip) Weigend, is reevaluated and subdivided into four still poorly documented, but clearly distinct subspecies (subspp. *rugosa*, *llaqtacochaensis* T.Henning, E.Rodr. & Weigend, *gracilipes* T.Henning, E.Rodr. & Weigend and *pygmaea* T.Henning, E.Rodr. & Weigend).

The floral biology of Loasaceae subfam. Loasoideae, centered around the stamen movement, is substance of the Chapters 5[d] to 8[g]. Chapter 5[d] attempts to classify the detailed pollination patterns observed within a broader theoretical framework, using data gained by field and greenhouse experiments. The interaction between these plants and their pollinators is exemplarily illustrated on *Nasa macrothyrsa* (Urb. & Gilg) Weigend pollinated by *Neoxylocopa lachnea* Moure 1951. Its significance is discussed in the context of the pollen presentation theory.

The conclusions of Chapter 5[d] are the basis for a study on the cost/benefit ratio of the complex floral behaviour for the plants conducted in Chapter 6[e]. A set of six species was chosen, representing different breeding systems and pollination syndromes, in order to reveal adaptations regarding these conditions and resulting shifts of floral traits. Two different lineages of Loasaceae subfam. Loasoideae (*Nasa* and *Caiophora*) were represented by three species each, to be able to detect possible phylogenetic constraints.

Chapter 7[f] experimentally outlines the mechanisms of the stamen movement in terms of the optimization of the pollen presentation by pollen packaging. Several aspects of the control of the plant over the amount of pollen offered at a given time and the presence, absence or actual abundance of pollinators are analyzed. Data from eight species from four genera of Loasaceae subfam. Loasoideae are included in this study to achieve an insight, generally valid across the genera of the subfamily.

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[c] Henning T, Rodríguez E, Weigend M. 2011. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae pro parte*, Loasaceae). *Botanical Journal of the Linnean Society* 167: 47-93.

[d] Weigend M, Ackermann M, Henning T. 2010. Reloading the revolver - male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales). *Biological Journal of the Linnean Society* 100. 124-131.

[e] Henning T, Ackermann M, Weigend M. 2012. Complex, but not cheap - floral rewards and floral behaviour in six species of Loasaceae. Submitted to *American Journal of Botany* manuscript ID: AJB-D-12-00023.

[f] Henning T, Weigend M. in prep. Total control - pollen presentation and floral longevity are modulated by light, temperature and visitation rates. To be submitted to *Plos One*.

Conversely to the commonalities in the mechanisms of the stamen movement determined in Chapter 7[f], Chapter 8[g] attempts to reveal differences in the detailed schedule of pollen presentation apart from the general process. The ability to perform a pollen presentation via a stamen movement is principally present in all genera of Loasoideae and can be predicted by the respective flower morphology. In turn, thigmonasty and minor differences in the detailed schedule of the pollen presentation can only be proved experimentally. All species available (45 species from 10 genera) are combarably treated with a standard experiment in this study (Chapter 8[g]). The results were statistically tested and distinct patterns observed are discussed in the context of pollination syndrome, phylogenetic placement and minor aspects of specific adaptations of single lineages.

In Chapter 9, a summary of the findings of the two main topics, systematics (Chapters 2[a] to 4[c]) and floral ecology (Chapters 5[d] to 8[g]), is provided. A comprehensive conclusion using all relevant findings of each aspect is given. The last paragraph of Chapter 9 furthermore tries to establish an explanatory background, linking these two themes, and it is discussed in how far they are mutually dependent. Finally, possible issues for future research are identified, which now can be addressed based on the results of the studies completed so far.

[g] Henning T, Weigend M. in prep. Stamen movement and pollen packaging in Loasaceae subfam. Loasoideae. To be submitted to *BMC Evolutionary Biology*.

2. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America *

Abstract

The monophyletic *Nasa poissoniana* group (Loasaceae, subfamily Loasoideae) is revised on the basis of extensive field studies, observations in cultivation and the revision of herbarium specimens. A core of taxa has been considered as closely allied on the basis of morphology in the past, but several additional taxa have been recognized as allied to this group on the basis of molecular data. One species, *N. raimondii*, formerly placed in the *N. stuebeliana* group, is now transferred to the *N. poissoniana* complex as a result of the molecular findings. *Nasa poissoniana* subsp. *glandulifera* is described as new to science on the basis of morphologically divergent collections of *N. poissoniana* from two northern Departments of Peru. Numerous new localities, often far from the previously known distribution area, are reported for several species. The *N. poissoniana* group has its center of diversity in the inner Andean valleys of southern Peru, quite unlike all other groups of *Nasa*, with centers of diversity in the Amotape–Huancabamba Zone in northern Peru and southern Ecuador. Descriptions, drawings and a distribution map and key to all taxa are provided, and detailed information on habitat and distribution is given for each species.

2.1 Introduction

Nasa Weigend, with over 100 species, is the largest genus of Loasaceae (Cornales; Weigend, 2004a; Weigend *et al*., 2004, 2006). Infrageneric systematics remain problematic in spite of the considerable morphological diversity. A large group of species, comprising approximately half of the taxa recognized, fall into the paraphyletic assemblage of series *Saccatae* Urb. & Gilg (Urban & Gilg, 1900), a group defined on the basis of its annual habit and spreading, white or yellow petals, but these characters are now known to be plesiomorphic for the genus and fail to define a monophyletic group (Weigend & Gottschling, 2006). Within this paraphyletic assemblage, three infrageneric groups can be recognized on the basis of morphological and/or molecular data, namely the *Nasa triphylla* (Juss.) Weigend group (Dostert & Weigend, 1999), the *Nasa stuebeliana* (Urb. & Gilg) Weigend group (Weigend & Rodríguez, 2003) and the *Nasa poissoniana* (Urb. & Gilg) Weigend group. Recent revisions are available for the first two groups, but the third has not been formally revised and all its constituent species have been published in separate publications. The first two groups have morphological synapomorphies and were thus recognized on the basis of morphological studies, but the *N. poissoniana* group lacks such clear morphological coherence and was recognized on the basis of molecular data (Weigend *et al*., 2004, Weigend & Gottschling, 2006). In addition, both the *N. stuebeliana* and *N. triphylla* groups have their centers of diversity in the Amotape–Huancabamba Zone in northern Peru and southern Ecuador, a region of extreme species' richness in

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many Andean plant groups [*Urtica* L. (Urticaceae; Weigend *et al*., 2005), *Ribes* L. (Grossulariaceae; Weigend *et al*., 2005; Weigend & Rodríguez, 2006), the *Passiflora lobbii* Mast. group (Passifloraceae; Skrabal *et al*., 2001) and *Iochroma* Benth (Solanaceae; Smith & Baum, 2006)]. The *N. triphylla* group is widespread and has one subspecies reaching southern Mexico, but its center of diversity is clearly in the Amotape–Huancabamba Zone. The *N. stuebeliana* group is largely restricted to the Amotape–Huancabamba Zone, with only two species found on the western slope of the Andes in central Peru. Conversely, the core members of the *N. poissoniana* group [*N. poissoniana* (Urb. & Gilg) Weigend, *N. vargasii* (J.F.Macbr.) Weigend, *N. ferruginea* (Urb. & Gilg) Weigend] were, until recently, only known from the inner Andean valleys of southern Peru, namely the Departments of Cuzco and Puno (Fig. 1). These three species are morphologically relatively easily recognized as allied, as is the recently discovered *N. weigendii* E.Rodr. (Rodríguez, 2009)

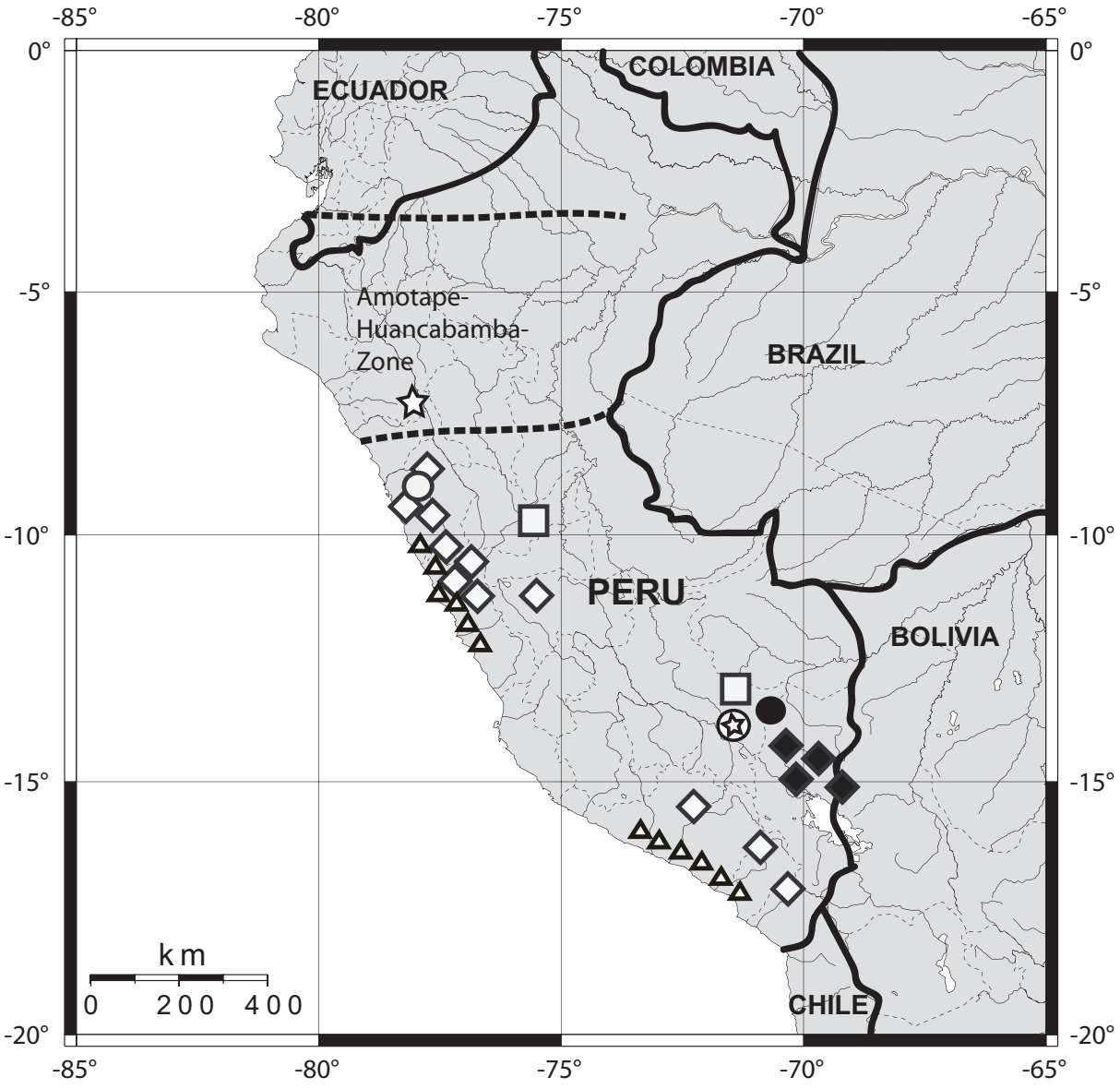

Figure 2.1: Distribution map for the species of the *Nasa poissoniana* complex: ☆, *Nasa weigendii*; ●, *Nasa poissoniana* subsp. *poissoniana*; ○, *Nasa poissoniana* subsp. *glandulifera* subsp. nov.; □, *Nasa vargasii*; ⊛, *Nasa raimondii*; ◆, *Nasa ferruginea*; ◇, *Nasa chenopodiifolia*; ▲, *Nasa urens*.

from northern Peru. *Nasa raimondii* (Standl. & F.A.Barkley) Weigend was formerly placed in the *N. stuebeliana* group (Weigend & Rodríguez, 2003) on the basis of the presence of broadly amplexicaul bracts, but is now transferred to the *N. poissoniana* group on the basis of the molecular data published in Weigend & Gottschling (2006). The two other species retrieved with *N. poissoniana* with the molecular data are morphologically quite divergent, namely *N. chenopodiifolia* (Desr.) Weigend, an extremely widespread and fast-growing weedy species, and *N. urens* (Jacq.) Weigend, a species restricted to the Loma formation, the coastal fog oases of the Peruvian and northern Chilean coast (Fig. 2.1). The present study provides updated descriptions and illustrations, a key and notes on the ecology and morphology of the members of the *N. poissoniana* group based on both extensive field studies and a revision of copious herbarium material.

2.2 Materials & Methods

Field studies were carried out in Peru in 1997–2009, leading to a total of 34 collections of species of the *N. poissoniana* complex. Specimens were prepared in the field following standard techniques and voucher sets were deposited in the local and national herbaria (USM, HUSA, HUT) in Berlin (B) and Munich (M). In addition, more than 160 specimens loaned from the following herbaria were revised: B, BM, BSB, CBGE, CUZCO, E, F, G, HAO, HUSA, HUT, K, LP, LPB, M, MO, NY, OXF, P, S, UC, US, USM, W, WISC and WU. All taxa recognized here have been brought into cultivation in the glasshouses at the Institut für Biologie, Freie Universität Berlin.

2.3 Results

2.3.1 Growth habit

All members of the group are ephemeral or annual. The plants usually branch strongly from the base with numerous lateral branches and the terminal shoots passing into flower in short sequence (Fig. 2.2 F). Overall plant size is typically 30–80(–110)cm (*N. urens*, *N. raimondii*, *N. ferruginea*, *N. poissoniana*, *N. weigendii*, *N. vargasii*). Dwarf specimens barely reaching 5cm and producing only one or two flowers/fruits are occasionally found, especially in *N. chenopodiifolia* and *N. urens*. The lower leaves are opposite, the upper leaves alternate, as in most Loasaceae. Branches usually terminate in monochasial inflorescences (Fig. 2.2 E), but dichasial inflorescences are also found in all taxa. Fruits reach maturity in the course of a few weeks and the life cycle of the plants is completed in c. 3.5–4 months from germination. The plants are entirely unable to regenerate vegetative growth from the base, even if cut back dramatically and with continued watering and fertilization in the glasshouse. Plants form numerous adventitious roots at the shoot base, gradually replacing the primary root. A shortly decumbent shoot base is sometimes present (*N. weigendii*).

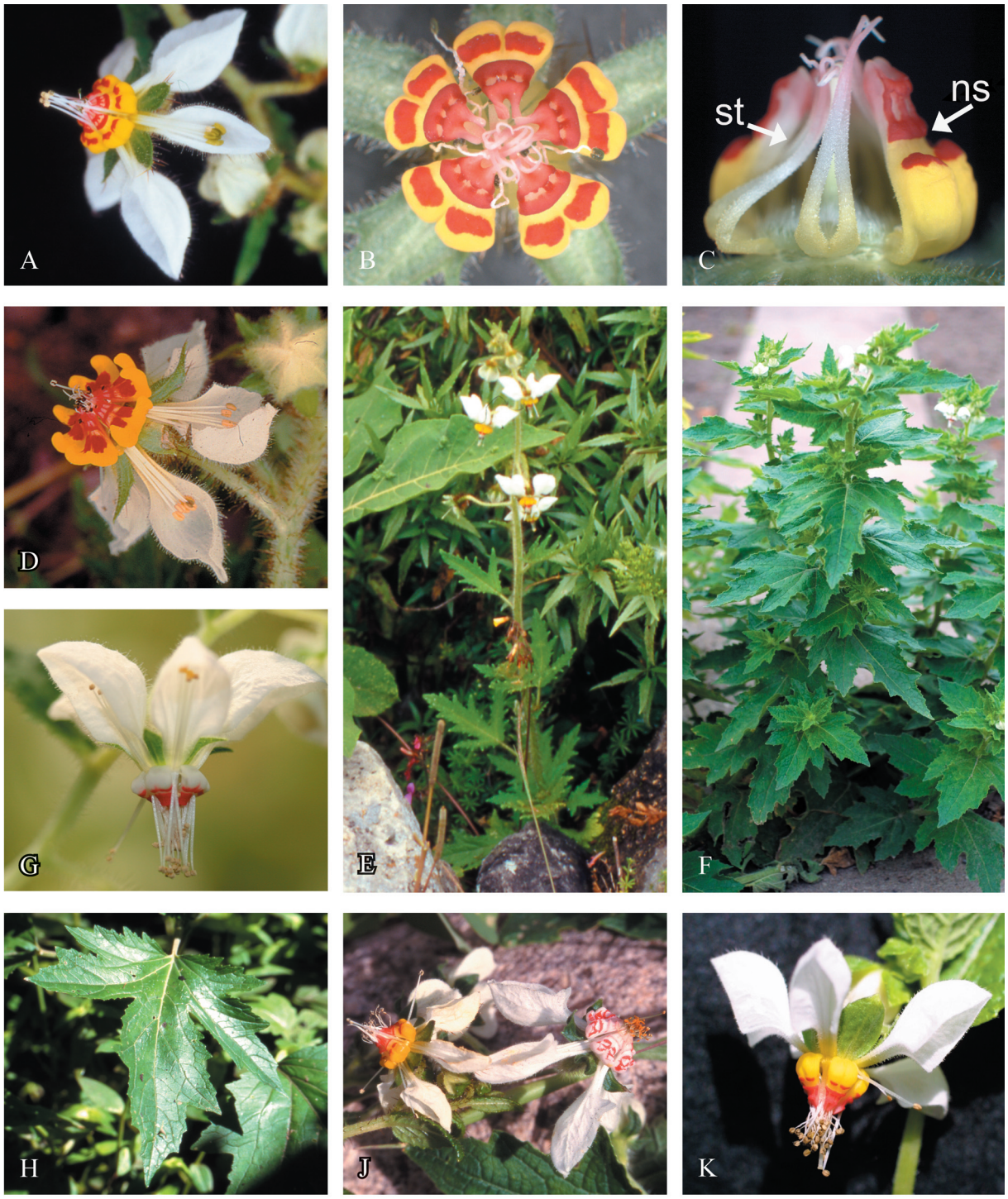

Figure 2.2: Morphology and habit of species of the *Nasa poissoniana* complex I. A–E, *Nasa poissoniana*: A, flower of *Nasa poissoniana* subsp. *glandulifera* subsp. nov. (photograph taken from cultivated plants from Weigend *et al.*, 8007); B, ditto, nectar scales, forming a revolver' seen from above; note the apical openings formed by each nectar scale (petals removed); C, ditto, lateral view; two nectar scales (ns) removed to show the free inner staminodes (st, two per scale) that close the scales ventrally and guide the pollinators to the nectar; D, flower of *N. poissoniana* subsp. *poissoniana*; note the typical horn-shaped nectar sacs of the southern subspecies (Weigend *et al.*, 2000/167); E, *Nasa poissoniana* subsp. *glandulifera* subsp. nov. in the natural habitat. F–H, *Nasa vargasii* (Weigend *et al.*, 5463): F, flowering plant in cultivation; G, flower; H, fully developed leaf. J–K, *Nasa raimondii* (Weigend *et al.*, 2000/289): J, flower in comparison with a flower of the sympatric *N. vargasii* (Weigend *et al.*, 2000/313); note the different petal size and coloration of the nectar scales; K, flower of *Nasa raimondii* at the end of the staminate phase.

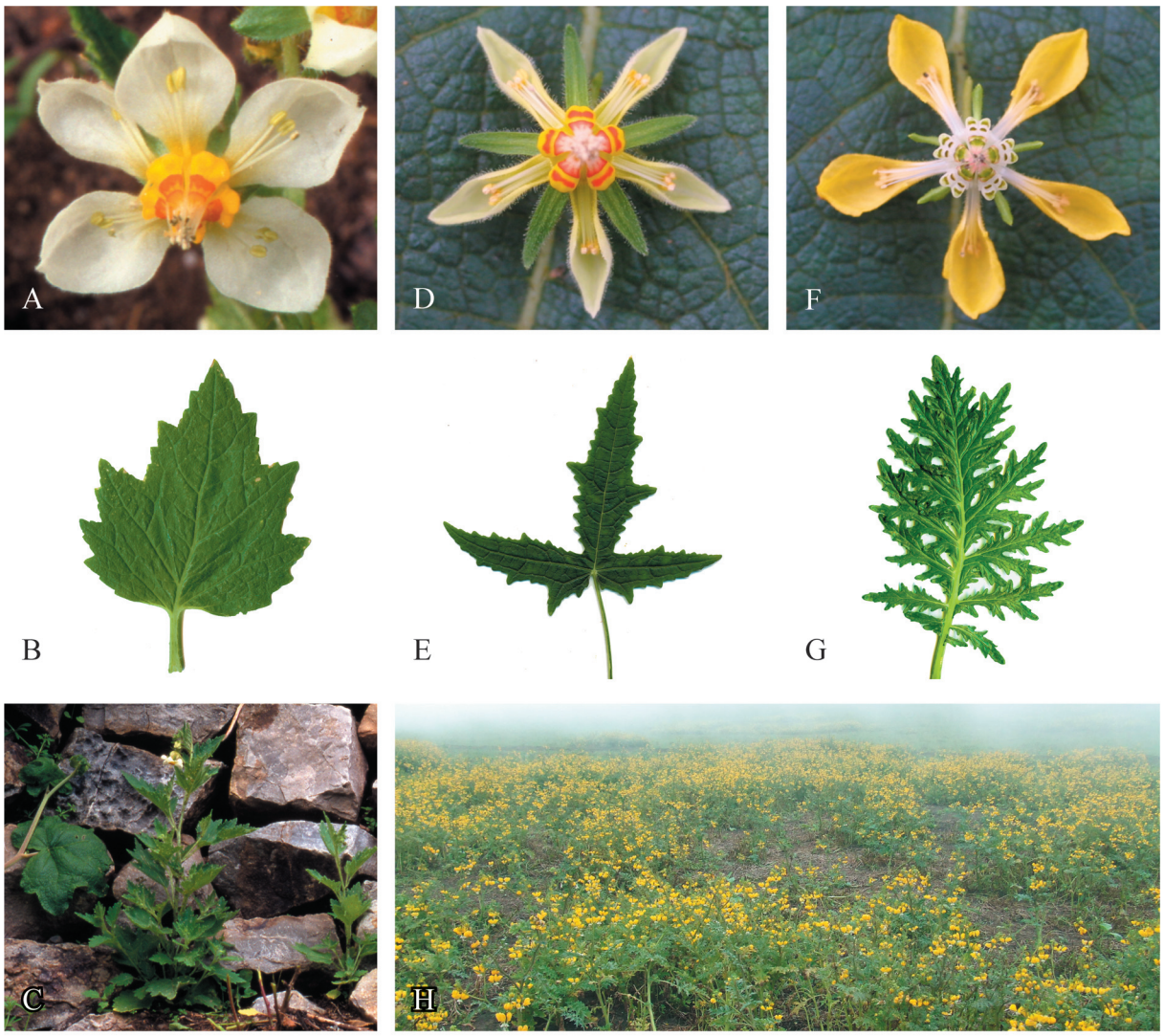

Figure 2.3: Morphology and habit of species of the *Nasa poissoniana* complex II. A–C, *Nasa chenopodiifolia* from Ancash (Weigend *et al.*, 97/176): A, flower; B, leaf; C, flowering plant on a stonewall around a crop plantation. D–E, *Nasa weigendii* in cultivation (Weigend & Schwarzer 7913-C): D, flower; E, subtrifoliolate leaf. F–H, *Nasa urens*: F, flower (photograph taken in cultivation from seeds from Weigend *et al.*, 7327); G, bipinnatifid leaf; H, large flowering stands of *Nasa urens* in the Lomas de Pasamayo (Department Lima).

2.3.2 Leaf morphology

The lamina of fully developed leaves is always petiolate and widely ovate in outline. The margin is usually lobed, sometimes irregularly three- or fivelobed, with irregularly lobulate lobes. In *N. raimondii*, leaf lobes are poorly developed and the margin is essentially irregularly dentate. In *N. vargasii*, the lamina is deeply subpalmately lobed and distantly serrate (Fig. 2.2 H). *Nasa weigendii* and *N. urens* are aberrant in leaf morphology: in *N. weigendii*, leaves are deeply lobed to truly trifoliolate (Fig. 2.3 E) and, in *N. urens*, leaves are deeply bipinnatifid with oblong to linear lobes (Fig. 2.3 G). The lamina size ranges from 20–110 mm × 15–90 mm (*N. chenopodiifolia*, *N. ferruginea*, *N. raimondii*) to 50–210 × 40–220 mm (*N. poissoniana*, *N. urens*, *N. vargasii*, *N. weigendii*). Apart from *N. raimondii*, bracts are not amplexicaul in the *N. poissoniana* group, unlike the bracts in most taxa of the *N. stuebeliana* group. Trifoliolate or pinnate leaves, as found in the *N. triphylla* group, are unknown in the *N. poissoniana* group, with the exception of *N. weigendii*.

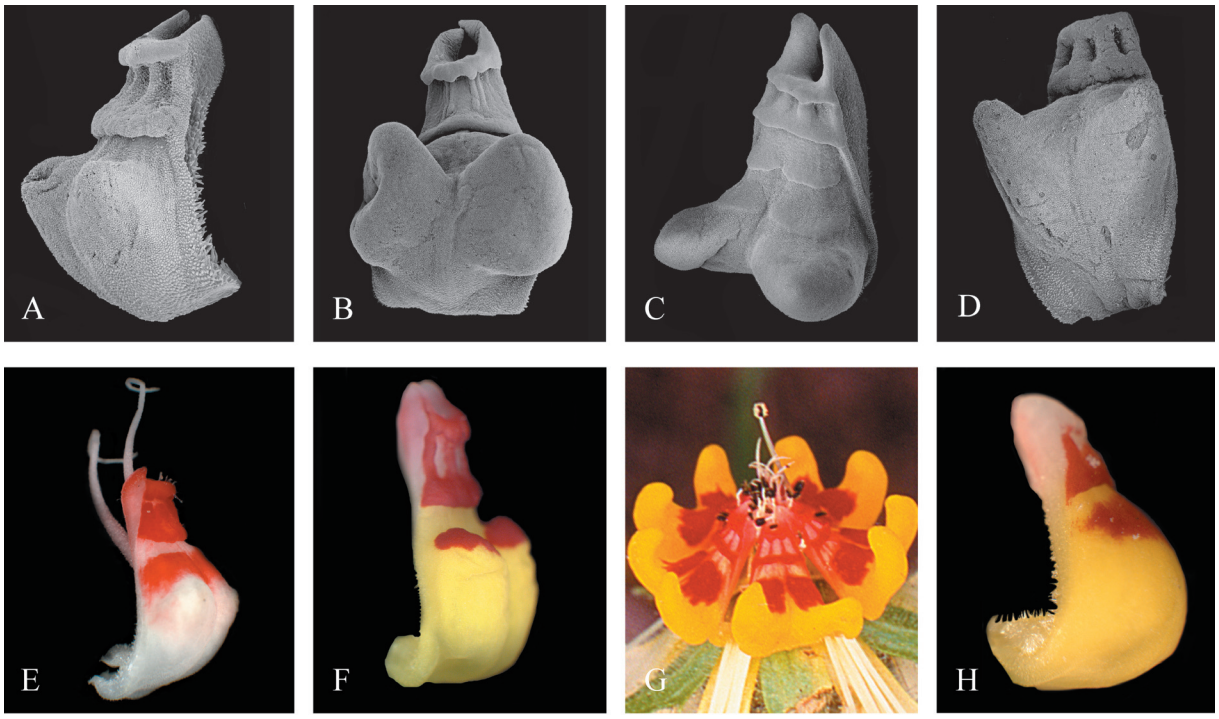

Figure 2.4: Morphology of nectar scales in the *Nasa poissoniana* complex. A, *Nasa chenopodiifolia*, dorsolateral view (Weigend *et al*., 97/176). B, *Nasa raimondii*, dorsal view (Weigend *et al*., 2000/208). C, *Nasa urens*: note the three horizontal calli on the back (Weigend *et al*., 5889). D–E, *Nasa vargasii*, form from Ambo (Weigend *et al*., 5463): D, scanning electron micrograph of liquid material from the original field collection; E, photograph of the scale of a cultivated specimen of the same origin; note the different size of the nectar sac-tips. F, *Nasa poissoniana* subsp. *glandulifera* subsp. nov., nectar sacs small and round (Weigend *et al*., 8007). G, *Nasa poissoniana* subsp. *poissoniana*, typical extremely horn-shaped nectar sacs of the southern subspecies (Weigend *et al*., 2000/167). H, Nectar scales of *N. weigendii*, with its indistinct nectar sacs (Weigend & Schwarzer 7913).

2.3.3 Flower morphology

Species of the *N. poissoniana* group have the tilt-revolver flowers typical of *Nasa* series Saccatae (Weigend & Gottschling, 2006). Petals are deeply boat-shaped (cymbiform) and uniformly snow-white (*N. chenopodiifolia*, *N. ferruginea*, *N. vargasii*; Figs 2.2, 2.3) to yellowish-white (*N. poissoniana*, *N. raimondii*; Fig. 2.2) or bright yellow (*N. urens*; Fig. 2.3 F). Floral scales are extremely variable both between and sometimes within species. The floral scales usually have a more or less rounded, ovoid lower back and distinct transverse calli (Fig. 2.4). However, in most species, two distinct dorsal 'horns' are occasionally found in the largest and best-developed flowers. This is particularly common in *N. poissoniana*, *N. urens* and *N. vargasii*, and usually absent in *N. chenopodiifolia*. Most species of the *N. poissoniana* group have yellow floral scales with one or several red or pink transversal and/or vertical calli on the back (*N. chenopodiifolia*, *N. ferruginea*, *N. poissoniana*, *N. raimondii*, *N. weigendii*). However, the floral scales of *N. vargasii* are red and white (Fig. 2.4 E) and the floral scales of *N. urens* are white with yellowish to greyish-green markings (transverse bands) on calli and nectar sacs (Fig. 2.4 C). The majority of taxa of the *N. stuebeliana* group have densely papillose floral scales with a velvety appearance with red as the dominant or only colour. This coloration and velvety appearance are absent in members of the *N. poissoniana* group.

2.3.4 Ecology and distribution

All species of this group are native to Peru. One species reaches into Bolivia (*N. ferruginea*) and one into northern Chile (*N. urens*). All species of the *N. poissoniana* group are found in open, often disturbed vegetation in seasonally dry habitats. They develop quickly during the wet season (December–April in the Andes, July–September on the coast). Most species are from inner Andean valleys, where they are commonly found in the undergrowth of Andean scrub communities and particularly on landslide/rockslide areas. *Nasa poissoniana*, *N. raimondii* and *N. vargasii* are largely restricted to the inner Andean valleys of central and southern Peru (Cuzco, Apurímac). Isolated outposts of two of the species have recently been discovered in northern Peru, with populations of *N. poissoniana* in Department Ancash and of *N. vargasii* in Department Junín. Despite this wide disjunction (c. 600–700 km), the specimens of *N. vargasii* from the north and from the south can only barely be told apart by the trained eye and are not segregated taxonomically here. The northern populations of *N. poissoniana* are taxonomically separated as a new subspecies, because they clearly differ from their southern counterpart by a more compact growth, more deeply lobed leaves, a densely glandular indumentum on the upper part, snow-white corollas and differently shaped nectar scales (Figs 2.2 A–D, 2.4 F, G). *Nasa ferruginea* is restricted to the eastern Cordillera of the Andes from Cuzco, Puno and northern Bolivia. Only *N. urens* and *N. chenopodiifolia* are essentially western Andean. *Nasa chenopodiifolia* is locally common in Andean scrub communities dominated by *Balbisia* Willd., *Echinocereus* Engelm. and *Mutisia* L.f. in southern Peru (Department Moquegua) and is otherwise a weed of disturbed sites, frequently entering cereal fields. *Nasa chenopodiifolia* has a wide altitudinal range, with most specimens from 2500 to 3500 m above sea-level (asl), but some specimens from the coastal Lomas of central Peru from 800 m asl. There are also some specimens of *N. chenopodiifolia* from inner Andean valleys in Ancash and Junín, which may, however, go back to dispersal by humans, as the species is a common weed (Fig. 2.3 C). *Nasa urens* is one of the most common and characteristic plant species of the Loma vegetation, where it can form monospecific stands several hundred square metres large (Fig. 2.3 H). It is found almost throughout the Peruvian Lomas and has also been reported from Lomas in the extreme north of Chile. Only one species, *N. weigendii*, is found in an inner Andean valley in the Amotape–Huancabamba Zone in northern Peru (Marañon valley near Chagual, Prov. Pataz, La Libertad; Fig. 2.1). The other species are restricted to the region south of the Amotape–Huancabamba Zone. For most groups in *Nasa*, the Amotape–Huancabamba Zone is the center of diversity and extremely narrowly endemic species abound there (for example, *Nasa* series *Carunculatae*, Weigend *et al.*, 2003; *N. stuebeliana* group, Weigend & Rodríguez, 2003; *N. triphylla* group, Dostert & Weigend, 1999). *Nasa weigendii* is the only narrowly endemic species of the *N. poissoniana* group; all other taxa are relatively widely distributed, usually over several Departments (Fig. 2.1).

2.3.5 Formal taxonomy

Key to the species of the *Nasa poissoniana* group

1. Corolla bright yellow, nectar scales white and greyish-green, leaves bipinnatifid (only coastal lomas).....2. *N. urens*
1*. Corolla (greenish-or yellowish-) white, nectar scales with red or yellow markings (mostly Andean habitats).....2
2. At least basal leaves truly trifoliolate, sepals two-thirds as long as petals and rounded .....4. *N. weigendii*
2*. Leaves lobed to subpalmately lobed, never with free leaflets, sepals ovate to subcircular and acuminate, less than one-half as long as petals.....3
3. Nectar scales white in lower half, red or pink at apex, petals 15–25 mm long.....7. *N. vargasii*
3*. Nectar scales yellow and red, petals 8–15(–20) mm long 4
4. Leaves deeply lobed (up to one-half to three-quarters of radius), margin serrate to dentate, nectar scales (occasionally) with two conspicuous, horn-shaped nectar sacs.....3. *N. poissoniana*
4*. Leaves only shallowly lobulate, lobules if present extending only one-third of radius, nectar scales without horn-like sacs.....5
5. Petals 15–20 mm long, capsule three times as long as wide.....6. *N. raimondii*
5*. Petals 8–10(–15) mm long, capsule either more compact or stretched 6
6. Petals white (8–)10–15 mm long, capsule c. four times as long as wide, plants dwarfed and unbranched, or branched from base, densely covered with short, yellowish setae .....1. *N. chenopodiifolia*
6*. Petals yellowish-white, 8–10 mm long, capsule c. twice as long as wide, branched only above, densely covered with dark reddish-brown setae (Peru: Cuzco, Puno; BolIvia: La Paz).....5. *N. ferruginea*

Species descriptions

1. *Nasa chenopodiifolia* (Desr.) Weigend, Rev. Peru. Biol. 13(1): 73 (2006) (Figs 2.3 A–C, 2.4 A, 2.5)

Basionym: *Loasa chenopodiifolia* Desr. in Lamarck, Encycl. 3: 580 (1791).
– Type: PERU, Lima 'herbier de Perou', Jos. de Jussieu s.n. (holotype P-JUSS!, Photo F, neg.nr. 38505).
= *Loasa xanthiifolia* Juss., Ann. Mus. Hist. Nat. 5: 26 (1804). Type: PERU, Lima, Dombey s.n. (holotype P-Juss!, fragment & photo F, neg.nr. 38504; isotype P!).
= *Loasa aspera* Ruiz & Pav, Flora Peruviae, et chilensis 5 (Anales Institute. Bot Cavanilles 16): 407. Table 441 b (1958). Types. Table 441 b, l.c. (lectotype designated in Weigend, 1998: 163); PERU, Lima, '*Loasa* de Huayaquil', Pavón 186 (epitype designated in Weigend, 1998: 163: G!, isotype: B†.).
= *Loasa fulva* Urb. & Gilg, Nova Acta Caes. Leop.- Carol. German. Nat. Cur. 76: 224 (1900). Types: Cultivated at Botanischer Garten Berlin-Dahlem anno 1846 (holotype B†, photo F, neg. nr. 10191); PERU, Department Lima: Santa Clara next to Lima–Oroya railway, 400–600 m, Weberbauer 1672 (neotype designated in Weigend, 1998: 163: F!, G!).
= *Loasa inconspicua* Urb. & Gilg, Nova Acta Caes. Leop.-Carol. German. Nat. Cur. 76: 221. 1900. Type: 'Lima, northern Peru', Cuming 1051 (holotype BM!; isotype E!).
= *Loasa leiolepis* Urb. & Gilg, Nova Acta Caes. Leop.-Carol. German. Nat. Cur. 76: 230. 1900. Types: Cultivated at Botanischer Garten Berlin-Dahlem anno 1843 (holotype B†, Photo F!, neg. nr. 10200); Peru, Department Lima: Santa Clara next to Lima–Oroya railway, 400–600 m, Weberbauer 1672 (neotype designated in Weigend, 1998: 163: F!, G!).

Description: Annual herb 10–110 cm tall. Stem terete, 2–7 mm thick at base, densely covered with reddishbrown setae 2–3 mm long and short glochidiate hairs (< 0.5 mm), decorated with dark green streaks. Leaves opposite below and alternate above, petioles 5–20 mm long, sparsely setose; lamina rhomboidal to widely ovate, 20–70 × 15–50 mm, margin lobulate, lobules dentate, base cuneate to truncate, apex acuminate; abaxial surface covered with red setae 2 mm long and short glochidiate hairs (c. 0.2 mm), adaxial surface covered with setae and scabrid hairs 0.3 mm long, venation pinnate. With 3–8 terminal inflorescences 5–26 cm long, with 5–12 deflexed flowers per branch, bracts ovate, lobulate to coarsely serrate, petiolate, 15–30 × 10–25 mm. Flowers pentamerous, rarely some tetramerous, pedicels 6–8 mm; calyx densely setose, tube clavate to narrowly conical, 5 × 2–3 mm, calyx lobes ovate acuminate, 2.5 × 1.5 mm. Petals white, cymbiform (2–)8–10(–15) mm long, (1–)4(–8) mm deep, base unguiculate and abruptly widened into two small, triangular teeth 2.5–5 mm from base, setose and set with glochidiate hairs on back. Nectar scales ovate, yellow, 2.5– 3 × 2 mm, base incurved, back with two inconspicuous nectar sacs or saccate as such, scale back with one red transversal callus, neck thickened, laterally protracted into two small erect wings 1 mm × 0.5 mm. Staminodia, 3–4 mm long, base slightly dilate, filiform above, papillose, white. Stamens in epipetalous fascicles of 7–20 each, filaments 4–5 mm long, white, anthers 0.5 mm long and wide, black. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect, cylindrical capsule with persistent calyx lobes, pedicel (3–)15–20 mm, capsule 5–25 mm long and 4 mm wide at apex, opening with three apical valves; seeds numerous, black, with reticulate testa.

Notes: It is highly plastic in plant, flower, fruit and seed size, ranging from tiny plants (< 5 cm) with one to two flowers to huge, widely branched individuals (> 100 cm tall) with countless, much larger flowers. This has led to the description of several taxa which appeared to be different in the herbarium, but only represent the extremes of a continuous range of variation. The oldest name, *N. chenopodiifolia*, fortunately refers to the most typical form of the species. The dwarf form with small flowers is what has been aptly described as *Loasa inconspicua*.

Habitat and distribution: *Nasa chenopodiifolia* is the most widespread taxon of the group and one of the most widespread species of *Nasa*. It has been reported from both the north and south-western side of the Andes, currently with an apparent distribution gap south of Lima, which may be a result of undercollection. As it is the only weedy species in the whole complex, its current distribution may be partly caused by dispersal by humans. The life cycle of the plant is rapid, with typical life spans (seed to seed) of 8–14 weeks, but dwarf specimens may reach maturity in as little as 4–6 weeks. *Nasa chenopodiifolia* is thus able to complete its life cycle even in frequently disturbed habitats and can rapidly establish itself as a weed.

Specimens examined: See appendix A1.

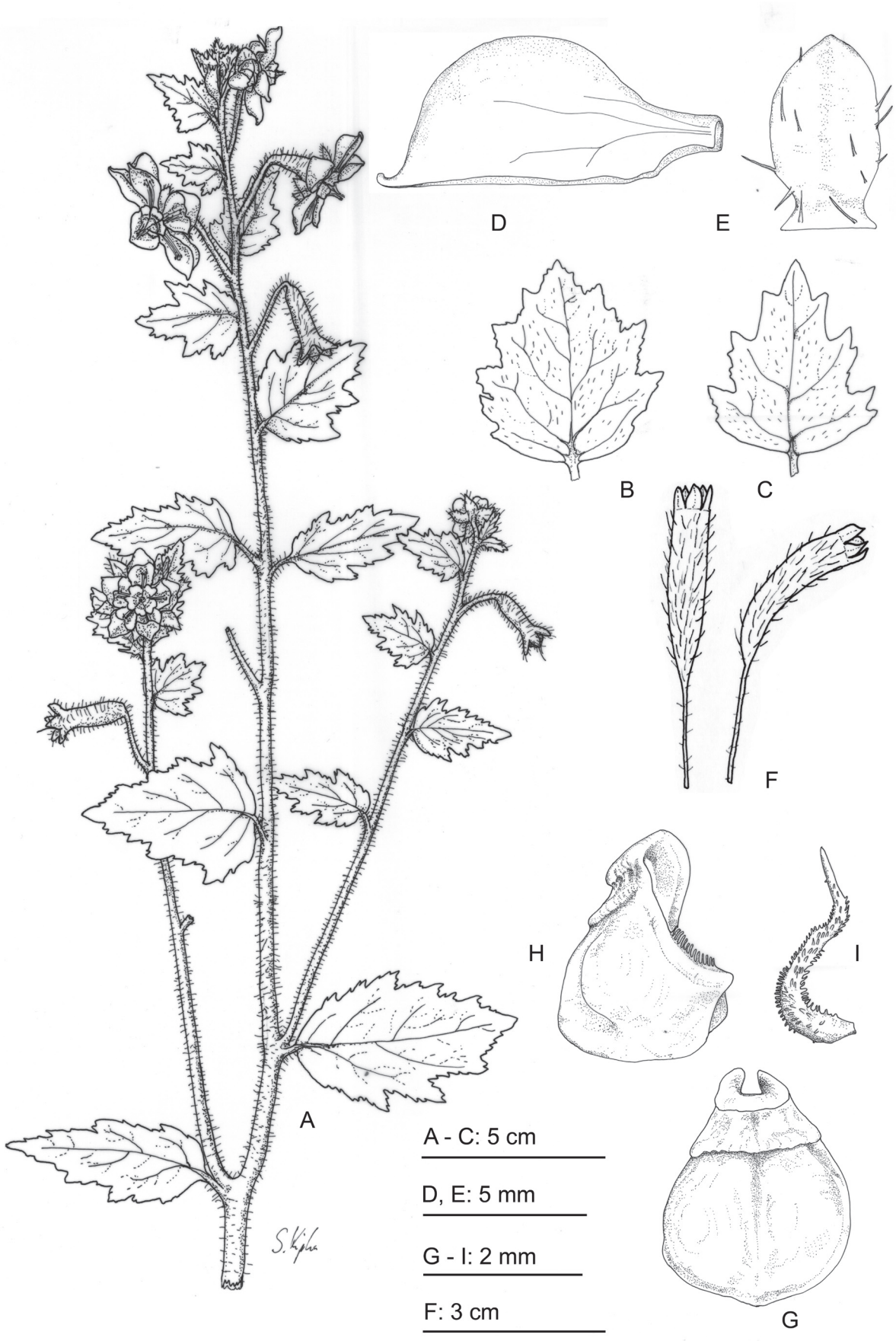

Figure 2.5: *Nasa chenopodiifolia*: A, flowering shoot; B, C, typical basal leaves; D, petal; E, sepal; F, fruits; G, floral scale, dorsal view; H, floral scale, lateral view; I, staminode. Drawn from Weigend *et al*. 5534 by S. Kipka.

2. *Nasa urens* (Jacq.) Weigend, Rev. Peru. Biol. 3(1): 83 (2006) (Figs 2.3 F–H, 2.4 C, 2.6)

Basionym: *Loasa urens*, Jacq., Obs. Bot. 2, 15. Table 38 (1767).
– Type. Table 38 in Jacq., Obs. Bot. 2. 1767 (lectotype designated in Weigend, 1998: 167); cultivated at Vienna, Jacquin s.n. (epitypes designated in Weigend, 1998: 167: W!, BM!).
= *Loasa hispida* L.f., Syst.veg. 12: 364 (1767), nom. superfl. Type: same as *Loasa* urens Jacq.
= *Loasa ambrosiifolia* Juss., Mem. Mus. Nat. Hist. Nat. 5: 26. Table 4 f.1 (1804). [as *ambrosiaefolia*] Types. Table 38 in Jacq., Obs. Bot. 2. 1784 (lectotype designated in Weigend, 1998: 167); cultivated at Vienna, Jacquin s.n. (epitypes designated in Weigend, 1998: 167: W!, BM!).
= *Loasa bipinnatifida* Ruiz & Pav., Flora Peruviae et Chilensis 5 (Anales Institute. Bot Cavanilles 16): 403. Table 439 (1958). Types. Table 439, l.c. (lectotype designated in Weigend, 1998: 167); Peru, Department Lima, Prov. Lima, Amancaes, Ruiz & Pavón s.n. (epitype designated in Weigend, 1998: 167: BM!).

Description: Annual herb (5–)15–60 cm tall. Stem terete, 2–7 mm thick at base, densely covered with reddish-brown setae 2.5–3.5 mm long and shorter (0.5 mm) glochidiate hairs. Leaves alternate above, petioles 5–25 mm long, setose; lamina rhomboidal to widely ovate in outline, 50–150 × 40–120 mm, bipinnatifid, dissected nearly to midvein, lobules narrow; base cuneate to truncate, apex acuminate; abaxial surface covered with red setae 2 mm long and short glochidiate hairs 0.1 mm long, adaxial surface covered with setae and slightly longer, scabrid hairs 0.2 mm long, venation pinnate. With one to five terminal, monochasial inflorescences 10–20 cm long, with 5–12 deflexed flowers per branch, bracts ovate (bi-)pinnatisect, subsessile, 15–30 × 10–25 mm. Flowers pentamerous, pedicels 10–15 mm; calyx densely setose, tube conical, 4 × 3 mm, calyx lobes ovate acuminate, 5 × 2 mm. Petals reflexed, bright yellow, deeply cymbiform, 20–25 mm long, c. 10mm deep, base unguiculate and abruptly widened into two
small triangular teeth 5 mm from base, setose and set with glochidiate hairs on back. Nectar scales with triangular back, much narrowed above, 8 × 7 mm, base incurved, back with two conspicuous nectar sacs about 2 mm in diameter, scale back with 3–5 red, green or reddish-green calli, neck thickened and slightly recurved, laterally protracted into two small erect wings 1 mm long and 0.5 mm wide. Staminodia 9–10 mm long, base slightly dilate, filiform above, papillose, white. Stamens in epipetalous fascicles of 15–20 each, filaments 13–15 mm, white, anthers 0.5 mm long and wide, black. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect, narrowly clavate to subcylindrical capsule with persistent calyx lobes, pedicel 30–35 mm long, capsule 15–20 × 10–15 mm, opening with three apical valves; seeds numerous, black, with deeply reticulate testa.

Notes: This is certainly the most ornamental species of the entire group and, although not the most widespread species, probably that with the highest abundance. In coastal lomas, it can form monospecific stands of hundreds of thousands of square metres, corresponding to thousands of individuals. With its ovate, bipinnatisect leaves and yellow flowers, it is

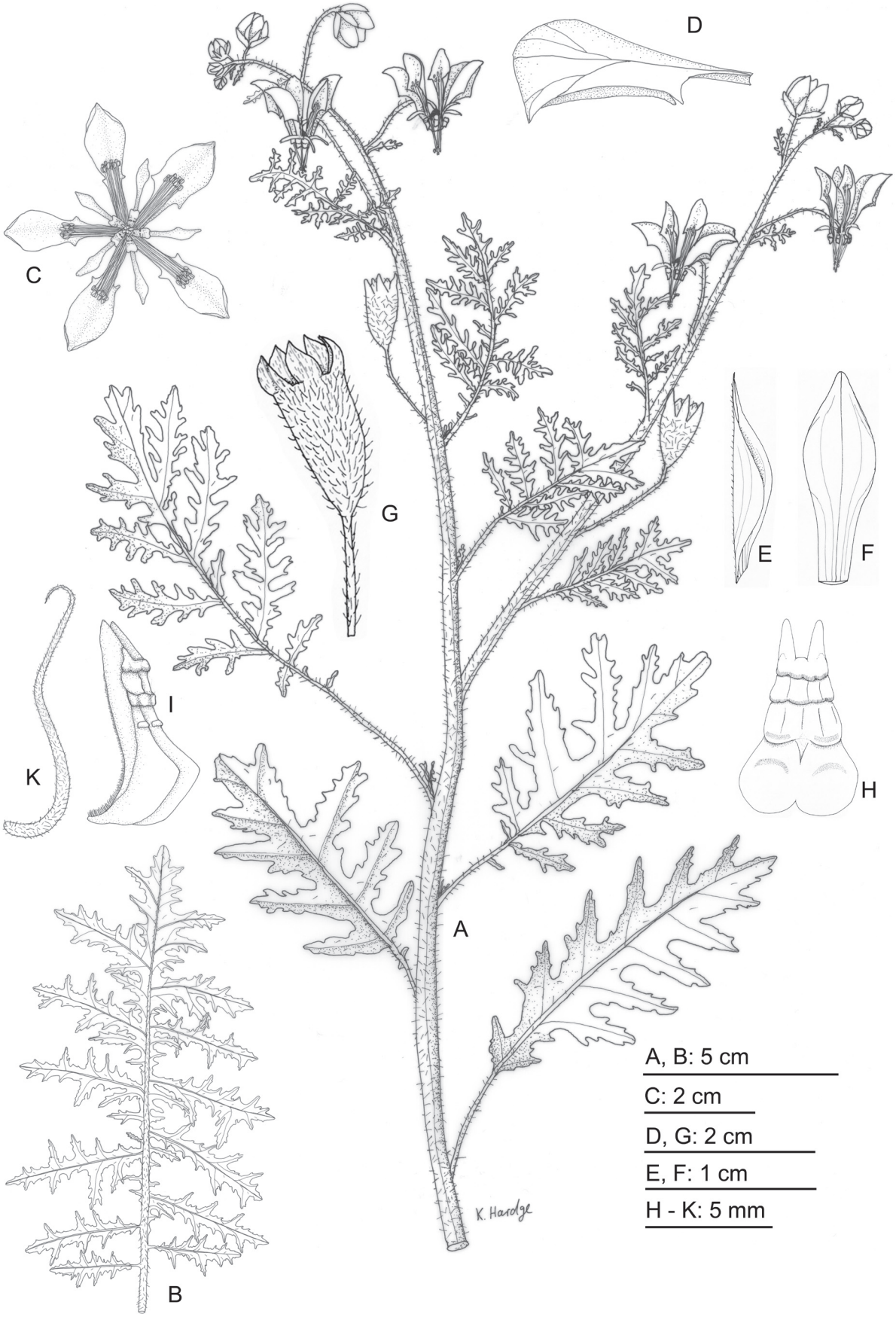

Figure 2.6: *Nasa urens*: A, flowering shoot; B, basal leaf; C, flower; D, petal; E, sepal, lateral view; F, sepal, dorsal view; G, fruit; H, floral scale, dorsal view; I, floral scale, lateral view; K, staminode. Drawn from Weigend *et al.* 7327 by K.Hardge.

also one of the most characteristic species of the genus and cannot be confused with other species of *Nasa*, or indeed any other plant species.

Habitat and distribution: The species occurs from Ancash in the north to northern Chile in the south, and is common in loma vegetation. Like most plants of the lomas, it flowers in August to October.

Specimens examined: See appendix A2.

3. *Nasa poissoniana* (Urb. & Gilg) Weigend, Rev. Peru. Biol. 13(1): 79. (2006)

Basionym: *Loasa poissoniana* Urban & Gilg, Nova Acta Acad. Caes. Leop.-Carol. Germ. Nat. Cur. 76: 226 (1900).
– Type: PERU. [Cuzco?] Without locality, Gay 1975 (holotype P!, Photo F, neg. nr. 38482).

Description: Robust, annual herb 40–80 cm tall. Stem terete, 4–10 mm thick at base, densely covered with reddish-brown setae 2.5–3.5 mm long, shorter glochidiate hairs (0.5 mm), decorated with numerous dark green stripes. Leaves opposite below and alternate above, petioles 20–45 mm; lamina rhomboidal to ovate, 50–120(–150) × 30–80(–100) mm, lobate to subpalmately lobed, lobes extending up to one-half to three-quarters of radius, lobes dentate or serrate, base truncate to subcordate, apex acuminate; both surfaces densely setose and glochidiate, abaxial surface covered with red setae 2 mm long and very short glochidiate hairs 0.1 mm long, adaxial surface covered with slightly longer red setae and scabrid hairs 0.2–0.5 mm long, venation pinnate. With two to five terminal, monochasial inflorescences 10–25 cm long, with 5–10 deflexed flowers per branch, bracts (narrowly) ovate, serrate to lobulate, shortly petiolate to subsessile, 15–55 × 10–40 mm. Pedicels 10–25 mm long; calyx densely red setose, tube conical, 5 × 4 mm, calyx lobes ovate-acuminate, 6 × 3 mm. Petals white to yellowish white, deeply cymbiform, 15–20 mm long, 10 mm deep, base unguiculate and abruptly widened into two small, triangular teeth c. 4 mm from base, setose and set with glochidiate hairs on back. Nectar scales with triangular back, much narrowed above, 6–7 × 4–5 mm, base incurved, back with confluent nectar sacs, 1.5 mm in diameter or two conspicuous, horn-shaped nectar sacs about 2 mm in diameter, scale back with one transversal and sometimes with two vertical calli, neck recurved, laterally protracted into two small, erect wings 1 × 0.5 mm, transverse calli and distal portion of sacs bright red, occasionally pink below callus and neck, remainder of the scale yellow. Staminodia 8 mm long, base slightly dilate, filiform above, papillose, white. Stamens in epipetalous fascicles of 15–20 each, filaments 8–12 mm long, white, anthers 0.5 mm long and wide, black. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect subcylindrical capsule with persistent calyx, pedicel 20–30 mm, capsule 20–30 × 9–10 mm, densely covered with red setae 3 mm long and scabrid hairs 0.5 mm long and decorated with 10 ± well-developed longitudinal ridges, opening with three apical valves; seeds numerous, black, with reticulate testa.

Key to the subspecies of *Nasa poissoniana*

1. Plants 50–80(–100) cm tall, corolla yellowish-white, leaves lobed up to one-half of radius, nectar scales with two conspicuous horn-shaped nectar sacs, glandular trichomes on shoot sparse (southern Peru, Department Cuzco)..... .....3.1 subsp. *poissoniana*

1*. Plants 40–60 cm tall, corolla snow-white, leaves deeply lobed (three-quarters of radius), nectar sacs not hornshaped, distal parts of the shoot densely covered with a glandular indumentum (northern Peru, Department Ancash, LaLibertad).....3.2 subsp. *glandulifera* subsp. nov.

3.1 *Nasa poissoniana* (Urb. & Gilg) Weigend, subsp. *poissoniana* (Figs 2.2 D, 2.4 G, 2.7)

= *Loasa cuzcoensis* Killip, Journ. Wash. Acad. Science 18: 91. (1928)
– Type: PERU. Department Cuzco, Prov. Cuzco: Cuzco, Herrera 1465 (NY!, F!, fragment G!, photo F, neg. nr. 63399).

Description: Plant 50–80 cm tall, stem 7–10 mm thick at base. Entire plant densely covered with reddish-brown setae, glandular trichomes sparse and largely restricted to the petals. Leaf lamina 50–120(–150) mm × 30–80(–100) mm, lobate to lobulate, lobes extending up to one-half of leaf radius; (1–)3–5 terminal inflorescences 10–25 cm long, with 5–10 deflexed flowers per monochasium, bracts 15–55 × 10–40 mm. Petals yellowish-white. Nectar scales 7 × 5 mm, back with two conspicuous, hornshaped yellow nectar sacs about 2 mm in diameter, scale back and calli red, occasionally pink below callus and neck.

Notes: The typical subspecies of *N. poissoniana* is quite common in the inner Andean valleys of Cuzco (Río Utcubamba), but has not yet been reported from elsewhere. It is readily differentiated from the other species growing in this area by the combination of red and yellow nectar scales (vs. red and white in *N. vargasii*), deeply lobed leaves and long cylindrical capsules (vs. shallowly lobed leaves and shorter capsules in *N. raimondii*) and long petals (> 15 mm vs. < 10 mm in *N. ferruginea*).

Specimens examined: See appendix A3.

3.2 *Nasa poissoniana* (Urb. & Gilg) Weigend, subsp. *glandulifera* T.Henning & Weigend subsp. nov. (Figs 2.2 A–C, E, 2.4 F, 2.8)

– Type: PERU: Department Ancash, Prov. Piscobamba, San Luis: road from Piscobamba to San Luis, 3000– 3500 m, 12.iii.2001, M. Weigend, K. Weigend, E. Rodríguez R., & M. Binder 5114 (holotype USM, isotypes B, HUT, M).

Diagnosis: Nasae poissonianae subsp. poissonianae affinis, sed ab ea habitu magis compacto, foliis profundius divisis, indumento in parte supera dense glanduloso, petalis niveis et saccis nectariferis non cornuatis differt.

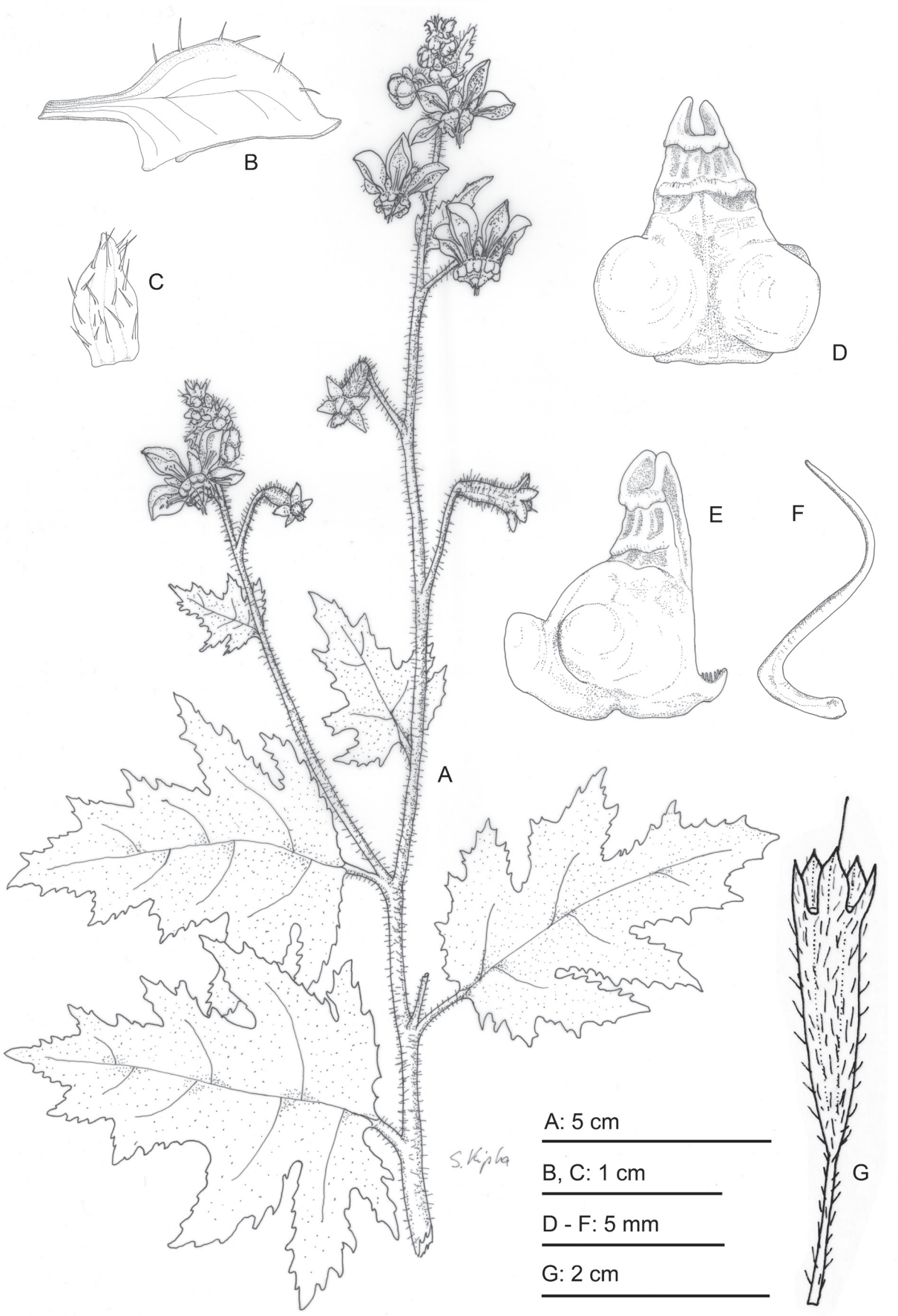

Figure 2.7: *Nasa poissoniana* subsp. *poissoniana* – typical form from Cuzco: A, inflorescence; B, petal; C, sepal; D, floral scale, dorsal view; E, floral scale, lateral view; F, staminode; G, fruit. Drawn from Weigen & Weigend 2000/208. Drawing made by S. Kipka.

Description: Plant 40–60 cm tall, stem 4–6 mm thick at base. Plants covered with reddish-brown setae and densely set with glandular trichomes, especially distally. Leaf lamina 50–90 × 45–80 mm, often subpalmately lobed, lower lobes of basal leaves showing an angle of 45° to the principal axis, lobes extending up to three-quarters of radius. Inflorescences 2–3, bracts 15–45 × 10–20 mm. Petals snow white. Nectar scales 6 × 4 mm, back with two confluent yellow nectar sacs c. 1.5 mm in diameter, transverse calli and distal portion of sacs bright red, section between callus and neck pink, remainder of scale yellow.

Notes: This new subspecies is now known from three collections from the two neighbouring Departments of Ancash and La Libertad. The collections are all from recent collecting trips to an area which had been traditionally ignored by botanists. The taxon may thus be much more common than the specimens cited here indicate. It has been documented from scree slopes and weedy places. It differs from the typical subspecies by its more compact growth, deeply lobed leaves, densely glandular indumentum in the upper part, snow-white petals and nectar sacs not hornshaped, but is evidently a northern outlier of that taxon.

Specimens examined (paratypes): PERU: Department. Ancash, Prov. Huari, Masín, 2530 m, 21.ii.2005 Cano *et al.* 15145 (USM); Department La Libertad, Prov. Pataz: road from Buldibuyo to Tayabamba, 3163 m, 24.iv.04, Weigend *et al.* 8007 (B, USM, HUT, HUSA, NY, MO, UC, K, P, BM) – cultivated at Berlin from this collection: Weigend *et al.* 8007-C (AAU, K, HUSA, HUT, UPS, B, FR, NY, M, MO, P, TEX, UC, USM, WISC).

4. *Nasa weigendii* E.Rodr., Arnaldoa 15: 21–29. (2008) (Figs 2.3 D, E, 2.4 H, 2.9)

– Type: PERU: Department La Libertad, Prov. Pataz, Alrededores de Pataz, 2610 m, 05.ii.2003, Sagástegui, Zapata, Rodríguez & Medina 17251 (holotype HUT, isotypes BSB, F, HAO, MO, NY, US, HUT, USM, W).

Description: Robust annual herb 40–60(–100) cm tall. Stem terete, 4–5 mm thick at base, densely covered with reddish-brown setae 2.5 mm long and shorter glochidiate hairs (0.5 mm), decorated with numerous dark green stripes. Leaves opposite below and alternate above, petioles 20–40(–80) mm; lamina rhomboidal to ovate, 50–90(–130) × 50–80(–160) mm, deeply lobed to truly trifoliolate, lower lobes of basal leaves showing an angle of 90° to the principal axis, lobes dentate or serrate, base cordate, apex acuminate; both surfaces setose especially along veins and set with hairs, abaxial surface set with red setae 2 mm long and short glochidiate hairs 0.1 mm long, adaxial surface covered with slightly longer red setae, scabrid hairs 0.2–0.5 mm long, and shorter glochidiate hairs, venation pinnate. With 2–5–(–10) terminal, monochasial inflorescences 15–30 cm long, with 7–10(–13) deflexed flowers per branch, bracts serrate to narrowly lobed, shortly petiolate to subsessile, 15–45 mm × 10–20 mm. Flowers pentamerous, pedicels 10–20 mm long; calyx densely red setose, tube conical, 3 × 3 mm, calyx lobes ovate–lanceolate, 4–7 × 1.5–2 mm. Petals yellowish-white, deeply cymbiform, 10–12 mm long, 3–4 mm deep, base unguiculate and widened into two small, triangular teeth 3–4 mm from base, set with few red setae and glochidiate hairs on back. Nectar scales with triangular back, much narrowed above, 4 × 2 mm, base

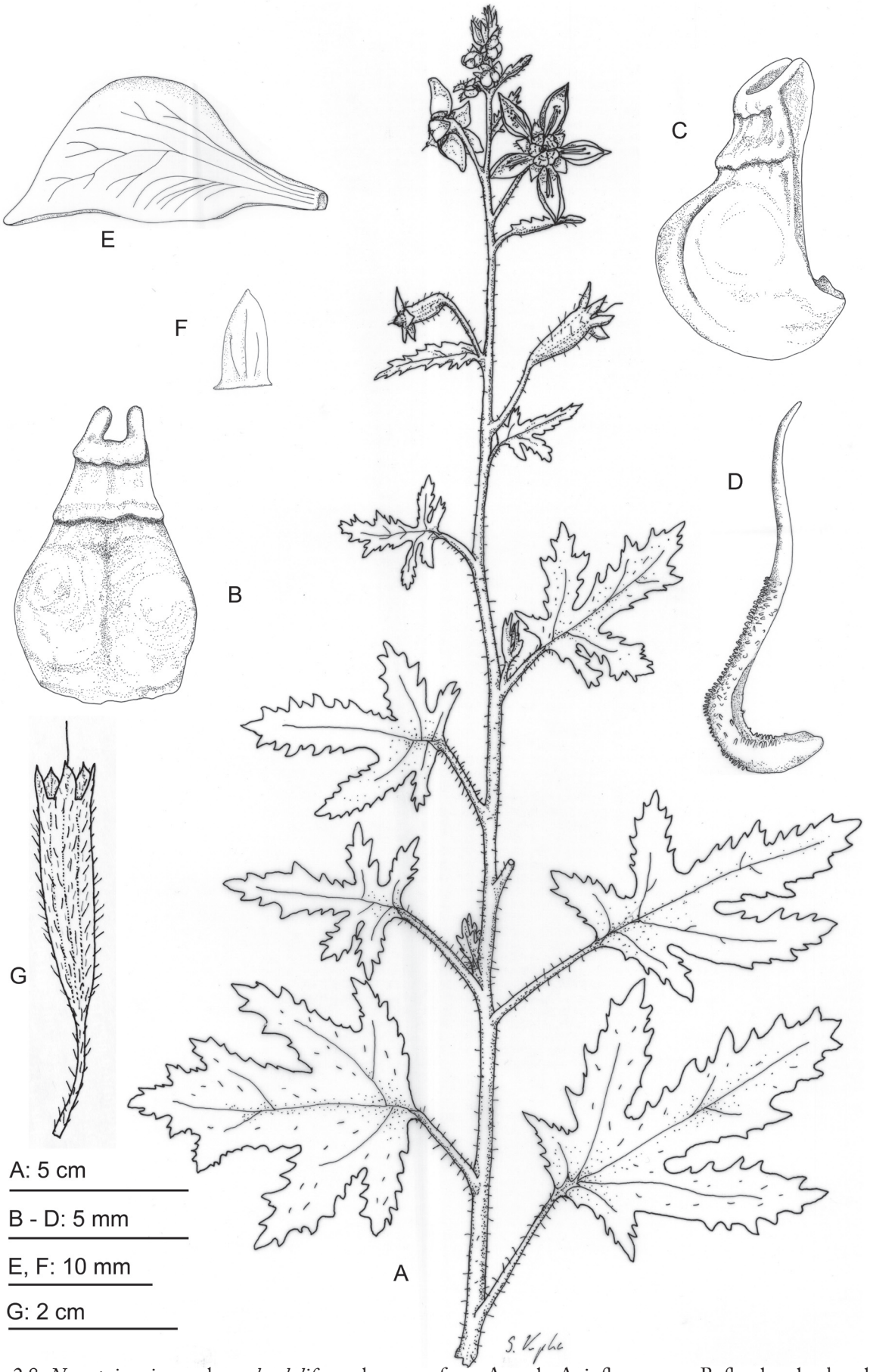

Figure 2.8: *Nasa poissoniana* subsp. *glandulifera* subsp. nov. from Ancash: A, inflorescence; B, floral scale, dorsal view; C, floral scale, lateral view; D, staminode; E, petal; F, sepal; G, fruit. Drawn from Weigend *et al*. 5114. Drawing made by S. Kipka.

incurved, back with two inconspicuous confluent nectar sacs or shallowly saccate as such about 1 mm in diameter, scale back with one transversal callus and two to four vertical calli, laterally protracted into two small, erect wings 0.5 × 0.5 mm, transversal, vertical calli and distal portion of the sacs red, neck and wings pinkish white, remainder of scale yellow. Staminodia two per scale, 5–6 mm long, base slightly dilate, 0.5 mm wide, filiform above, papillose, pinkishwhite. Stamens numerous, in five epipetalous fascicles of 15–20 each, filaments 5–7 mm long, white, anthers 0.5 mm long and wide, white. Ovary inferior, with three parietal placentae and numerous ovules. Fruit a clavate to subcylindrical capsule with persistent calyx, pedicel erect, 20–25 mm long, capsule 15–20 mm long and 5–7 mm wide at apex, densely covered with red setae 3 mm long and glochidiate hairs < 0.5 mm long, opening with three apical valves; seeds numerous, black, with reticulate testa.

Notes: *Nasa weigendii* is the only species in the *N. poissoniana* complex with trifoliolate leaves, a leaf type otherwise only found in the *N. triphylla* group. However, the flower and fruit morphology clearly characterize *N. weigendii* as a member of the *N. poissoniana* complex. Most importantly, the short, cylindrical capsules densely covered with blackish brown setae are common in the *N. poissoniana* complex, but absent in the *N. triphylla* complex (in which fruits are mostly conical or hemispherical and sparsely setose and/or the setae are white or yellowish).

Distribution and habitat: *Nasa weigendii* is so far only known from two collections from two neighbouring provinces of the Department La Libertad. This region had not been investigated by botanists to any major extent until recently, when a major collection trip was undertaken under the leadership of Abundio Sagástegui Alba from Trujillo, together with several colleagues, and another trip was led by the second author of this paper. No other major collecting trips have been undertaken in this area and it can be assumed that the species may be more common than the collections cited here indicate.

Specimens examined: See appendix A4.

5. *Nasa ferruginea* (Urb. & Gilg) Weigend, Rev. Peru. Biol. 13(1): 74. (2006) (Fig. 2.10)

Basionym: *Loasa ferruginea* Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. Germ. Nat. Cur. 76: 225. (1900).
– Type: PERU Department Puno, Prov. Agapata, Agapata, Lechler 1877 (lectotype designated in Weigend, 1998: 164: GOET!; isolectotypes K, B†, photo F!, neg. nr. 10187).

Description: Robust, annual herb 20–50 cm tall. Stem round, 3–7 mm thick, densely covered with reddishbrown setae 1.5–2 mm long, shorter scabrid hairs (0.5 mm), also decorated with numerous dark green stripes. Leaves opposite below and alternate above, petioles 15–20(–25) mm; blades widely ovate, sometimes trilobate, 40–60 mm long, 30–50 mm wide, membranaceous to subchartaceous, base cuneate to truncate, apex acuminate, margin

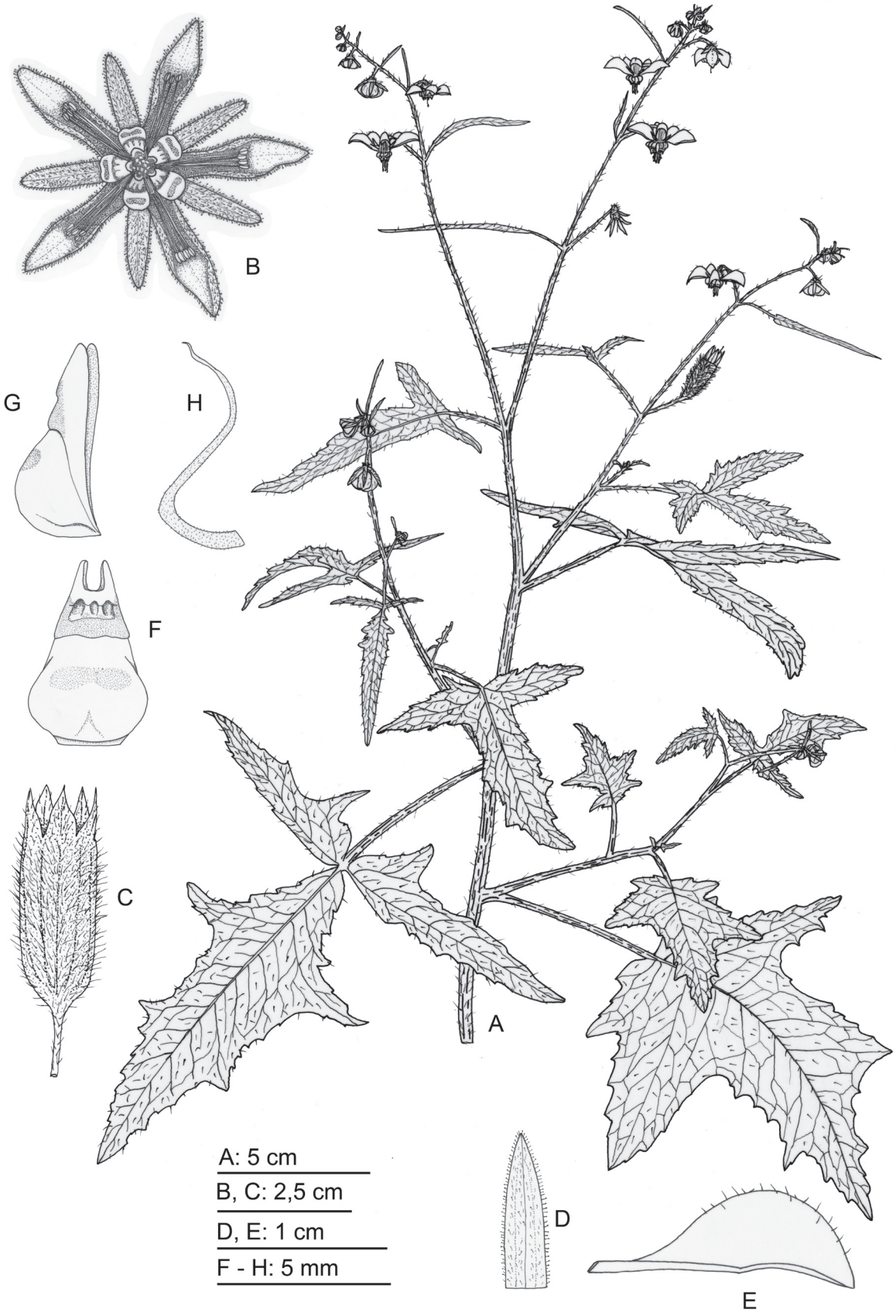

Figure 2.9: *Nasa weigendii*: A, flowering shoot; B, flower (note the lanceolate calyx lobes); C, capsule; D, sepal; E, petal; F, floral scale, dorsal view; G, floral scale, lateral view; H, staminode. Drawn from Weigend & Schwarzer 7913 and cultivated plants raised from seeds of this collection. Drawing made by T. Henning.

serrate, lobes, if present, extending to one-third of the radius, both surfaces densely hairy, abaxial surface covered with pale setae 2 mm long and short glochidiate hairs 0.1 mm long, adaxial surface covered with slightly longer pale setae and scabrid hairs 0.2–0.5 mm long, venation pinnate. With one to three terminal, monochasial inflorescences 10–25 cm long, with up to seven pendant flowers per branch, bracts ovate, shortly petiolate to nearly sessile, 10–30(–60) mm long and 10–20(–40) mm wide. Pedicels 8–10 mm long; calyx very densely red setose, tube conical, 3 mm long, 2 mm wide, calyx lobes five, ovate acuminate, 2.5 mm long, 1.5–2 mm wide. Petals five, yellowish white, cymbiform, 8–10 mm long, 3 mm deep, base unguiculate and abruptly widened into two small, triangular teeth 3 mm from base, setose and set with glochidiate hairs on back. Nectar scales with ovate back, narrowed above, yellow, 2.5 mm long and 1.5 mm wide near base, base incurved, back saccate, scale back with one red, transversal callus, neck thickened, slightly recurved, red, without filaments, laterally protracted into two tiny, erect wings < 1 mm long and < 0.5 mm wide. Staminodia two per scale, 5 mm long, base slightly dilate, 1 mm wide, filiform above, papillose, white. Stamens numerous, in five epipetalous fascicles of 15–20 each, filaments 8–9 mm long, white, anthers 0.5 mm long and wide, black. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect, clavate to subcylindrical capsule with persistent calyx, pedicel 20–25 mm long, capsule 15–20 × 8–10 mm, densely covered with reddishbrown setae 2.5–3.5 mm long, shorter scabrid hairs and decorated with 10 longitudinal ridges, opening with three apical valves; seeds numerous, black, with reticulate testa.

Notes: As the name indicates, *N. ferruginea* is characterized by a dense reddish-brown indumentum, giving the plant a rust-red appearance. Unlike the other species of the group, the plants are stiffly erect and branched only above. Together with the short petals (< 10 mm), the species is thus readily distinguished from its closest ally, *N. poissoniana*.

Habitat and distribution: *Nasa ferruginea* grows in rather wet situations, preferably in open soil under rocks and along water courses in (former) cloud forest areas. The two populations seen in the field (by the second author) were small and consisted of less than 10 individuals. This may be typical for the species and is also found in other cloud-forest species of *Nasa*. It is found on the eastern and northern slopes of the Eastern Cordillera, where it is the only species of the group and the only white-flowered species of *Nasa*. The other species are restricted to inner Andean valleys and west- or south-facing slopes.

Specimens examined: See appendix A5.

6. *Nasa raimondii* (Standl. & F. A. Barkley) Weigend, Rev. Peru. Biol. 13(1): 80. (2006) (Figs 2.2 J, K, 2.4 B, 2.11)

Basionym: *Loasa raimondii* Standl. & F. A. Barkley, Bull. Torrey Bot. Club 74: 82. (1947). – Type: PERU: Department Cuzco, Prov. Urubamba: Hacienda Tuncapata Stihit, Vargas 2672 (holotype F!; isotype UC!).

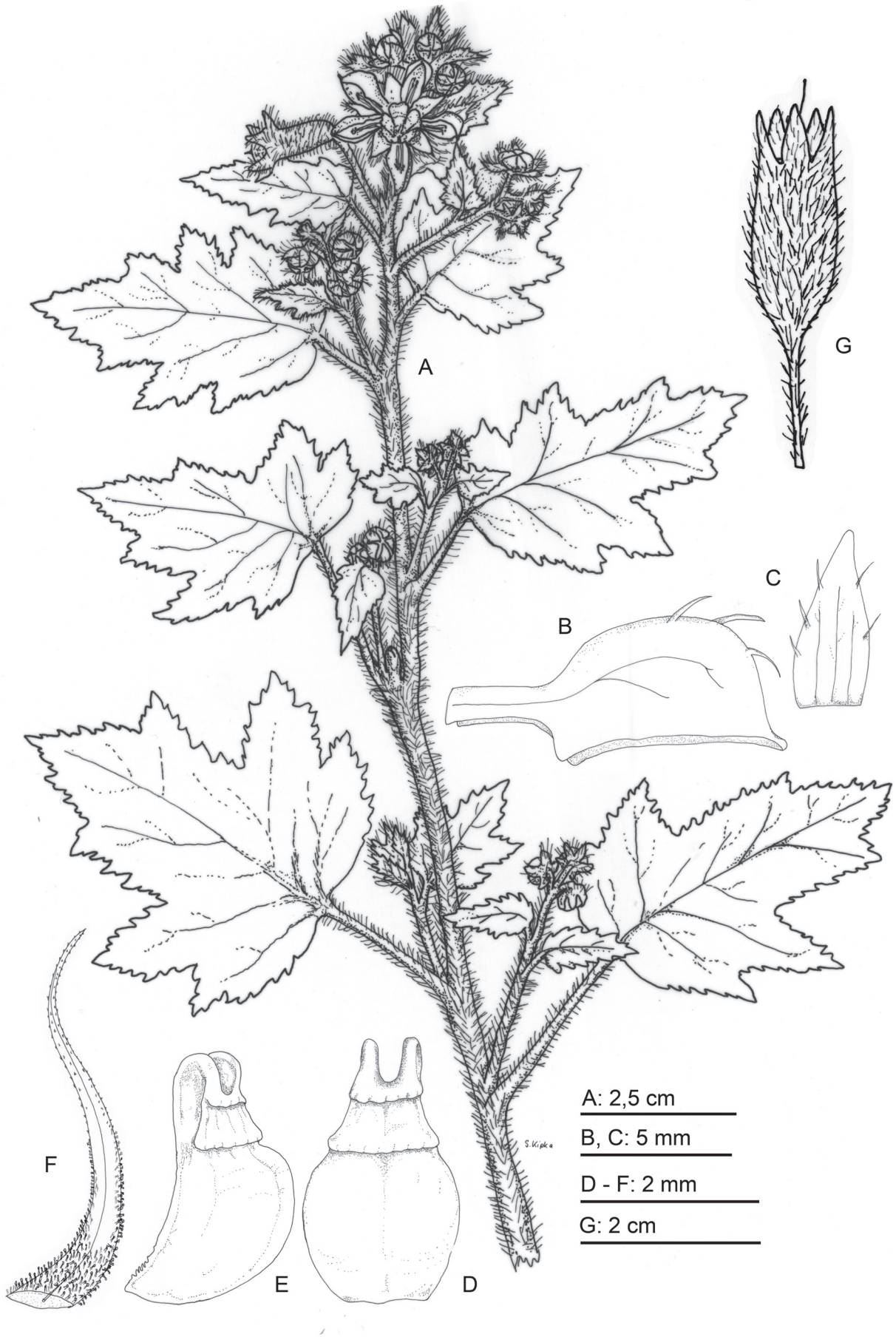

Figure 2.10: *Nasa ferruginea*: A, flowering shoot; B, petal; C, sepal; D, floral scale, dorsal view; E, floral scale, lateral view; F, staminode; G, fruit. Drawn from Weigend & Weigend 2000/199 by S. Kipka.

Description: Robust, annual herb 20–50(–160) cm tall. Stem terete, 3–4(–10) mm thick at base, covered with reddish-brown setae 2.5–3.5 mm long, shorter glochidiate hairs (0.5 mm), sometimes with glandular trichomes and multicellular hairs 1–1.5 mm long, decorated with numerous dark green stripes. Leaves opposite below and alternate above, petioles 20–45 mm; lamina widely ovate, 40–75 × 40–60 mm, margin serrate, sometimes with one to two shallow lobes on each side, lobes extending up to one-fifth of radius, base rounded to truncate, apex acuminate; both surfaces densely hairy, abaxial surface covered with red setae 2 mm long and short glochidiate hairs 0.1 mm long, adaxial surface covered with slightly longer red setae and scabrid hairs 0.2–0.5 mm long, venation pinnate. With three to five terminal, monochasial inflorescences 10–25 cm long, with 5–10 deflexed flowers per branch, bracts (narrowly) ovate, shortly petiolate to subsessile, 15–55 × 10–40 mm, flowers pentamerous, pedicels 7–10 mm long; calyx densely red setose, tube conical, 5 × 4 mm, calyx lobes ovate acuminate 6 × 3 mm. Petals yellowish-white, deeply cymbiform, 10–15 mm long, 10 mm deep, base unguiculate and abruptly widened into two small, triangular teeth 4 mm from base, setose and set with glochidiate hairs on back. Nectar scales with ovate back, narrowed above, yellow, 7 × 5 mm, base incurved, back with two conspicuous nectar sacs about 2 mm in diameter, scale back with one red, transversal callus, neck thickened, slightly recurved, red, without filaments, laterally protracted into two small, erect wings 1 × 0.5 mm. Staminodia 8 mm long, base slightly dilate, 1 mm wide, filiform above, papillose, white. Stamens in epipetalous fascicles of 15–20 each, filaments 8–9 mm long, white, anthers 0.5 mm long and wide, black. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect, shortly subcylindrical capsule with persistent calyx, pedicel 20–25 mm, capsule 20–30 × 9–10 mm, densely covered with reddish-brown setae 2.5–3.5 mm long, shorter glochidiate hairs (0.5 mm), glandular trichomes and multicellular hairs 1–1.5 mm long, opening with three apical valves; seeds numerous, black, with reticulate testa.

Notes: *Nasa raimondii* is frequently confused with *N. poissoniana* and *N. vargasii*, but clearly differs in having broadly amplexicaul bracts, a character otherwise found only outside the group in distantly related taxa (*N. stuebeliana* group). In the living state, its leaves are shiny and bright green, whereas the other species have dull, usually dark green leaves. It also has smaller flowers, more compact capsules, a sparser indumentum and only shallowly lobulate leaves.

Habitat and distribution: *Nasa raimondii* was until recently considered as endemic to the Department of Cuzco, but the collections cited here show that its range extends much further north into the Department of Huancavelica. The flora of Apurímac, Ayacucho and Huancavelica is still very incompletely documented in herbaria and the species may be common across the region. The species is found naturally in open places in the moister, upper parts of Andean scrub communities, but is now most common as an agricultural and roadside weed, sometimes forming large populations in, for example, potato fields.

Specimens examined: See appendix A6.

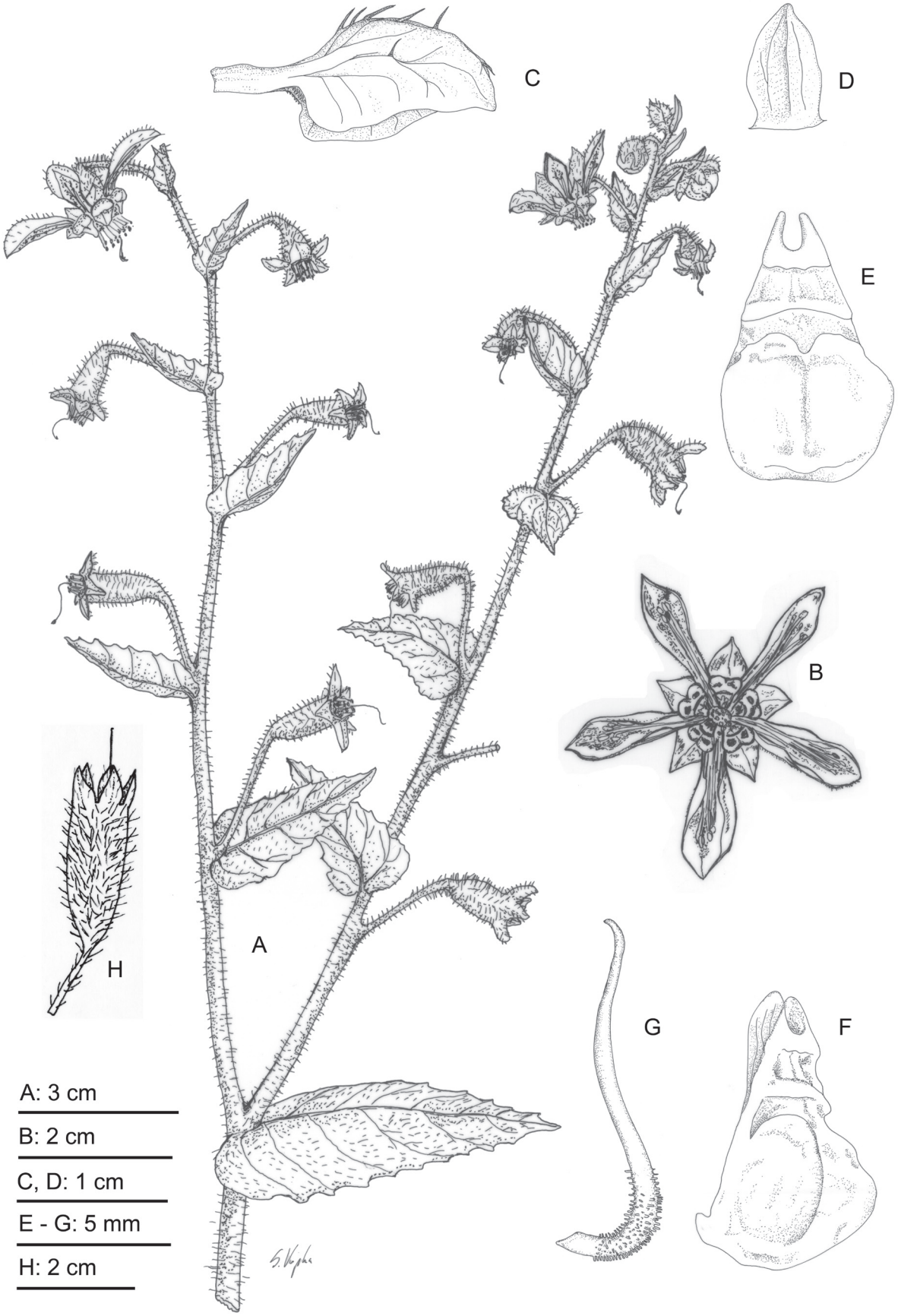

Figure 2.11: *Nasa raimondii*: A, flowering shoot; B, flower; C, petal; D, sepal; E, floral scale, dorsal view; F, floral scale, lateral view; G, staminode; H, fruit. Drawn from Weigend & Weigend 2000/289 by S. Kipka.

7. *Nasa vargasii* (J.F.Macbr.) Weigend, Rev. Peru. Biol. 13(1): 83. (2006) (Figs 2.2 F–H, J, 2.4 F, 2.12)

Basionym: *Loasa vargasii* J.F.Macbr., Field Mus. Nat. Hist. 13: 166. (1941).
– Type: PERU. Department Apurimac, Prov. Abancay: Río Pachaca, 2000 m, Stork, Horton & Vargas 10531 (UC!, fragment & Photo F!, neg. nr. 10531).

Description: Robust, annual herb 50–70(–120) cm tall. Stem terete, 5–15 mm thick at base, sparsely to densely covered with yellowish to pale brown setae 2.5–3.5 mm long, shorter scabrid hairs (0.5 mm), decorated with numerous dark green stripes. Occasionally glochidiate hairs (< 0.2 mm) and scattered uniseriate gland-tipped trichomes (< 1 mm) appear on the distal parts of the shoot system (including the pedicels and petals). Leaves opposite below and alternate above, petioles 40–60(–75) mm; lamina rhomboidal to widely ovate, 100–135(–210) × 80–120(–220) mm, base truncate, lobes rectangular, acuminate, up to 45–90 × 30–55 mm, distantly serrate, lamina apex acuminate, subpalmately lobed up to two-thirds of radius, both surfaces from almost glabrous to hairy, abaxial surface covered with pale setae 2 mm long and short glochidiate hairs 0.1 mm long, adaxial surface covered with slightly longer red setae and scabrid hairs 0.2–0.5 mm long, venation pinnate. With one to three terminal, monochasial inflorescences 20–60 cm long, with 8–13 deflexedflowers per branch, frondose-bracteose bracts petiolate, widely ovate and lobed, 20–50 × 20–35 mm, narrowly ovate and serrate to entire above, 10–50 × 3–20 mm. Flowers pentamerous, pedicels 5–25 mm long; ovary densely covered with yellowish to white/pale setae, setae 2–4 mm long, and scabrid trichomes < 0.1 mm long, tube conical, 5 × 5 mm, calyx lobes ovate to subcircular, 4–7 × 3–4 mm. Petals white, deeply cymbiform, 15–25 mm long, 5–8 mm deep, base unguiculate and abruptly widened into two small, triangular teeth 10 mm from base, setose and set with glochidiate hairs on back. Nectar scales with triangular back, much narrowed above, yellow, 7 × 5 mm, base incurved, back with two conspicuous nectar sacs about 2 mm in diameter, scale back with one red, transversal callus, neck thickened, slightly recurved, red, laterally protracted into two small, erect wings 1 × 0.5 mm. Staminodia 8 mm long, base slightly dilate, 1 mm wide, filiform above, papillose, white. Stamens in epipetalous fascicles of 15–20 each, filaments 10–16 mm long, white, anthers 0.5 mm long and wide, yellow. Ovary inferior, with three parietal placentae and numerous ovules. Fruit an erect, narrowly cylindrical capsule with persistent calyx, pedicel 20–30 mm, capsule 25–40 × 5–10 (–15) mm, densely covered with yellow setae 2.5 mm long and scabrid hairs 0.5 mm long and (usually) decorated with 10 well-developed longitudinal ridges, opening with three apical valves; seeds numerous, black, with reticulate testa.

Notes: This species is the only species with white and red nectar scales in the group; it is further characterized by narrow petals and large, deeply lobed leaves. As the coloration of flowers is often not recognizable in dried specimens, some collections can be confused with *N. poissoniana* and *N. raimondii* and vice versa. *Nasa raimondii* can be further distinguished by its only shallowly lobulate leaves and short capsules. *Nasa poissoniana* may be difficult to differentiate in the herbarium, but the margins of the basal leaves of *N. vargasii* are usually

Figure 2.12. *Nasa vargasii*. Southern form from Apurimac (Weigend & Weigend 2000/338): A, inflorescence; B, basal leaf; K, fruit from Cuzco (Huamantupa *et al*., 4075); L, fruit from Apurimac. Northern form from Huanuco (Weigend *et al*., 5463): C, habit; D, basal leaf; E, petal; F, sepal; G, floral scale, dorsal view; H, staminode; I, floral scale, lateral view; M, fruit. Drawing made by S. Kipka.

less deeply serrate, and the southern, sympatric form of *N. poissoniana* has two distinct horn-shaped nectar sacs on the scales, which are absent in *N. vargasii* and which are easy to recognize in the herbarium.

Habitat and distribution: *Nasa vargasii* is common in steep scree slopes, especially under trees of *Schinus molle* L. (Anacardiaceae). The species was originally described from southern Peru, but we now also know some highly disjunct populations from Department Huánuco in central Peru. Despite this huge distribution gap (c. 700 km), it is difficult to tell the specimens apart, with northern populations differing marginally in a more compact habit, higher densities of gland-tipped hairs (especially towards the inflorescence), prominently developed longitudinal ridges on the capsules and marginally smaller flowers. However, herbarium specimens cannot be reliably told apart, with some southern specimens indistinguishable from the northern 'form'. We therefore treat all collections of this species as a single taxon.

Specimens examined: See appendix A7.

3. Two new species of *Nasa* (Loasaceae) from Andean South America*

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http://www.mapress.com/phytotaxa/content/2011/f/p00026p008f.pdf

* published as: Henning T, Weigend M. 2011. Two new species of *Nasa* (Loasaceae) from Andean South America. *Phytotaxa* 26: 1-8.

4. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae pro parte*, Loasaceae)*

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DOI: 10.1111/j.1095-8339.2011.01164.x

* published as: Henning T, Rodríguez E, Weigend M. 2011. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae pro parte*, Loasaceae). *Botanical Journal of the Linnean Society* 167: 47-93. DOI: 10.1111/j.1095-8339.2011.01164.x

5. Reloading the revolver - male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales)*

Abstract

Reward partitioning and replenishment and specific mechanisms for pollen presentation are all geared towards the maximization of the number of effective pollinator visits to individual flowers. An extreme case of an apparently highly specialized plant–pollinator interaction with thigmonastic pollen presentation has been described for the morphologically complex tilt-revolver flowers of *Caiophora arechavaletae* (Loasaceae) pollinated by oligolectic *Perditomorpha pampeana* (formerly *Bicolletes pampeana* - Colletidae, Hymenoptera). We studied the floral biology of *Nasa macrothyrsa* (Loasaceae) in the field and in the glasshouse, which has very similar floral morphology, but is pollinated by polylectic *Neoxylocopa* bees (Apidae, Hymenoptera). We investigated the presence of thigmonastic anther presentation, visitor behaviour (pollinators and nectar robbers), co-ordination of pollinator visits with flower behaviour and the presence of nectar replenishment. The aim of this study was to understand whether complex flower morphology and behaviour can be explained by a specialized pollination syndrome, or whether alternative explanations can be offered. The results showed that *Nasa macrothyrsa* has thigmonastic pollen presentation, i.e. new pollen is rapidly (<< 10 min) presented after a pollinator visit. Nectar secretion is independent of removal and averages 7–14 μL h–1. The complex flowers, however, fail to exclude either native (hummingbirds) or introduced (honeybees) nectar robbers, nor does polylectic *Neoxylocopa* actively collect the pollen presented. The findings do not support a causal link between complex flower morphology and functionality in Loasaceae and a highly specialized pollination. Rapid pollen presentation is best explained by the pollen presentation theory: the large proportion of pollinators coming shortly after a previous visit find little nectar and are more likely to move on to a different plant. The rapid presentation of pollen ensures that all these valuable 'hungry pollinators' are dusted with small pollen loads, thus increasing the male fitness of the plant by increasing the likelihood of siring outcrossed offspring.

5.1 Introduction

Floral rewards are crucial for pollinator attraction, and pollen and nectar are the most common rewards for pollinators. To encourage repeated visits, rewards must be replenished at ecologically relevant frequencies (Engel & Irwin, 2003; Castellanos *et al.*, 2006). Nectar is commonly replenished in flowers and may be available throughout anthesis, in spite of repeated removal by flower visitors (Aizen & Basilio, 1998; Valtueña *et al.*, 2007), and

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overall nectar production may be adjusted to higher and lower visitation rates (Vickery & Sutherland, 1994; Castellanos *et al.*, 2002; Hernández-Conrique *et al.*, 2007). In contrast, pollen cannot be replenished, as the number of anthers present and the amount of pollen contained in them is fixed long before anthesis. However, particular mechanisms have evolved to improve male fitness by increasing the likelihood of pollen transfer to receptive stigmas. Pollen presentation theory explains pollen presentation in terms of male fitness (Lloyd & Yates, 1982; Harder & Thomson, 1989, Thomson 2003, Castellanos *et al.*, 2006). One crucial aspect is that not all pollen can be removed by an individual flower visitor. This can be ensured by, for example, sequential maturation of anthers of individual flowers ('packaging'), or by providing the anther only with a small opening, releasing pollen in small portions ('dispensing'; Lloyd & Yates, 1982; Harder & Thomson, 1989; Thomson *et al.*, 2000). A particularly complex case of pollen partitioning by packaging has been described from the largely neotropical Loasaceae subfamily Loasoideae. These plants have proterandrous tilt-revolver flowers with 80–100(–120) stamens in five groups, with the stamens initially enclosed in pockets formed by the petals (Fig. 5.1 A; Weigend & Gottschling, 2006). The five stamen groups alternate with five boat-shaped staminodial complexes. Staminodial complexes consist of an outer, deeply boat-shaped nectar scale (open at the apex and towards the inside of the flower) and two free staminodes closing the inside of the scale. Seen from above, the apical openings of these floral scales form a circle like the holes in the drum of a revolver (Fig. 5.1 E). Individual anthers mature over several days and one-by-one move into the center of the flower, where they dehisce and present their pollen (Fig. 5.1 B, C). Nectar is secreted into the scales (Fig. 5.1 F), and bees probe each scale to harvest its nectar. Most importantly, in *Caiophora arechavaletae* (and some other species of *Blumenbachia* and *Caiophora*), stamen movement can also be triggered by nectar-harvesting bees (Schlindwein & Wittmann, 1997b; Schlindwein, 2000). Only oligolectic females of the short-tongued bee *Perditomorpha pampeana* (Colletidae) pollinate these flowers. These bees return to the individual flower shortly after the first visit (c. 5 min) to harvest the pollen presented as a result of the previous manipulation of the nectar scales (Schlindwein & Wittmann, 1997b). The complex floral morphology, the difficult access to the nectar, frequent documentation of colletids as pollinators and, especially, the triggered pollen presentation seem to point to a specialized (and possibly exclusive) relationship between tilt-revolver flowers and short-tongued, oligolectic colletid bees (Schlindwein & Wittmann, 1997b; Schlindwein, 2000). However, Ackermann & Weigend (2006) and Weigend & Gottschling (2006) reported a wide range of bees and other insects as flower visitors for different species of Loasaceae with tilt-revolver flowers from several distantly related genera, casting doubt on a direct causal link between complex flowers and specialized pollination. The Andean genus *Nasa* is the largest genus of Loasaceae (> 100 species), and about one-half of its species have tilt-revolver flowers as described above (Weigend & Gottschling, 2006). It is unknown whether species of *Nasa* also possess triggered (thigmonastic) stamen movement, whether nectar is replenished in these tilt-revolver flowers and whether nectar replenishment (if present) is triggered by flower visits. We therefore investigated the floral function and ecology of *Nasa macrothyrsa*, a shrubby species, narrowly endemic to the provinces of San Miguel and Contumazá, Department of Cajamarca, in northern Peru (Weigend, Henning & Schneider, 2003). Its flowers are larger but structurally similar to those of *C. arechavaletae*. In contrast with

C. arechavaletae, *N. macrothyrsa* is mainly visited by large carpenter bees (*Neoxylocopa lachnea* Moure, 1951, observed in several populations across the distribution area of the species; Ackermann & Weigend, 2006) and produces a large amount of nectar (under visitor exclusion 75.13 ± 28.98 µL nectar per flower, 32.39 ± 10.61% sugar, 24.04 ± 11.51 mg sugar per flower; Ackermann & Weigend, 2006). We investigated whether this species, with carpenter bees as presumably less specialized pollinators (Raju & Rao, 2006), showed the same complex pattern of reward partitioning and presentation, and whether the pollen/reward replenishment was paralleled by correspondingly timed flower visits. We aimed at a detailed picture of flower behaviour. How long are the phases of anthesis in *N. macrothyrsa* and are its flowers self-pollinated? Is stamen movement also thigmonastic? Is nectar replenished, and is nectar replenishment influenced by flower visits? What is the timing of flower visits? Does reward accumulation (pollen and nectar) in some way correspond to the observed visitor frequencies?

5.2 Materials & Methods

Plant material and cultivation. Observations on the flowers of *N. macrothyrsa* were made on plants in their natural habitat [Peru: Depto. Cajamarca, Prov. Contumazá, Contumazá; on a rocky slope next to the road, surrounded by cloudforest remnants. 2681 m, 12.vi.2008, T. Henning & J. Schulz 32 (USM)]. Seeds collected on a previous collection trip had been used to raise plants of this species in the glasshouse [voucher: M. Weigend *et al.* 7471 (B, M, USM, HUT)]. Seeds were sown into standard sowing soil (Compo Sana). Seedlings were pricked out as soon as the cotyledons were fully developed into 5-cm clay pots filled with the same soil, and then repotted into successively larger clay pots [potting soil: two parts mature leaf compost, one part peat, fertilized with a mixed inorganic–organic fertilizer (Garten- and Gemüsedünger, ASB Grünland H. Aurenz GmbH) and basalt powder (Neudorffs UrgesteinsMehl, W. Neudorff GmbH KG)]. Plants were kept outdoors in light shade during the summer (May–October) and moved into the glasshouse in winter (November–April). Flowering took place between November and February when the plants were kept in the glasshouse with artificial lighting (12 h, high-pressure sodium lamps: Philips SON-T AGRO 400 W) and temperatures of 10–15 °C at night and 20–25 °C during the day (closely resembling the temperature regime in the natural habitat).

Glasshouse experiments. Flower longevity was investigated by marking individual flowers and recording the phases of anthesis (incipient anthesis, staminate phase, carpellate phase, shedding of petals/anthers, N = 20). Overall stamen number per flower was recorded (N = 29). Self-pollination was investigated by marking individual flowers and then either leaving them unmanipulated (N = 30, selfing) or hand-pollinating by dehisced anthers from other flowers (N = 28, pollination) and subsequently recording fruit set. Flowers were not bagged, as experiments were carried out in winter (November–February) without pollinator activity. Nectar replenishment was studied in flowers in the middle staminate phase. At the beginning of the experiment, the entire amount of nectar present in each flower was removed by insertion of graded microcapillaries (microcapillaries: 5-, 10- and 25-µL Duran Ringcaps, Hirschmann Laborgeräte, Eberstadt, Germany) into the nectar

scales. The amount of nectar replenished was then studied by harvesting and measuring the nectar five times after fixed intervals, once at the end of the experiment. Sugar concentrations were calculated from Brix measurements made for each flower separately with a hand-held refractometer (neoLab-Handrefraktometer Universal; 10–80% Brix). A first experiment was carried out in December 2005 (five intervals of 30 min, total 150 min, N = 18; one interval of 150 min, N = 8); a second experiment was carried out on the same plants in December 2008 (five intervals of 60 min, total 300 min, N = 26; one interval of 300 min, N = 16). Mean nectar production was calculated for each interval (30 min and 60 min) and compared with the longer interval (150 min and 300 min). Stamen movement was studied in December 2008 by marking flowers and cutting off mature stamens already present in the center of the flower 1 h before the first stimulation experiment. Twenty flowers were used for the experiment with a control group of ten flowers. Five consecutive 30-min intervals between stimuli were chosen, based on field observations indicating an average interval between two visits to individual flowers of c. 25 min. Stamen movement was triggered by slight bending of all five nectar scales outwards with a dissecting needle, thus imitating a pollinator visit. Stamen movement from the reflexed into the upright position takes c. 1.5–3 min, so that the 30-min interval between stimuli was further subdivided into 5-min subintervals for recording purposes. Autonomous stamen movement was observed in an unmanipulated control group. Each flower and every flower manipulation were treated statistically as a single event, comparable with a single pollinator visit in the natural habitat.

Field observations. Field observations were carried out in June 2008 on a large population (several dozen plants with several hundred open flowers) near Contumazá (see above). Flowers were individually marked and flower visits were recorded. A total of 30 flowers was watched during two observation periods of 150 min each (12 June 2008, 14:30–17:00 h; 13 June 2008, 10:00– 12.30 h). Visitor intervals and the type of visitor were recorded. If a flower was visited twice within the same minute, an interval of 30 s was arbitrarily assigned. Voucher specimens of visiting insects were caught at the end of the observation period for determination purposes. Hummingbird visits were recorded without determination of species or vouchering.

Statistics. In stamen movement, the triggered flowers and the control group are independent samples and the data (number of stamens moved at a given time) are not normally distributed. The general tendency of stamen movements was tested for significance using the nonparametric Mann–Whitney U-test. Nectar secretion was tested for significant differences by comparing only the total amount of nectar per flower secreted during the time of the experiment. The data were first tested for normality using the Kolgomorov–Smirnov test (normal distribution given in all cases). Then, a *t*-test was performed to compare the nectar values produced per flower in the repeatedly probed and control flowers. The resulting *P* values (exact significance, two-tailed) are given in parentheses. Datasets were prepared using Microsoft Excel (Microsoft Corp.); statistical analyses were performed using SPSS 9.0 (SPSS Inc.) for Windows. Graphs were calculated with SigmaPlot 8.0 (SPSS Inc.) and graphically processed with Adobe Illustrator CS (Adobe Systems Inc.).

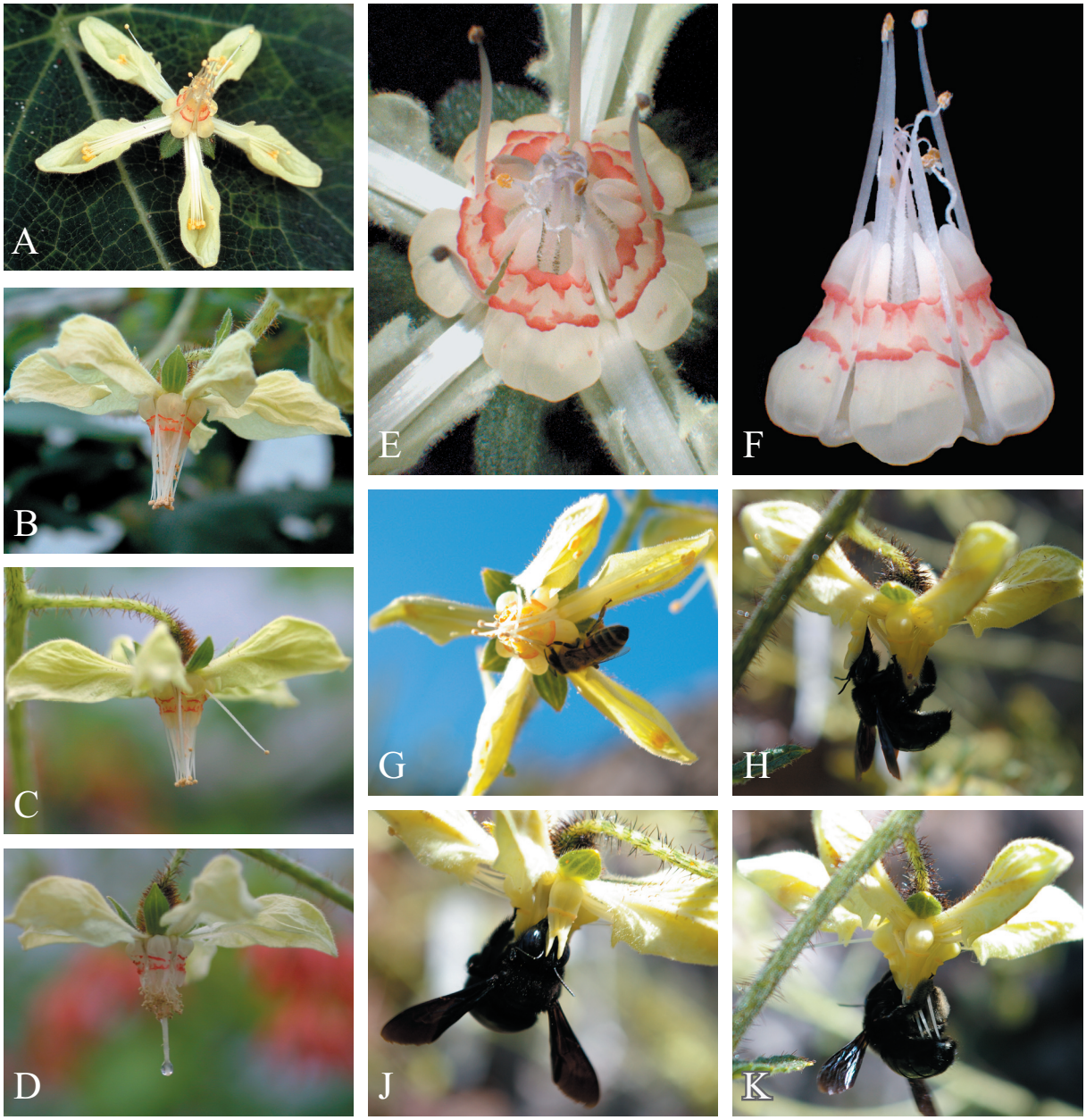

Figure 5.1: Flower of and flower visits on *Nasa macrothyrsa*. A, Flower, note the numerous stamens reflexed into the hooded petals. B, Flower in the staminate phase, with red and white floral scales and stamens in the center of the flower. C, Stamen moving from the petal on the upper right into the flower center. D, Nectar overflowing (along style) in the absence of flower visitors. E, Staminodial complexes with apical openings of the floral scale (access to the nectar). F, Staminodial complexes, lateral view, nectar visible through semi-transparent nectar scales. G, Honeybee robbing nectar, holding on to the petal base and accessing nectar scale from the side without touching anthers or stigma. H,J, Carpenter bee holding onto other nectar scales and inserting proboscis into one floral scale to remove nectar. K, Abdomen of carpenter bee dusted with pollen; note the filaments in the center of the flower and the triggered stamen moving from the left.

5.3 Results

Flower longevity and self-pollintaion. Unmanipulated flowers were open for c. 5 days (5.21 ± 0.58 days) and were in the staminate phase for c. 4 days (3.83 ± 0.42 days). Individual flowers possessed > 100 stamens each (104.69 ± 11.32). Flowers were not self-pollinated; unpollinated flowers set no fruit in the glasshouse (N = 30, all abortive), but 85% of hand-pollinated flowers set fruit (N = 28, resulting in 25 capsules with viable seed).

Nectar replenishment. Unmanipulated flowers in the middle staminate phase contained 84.23 ± 33.34 µL of nectar with a concentration of 44.38 ± 3.72% (30-min experiment), and 17.56 ± 7.48 µL of nectar with a concentration of 53.96 ± 5.77% (60-min experiment). Nectar replenishment was found to take place in both experiments (2005, 2008), but nectar secretion was clearly higher in 2005 than in 2008. In the 30-min experiment, individual flowers produced 0.15 ± 0.04 µL of nectar per minute with a concentration of 25.17 ± 2.73%, corresponding to 0.04 ± 0.02 mg sugar per minute. In the 60-min experiment, individual flowers produced 0.07 ± 0.02 µL of nectar per minute with a concentration of 22.86 ± 2.47%, corresponding to c. 0.02 ± 0.005 mg sugar per minute. There was no significant difference in nectar secretion between the flowers sampled at intervals of 30 and 60 min compared with the flowers sampled after 150 and 300 min (60 min: N = 25, blind = 16, T = 0,794, d.f. = 39, *P* = 0432; 30 min: N = 18, blind = 8, T = 0,793, d.f. = 24, *P* = 0435). Accumulated nectar measured at the beginning of the experiment had a much higher sugar concentration than the freshly secreted nectar produced during the experiment. Nectar production/replenishment appears to be independent of nectar removal in the time frame relevant to this study. Indeed, flowers do not cease to produce nectar if nectar is not removed, and floral scales eventually overflow with nectar dripping out of the flower (Fig. 5.1 D).

Thigmonastic stamen movement. Autonomous stamen presentation was studied in unmanipulated flowers in the glasshouse (5 × 30-min intervals on 10 flowers = 50; Fig. 5.2). An average of 2.44 ± 0.72 stamens every 30 min (one interval) moved into the center of the flower. Simulated pollinator visits (tilting of the floral scales in intervals of 30 min,

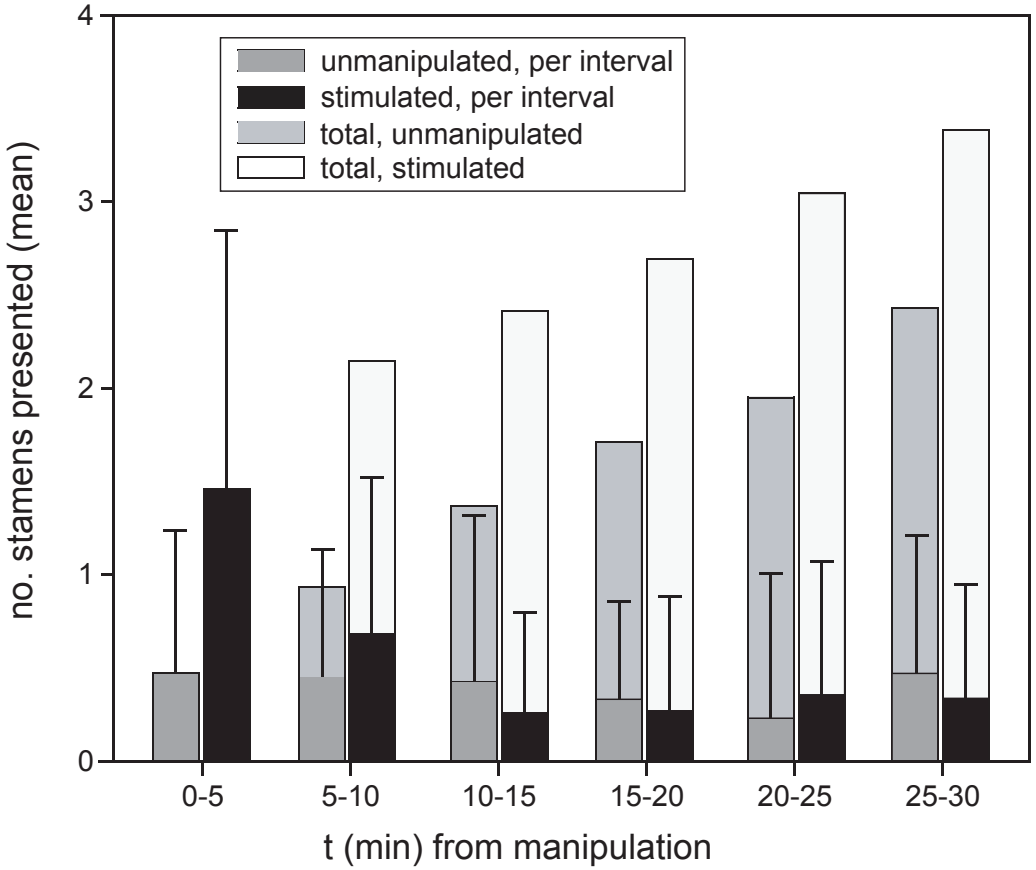

Figure 5.2: Autonomous and triggered stamen movement in flowers of *Nasa macrothyrsa*. Dark bars, number of stamens moving per stimulus and interval; light bars, sum of stamens present in the center of the flower after each interval at a given interval after the stimulus.

5 × 30-min intervals on 20 flowers = 100) led to a highly significant increase in stamen presentation (P = 0.007, Mann–Whitney-U-test), with an average of 3.39 ± 0.79 stamens every 30 min (Fig. 5.2). Most of the thigmonastic stamen movement occurred soon after the stimulus (63% in the first 10 min).

Field observations. During the observation period, a total of 136 individual flower visits were recorded, with *Neoxylocopa* as the most frequent visitor (77 visits, 52.03%, Fig. 5.1 H–K), followed by (non-native, introduced) honeybee *Apis mellifera* (59 visits, 39.86%, Fig. 5.1 G) and hummingbirds (12 visits, 8.11%). *Apis* and hummingbirds usually failed to touch anthers or stigma (and did not collect pollen in the flowers) and are unlikely to pollinate. Only *Neoxylocopa* bees collected nectar as described for *Perditomorpha* bees in *C. arechavaletae* (Schlindwein & Wittmann, 1997b), that is by inserting their proboscis into each floral scale (Figs 5.1 H, I) whilst pollen was deposited on their abdomen (Fig. 5.1 K). *Neoxylocopa* did not actively remove pollen from the anthers. All flowers were visited at least twice during the observation period, with 4.93 ± 2.43 visits per flower and intervals between visits ranging from 0.5 to 105 min, with a mean interval of 24.78 ± 26.55 min between visits (Fig. 5.3). However, data were heavily skewed towards shorter intervals, and the median time lapse between visits to individual flowers was only 11 min because of a very high percentage of bee visits after less than 10 min (48.31% total; 60.61% in *Neoxylocopa*, 48.93% in *Apis*, 33.30% in hummingbirds).

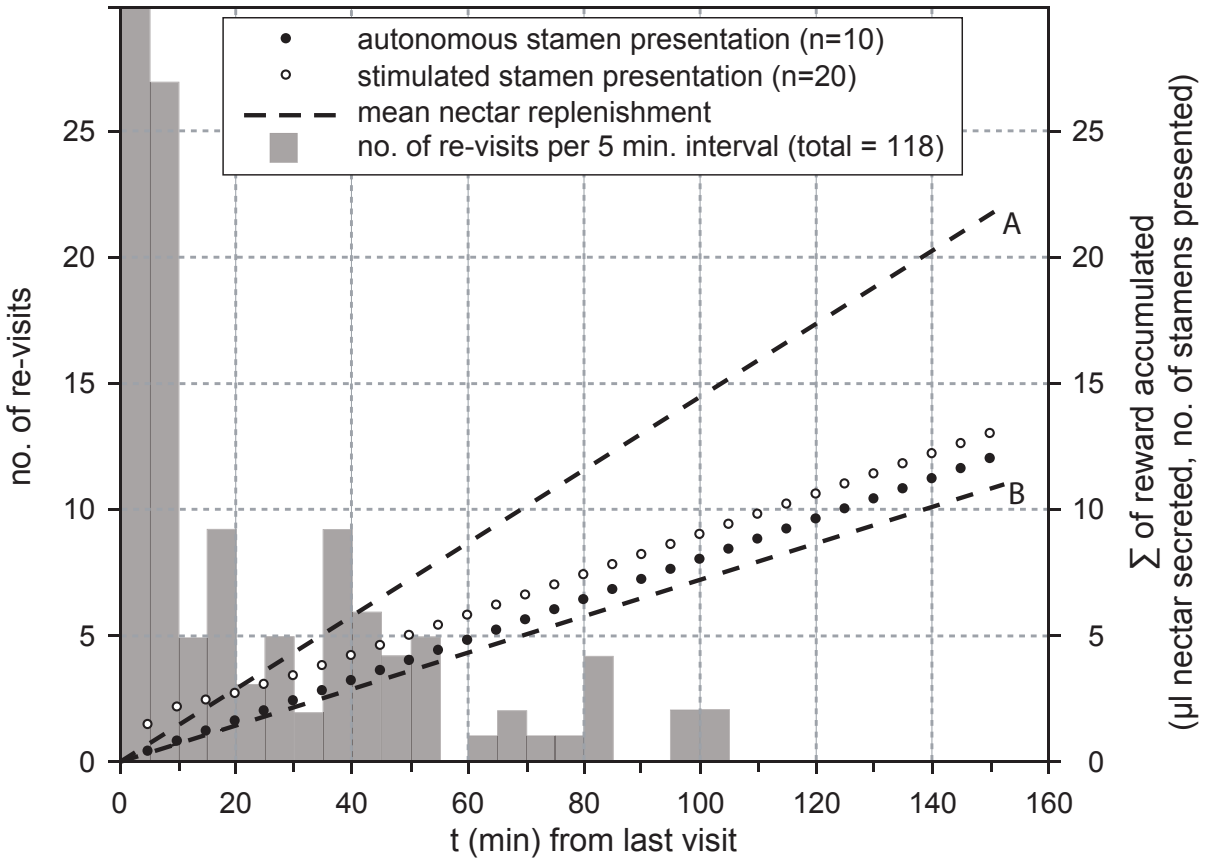

Figure 5.3: Flower visits and reward accumulation. Absolute number of second visits to individual flowers and reward available per flower at a given time (t) after previous visit: nectar A, 30-min experiment (2005); nectar B, 60-min experiment (2008). The majority of visitors come very soon after a previous visit and (presumably) reward depletion.

5.4 Discussion

The flowers of *N. macrothyrsa* require pollination to set fruit. The field data presented here, in conjunction with the data from other populations presented in Ackermann & Weigend (2006), show that *Neoxylocopa lachnea* is the principal flower visitor and likely pollinator of *N. macrothyrsa*. There is thus no exclusive relationship between tilt-revolver flowers and Colletid bees. *Neoxylocopa* bees expertly manipulate the complex flowers, extracting nectar from all five nectar scales, whilst their abdomen is dusted with pollen from the mature anthers in the center of the flower. Conversely, honeybees and hummingbirds evidently act as nectar robbers, without effecting pollination. The flowers show a highly complex pattern of reward/pollen partitioning and timing: the numerous anthers are autonomously (slowly) or thigmonastically (more rapidly) presented in the center of the flower, the nectar is distributed over five nectar scales, which increases handling time for the visitor, and nectar is continuously replenished in the five nectar scales. The nectar replenished after visits has a relatively low sugar concentration of c. 22–25%, compared with accumulated nectar in flowers that have not recently experienced a visit.

The flowers of *N. macrothyrsa* have an extended staminate phase (four-fifths of anthesis duration), especially when compared with polyandrous flowers of other genera of Loasaceae with less complex floral morphology: In *Mentzelia* L., flowers are usually open for a single morning with all pollen (from a similarly large number of anthers) dispensed in a few hours (subfamily Mentzelioideae; Weigend, 2007). *Nasa macrothyrsa* pollen is presented in small portions (a few anthers at a time), and this can be considered as an extreme case of 'packaging'. Taking the length of the staminate phase from the glasshouse (3.83 ± 0.41 days, c. 12 h daylight, similar to natural conditions) and superimposing the visitor frequency in the field (average interval 24.78 ± 26.55 min), we would expect an average of over 100 flower visits overall for each flower during the staminate phase alone (multiplying visitor frequency from the field with floral longevity from the glasshouse), nearly half by the legitimate pollinator *Neoxylocopa*. As a result of sequential anther maturation and rapid thigmonastic pollen presentation, the vast majority of visitors will be dusted with small amounts of pollen throughout the staminate phase. Harder & Wilson (1994) argued that 'the most effective (pollen) dispensing schedule allows dynamic adjustment of removal to the prevailing frequency of visits experienced by individual plants' – this is clearly the case in *N. macrothyrsa*, where anther presentation is triggered by flower visits. Conversely, the carpellate phase in *N. macrothyrsa* is only c. 1 day and, from experimental pollination in the glasshouse, it would seem that a single pollinator visit will be sufficient for full seed set. Pollen presentation in *N. macrothyrsa* can be strongly accelerated by pollinator visits, ensuring the rapid replenishment of pollen during periods of high visitor activity. In the field, most (60.61%) flowers visited by *Neoxylocopa* received a second visit within the first 10 min. Thigmonastic pollen presentation ensures that fresh pollen is already available after this short time period, but visitors will only receive a very minor amount of nectar (< 0.7–1.5 mL). Overall attractiveness (i.e. amounts of pollen and nectar available in the individual flower) gradually increases with time from the last visit as a result of reward accumulation (Fig. 8.3), ensuring that some of the flowers of *N. macrothyrsa* visited by *Neoxylocopa* offer a very large amount of nectar and pollen, making it a rewarding resource.

In particular, nectar is then available in large quantities and at higher sugar concentrations. Pollinators encountering little or no nectar in an individual flower appear to be more likely to leave the inflorescence or the plant concerned and move on to a different individual (Cresswell, 1990; Biernaskie *et al.*, 2002; Johnson *et al.*, 2004; Jersákova & Johnson, 2006). Thus, the 'best' pollinators for *N. macrothyrsa* are those visitors coming soon after a previous flower visit, as they will receive little nectar and are thus more likely to move on to a different plant. Moreover, bees receiving a heavy pollen load from individual flowers are much more likely to groom (i.e. move the pollen into the scopae for transport) than bees receiving a smaller pollen load (Harder, 1990a). Pollen is then no longer available for pollination. Thus, depositing small amounts of pollen on all visitors is the most efficient way of dispensing pollen in terms of male fitness. Small amounts of pollen presented whilst nectar is depleted should be most likely to sire out-crossed offspring because of these idiosyncrasies of bee behaviour. Accelerated pollen presentation will be advantageous, irrespective of pollinator species, in a reward-limited system, and probably increases male fitness.

Schlindwein & Wittmann (1997b) viewed the parallelism between pollen presentation and bee behaviour in *P. pampeana* on *C. arechavaletae* as an extreme case of a specialized pollination syndrome. The data presented here argue that this may be a classical case of asymmetric specialization, which is probably common in plant–pollinator systems (Vázquez & Aizen, 2004). *Perditomorpha pampeana* is apparently specialized on *C. arechavaletae*, but *C. arechavaletae* is probably not dependent on *P. pampeana*. Evidence from the cultivation of other similar species of *Caiophora* shows that European bumblebees (*Bombus*) and honeybees (*Apis*) are perfectly capable of learning to manipulate the complex tilt-revolver flowers and of efficiently pollinating these flowers. There is no need to explain the complex morphology and behaviour of tilt-revolver flowers in general by the specialized behavioural pattern of particular species of Colletid bee (as presumably highly specialized pollinators). Even unspecialized and ineffective visitors, such as introduced honeybees, are not successfully excluded and are perfectly capable of exploiting floral rewards in spite of the complex flower morphology. We argue that thigmonastic pollen presentation in tilt-revolver flowers is a mechanism to increase male fitness by making maximum use of the 'hungry pollinator'. Which animal species ultimately becomes the dominant visitor/pollinator species is determined by the complement of potential pollinator species present in a given locality and the competition between them (Herrera, 2005). Complex reward partitioning and timing, together with rapid and repeated pollen presentation, probably ensure a high degree of outcrossing, even under interference from native (hummingbirds) and introduced (honeybees) nectar robbers, as in the system studied here.

6. Complex, but not cheap - floral rewards and floral behaviour in six species of Loasaceae*

Abstract

Autonomous and thigmonastic pollen presentation via anther movements is found in several taxa of Loasaceae. It represents a complex mechanism for pollen partitioning. Breeding systems, pollen and ovule numbers and nectar are here quantified for six species of Loasaceae (3 each from the genera *Nasa* and *Caiophora*), representing different breeding systems and pollination modes. We investigated the presence and timing of thigmonastic pollen presentation and possible links between pollination mode and cost (using P/O ratios and nectar as proxies). All species were found to be facultatively autogamous or facultatively xenogamous. P/O ratios range from 3044 to 8743 and are very high for these breeding systems. Stamen thigmonasty is confirmed for all six species and thigmonastic pollen presentation is dramatically increased (1 to 10 × 5 min after a visit, 1.5 to 3.2 × across a 30 min interval) compared to autonomous presentation. However, differences in nectar (0.43–75.13 µl/flower) and nectar sugar (0.23–24.04 mg/flower) are higher than differences in pollen/flower and nectar seems to be adjusted more strongly to pollination mode. The amount of pollen per flower and P/O ratios are more conserved and likely under a phylogenetic constraint. Species pollinated by pollen collecting short-tongued bees have the lowest P/O ratios and the smallest amounts of nectar (0.23–1.77 mg sugar/flower), making this pollination mode the by far most economical one. Pollination by hummingbirds and long-tongued bees is much more "expensive" in terms of nectar production (8.45–24.04 mg/flower) and correlated to considerably higher P/O ratios. Pollen presentation is particularly rapid in flowers pollinated by short-tongued bees (accelerated 5–10 times compared to 1–3 times in other pollination modes). The data here presented confirm that Loasaceae adjust the amount and type of nectar offered to different pollinators and show for the first time that they are also able to adjust the amount of pollen to different pollination modes. Pollen dispensation is moreover dramatically accelerated under high visitation frequencies. In spite of the complex pollen presentation scheme, P/O ratios are high in thigmonastic Loasaceae and the mechanism is not a cheap one.

6.1 Introduction

The effective transfer of pollen to receptive stigmas in order to sire offspring is central to pollination biology. The effectiveness of pollen transfer is one crucial component of male fitness and different pollination syndromes diverge dramatically in their efficiency. Abiotic (e.g., wind) pollination is considered as a particularly wasteful mechanism, whereas autogamy is perceived as a parsimonious strategy (Cruden 1977, 2000). The

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relative amount of pollen required for successful pollination can be approximated by the pollen-ovule ratio (P/O ratio) and "the more efficient the transfer of pollen is, the lower the P/O ratio should be" (Cruden 1977, p. 32). A correlation between breeding system and P/O ratios has been reported, with obligately xenogamous species having generally higher P/O ratios than their facultatively xenogamous and autogamous relatives (e.g. Aedo *et al.*, 2005, 2007; Cruden, 2000). P/O ratios may additionally be influenced by, e.g., the size of receptive surfaces or pollen bearing areas of pollinators, duration of stigma receptivity, seed size, and other ecological factors (Cruden 2000, Cruden & Miller-Ward, 1981; Uma Shaanker & Ganeshaiah, 1984; Small, 1986; Castellanos *et al.*, 2006). Several studies have confirmed the validity of Cruden´s conclusion (Cruden, 1977) of the predictability of breeding systems based on the correlation of breeding system and P/O ratios (McDade, 1985; Gallardo *et al.*, 1994; Lopez *et al.*, 1999; Wyatt *et al.*, 2000; Jürgens *et al.*, 2002; Wang *et al.*, 2004). With increased specialization in plant-pollinator interaction (and hence increased pollinator efficiency) a decreased P/O ratio can sometimes be observed, minimizing the energetic investment when more efficient pollination is likely achieved via specialization (Schemske, 1981; Ramirez & Seres, 1994; Lopes & Machado, 1999; Torres & Galetto, 1999; Löhne *et al*,. 2004; Leite & Machado, 2007; Almeida Barreto & Freitas, 2007). Pollen is after nectar the second important floral reward for flower visitors,. In many pollination syndromes flower visitors actively take up only the nectar (e.g., ornithophily), but in others (often in melittophily) the visitors also actively harvest and collect pollen, which is thus no longer available for pollination. Oligolectic bees are often highly effective pollinators (Schlindwein, 2004), but oligolectic bees also collect pollen for their brood. It is unclear in how far this secondary use of pollen as a reward might reduce the benefit obtained from specialized pollination - this might enforce an increased P/O ratio to offset pollen loss through consumption. Thus, while pollination by oligolectic bees is an effective strategy, it remains unclear whether it is also efficient, i.e., whether the resource investment of the plant is indeed smaller, as compared to other pollination modes. However, there seem to be no clear trends as to differences in the efficiency of different animal pollinator groups as such. In a comprehensive study on the genus *Penstemon* (Scrophulariaceae) Castellanos *et al.* (2006) found no differences in the P/O ratios of bee versus hummingbird pollinated taxa, and thus an effect of pollen collection by bees does not become visible from their data.

In Loasaceae subfam. Loasoideae an extremely intricate system of specialized pollination by short-tongued bees has been demonstrated by Schlindwein & Wittmann (1997b). The complex flower morphology of these plants was first investigated in detail by Urban (1886, 1892) and then again in the 20th century (Brown & Kaul, 1981). The flowers have usually boat-shaped, spreading (Fig. 6.1 A–C, E, F) to reflexed (Fig. 6.1 D) petals into which the antepetalous stamen bundles are initially reflexed (Fig. 6.1 G). In the course of the anthesis (staminate phase) the stamens individually move into an erect position (Fig. 6.1 G, K–M), anthers dehisce and present their pollen. In addition to the autonomous anther movement Schlindwein & Wittmann (1997b) demonstrated that stamen movement in *Caiophora arechavaletae* Urb. can be triggered by the pollinator through manipulation of the floral scale (Fig. 6.1 K–M) when extracting nectar. Thigmonastic stamen presentation was interpreted as part of a complex floral behaviour related to specialized pollination by oligolectic colletid bees. However, while pollination by short-tongued colletid bees may be common in Loasaceae, a wide range of different animal pollinators are now known for the

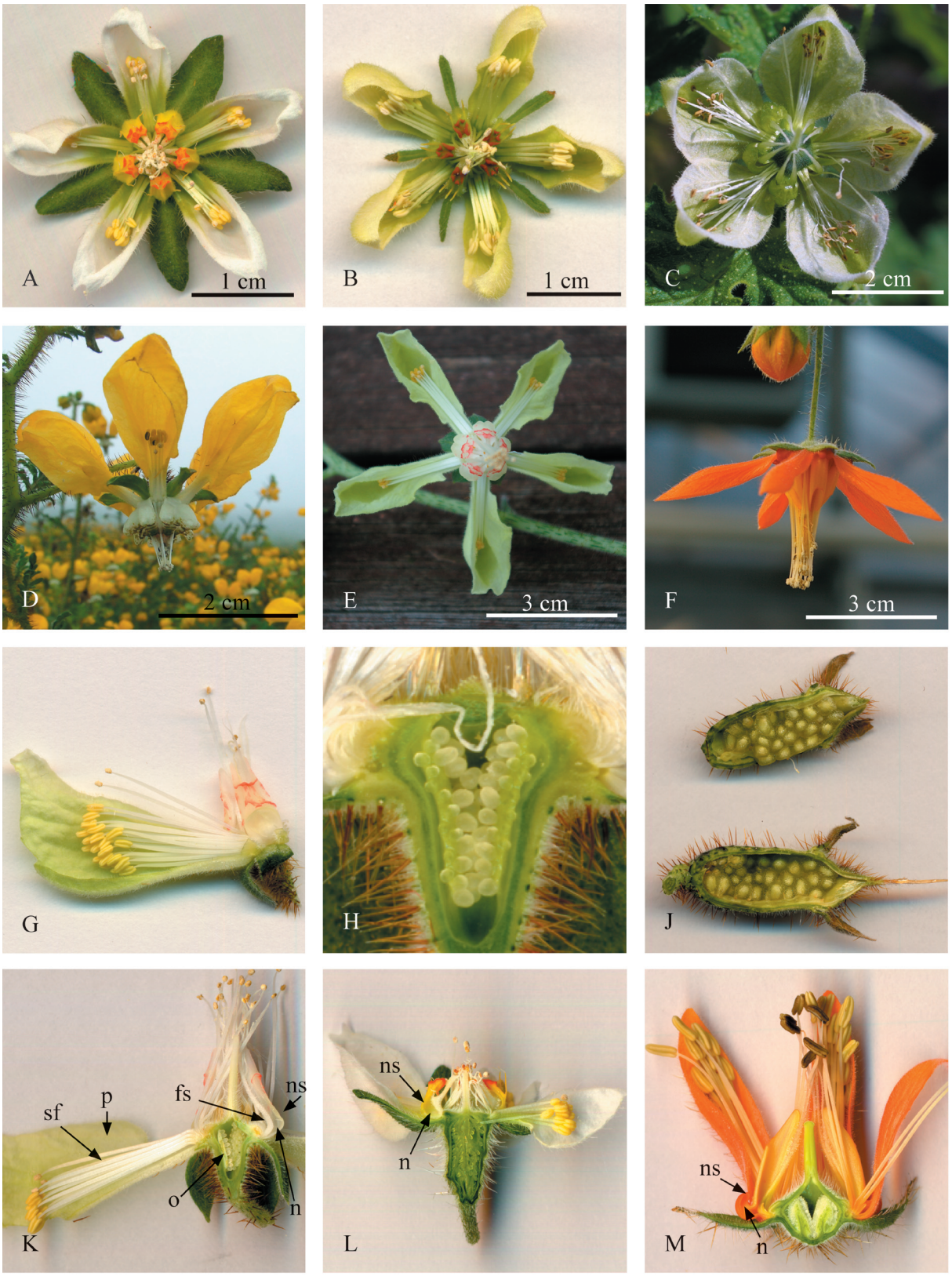

Figure 6.1: Floral morphology of species examined. A-F: Flowers, note the staminodial complexes and mature stamens in the center and the immature stamen bundles reflexed into the petals. A: *Caiophora arechavaletae*, B: *C. stenocarpa* , C: *C. carduifolia*, D: *Nasa urens*, E: *N. macrothyrsa* and F: *N. ranunculifolia*. G–M: *N. macrothyrsa*. G: reflexed stamen fascicle. H. Longitudinal section through a receptacle, note the numerous ovules of the two visible (out of three) placentae. J: Longitudinal section through an almost mature fruit with numerous, fully developed seeds. K–M. Longitudinal sections through the flowers. of K: *N. macrothyrsa*, L: *C. arechavaletae* and M: *N. ranunculifolia*. Note the different sizes and orientations of the flower structures that guide the flower visitors to nectar and pollen. fs: free inner staminodes, n: nectar reservoir, ns: nectar scales, o: ovules, p: petal, sf: stamen fascicle.

family (Keeler, 1981; Cocucci & Sersic, 1998; Ackermann & Weigend, 2006; Weigend & Gottschling, 2006). Weigend *et al*. (2010) offer a functional explanation for thigmonastic pollen presentation in Loasaceae subfam. Loasoideae. They showed that stamen movements can also be triggered in flowers of *Nasa macrothyrsa* (Urb. & Gilg) Weigend, but their main pollinators, male and female carpenter bees of *Neoxylocopa lachnea* (Moure 1951), only take up nectar (on these flowers, Weigend *et al*. 2010). Since nectar is secreted continuously, the small amount of nectar received at a second visit (soon after a previous visit) will likely encourage the bees to move on to different inflorescences or plants. Weigend *et al*. (2010) argue that rapid thigmonastic stamen presentation in Loasoideae is thus advantageous, irrespective of pollinator species, since it ensures the availability of pollen in flowers recently depleted of nectar and thus makes optimal use of the "hungry pollinators": Flies, bees and hummingbirds that visit flowers that have been recently visited and where nectar is depleted (but fresh pollen is already available) are more likely to move on to different plants than to other flowers on the same plant (Cresswell, 1990; Irwin & Brody, 1998; Biernaskie *et al*., 2002; Johnson *et al*., 2004; Jersákova & Johnson, 2006). Accordingly, rapid pollen presentation will likely increase male fitness, irrespective of the degree of pollinator specialization and irrespective of whether the flower visitors collect pollen, or nectar, or both.

Pollen presentation theory (PPT, Percival, 1955; Lloyd & Yates, 1982; Harder & Thomson, 1989; Thomson 2003; Castellanos *et al*., 2006) argues that pollen release in plants may be adjusted to the frequency of flower visits in order to optimize male function. A selection for increased male fitness in hermaphrodite flowers may favour a protracted period of pollen donation with pollen released in minor amounts by dispensing or packaging (Harder & Thomson, 1989). Thomson *et al*. (2000) and Castellanos *et al*. (2006) also show that pollen release is more rapid in bird-pollinated taxa of *Penstemon* than in bee-pollinated taxa, which release pollen more gradually. It is assumed that this mechanism evolved in order to make optimal use of the less frequent visits by hummingbirds and their larger carrying capacity. However, based on the same model system, it has also been shown that – surprisingly – bumblebees and hummingbirds removed similar amounts of pollen per visit and had similar pollen transfer efficiencies per visit (Castellanos *et al*., 2003). Loasoideae have an unusual degree of control over pollen dispensation, since they actively react to pollinator visits by presenting new stamens. They thus represent an ideal model system for investigating the pollen presentation theory, since – unlike most plants – they should be able to adjust the number of anthers presented per unit of time to optimize pollen carryover. Loasoideae have three main pollinator groups (short-tongued and long-tongued bees and hummingbirds) and differ in their selfing ability (Ackermann & Weigend, 2006). It has been shown that nectar amount and composition is strongly correlated to pollinator group (Ackermann & Weigend, 2006), but nothing is known about possible differences in the amount of pollen offered by the flowers respectively the P/O ratios in different species, nor whether there are differences in their stamen movement patterns.

The present study wants to investigate floral behaviour and rewards in relation to pollination mode and breeding system. Six different species from two genera of Loasoideae are here studied. Three species of *Caiophora* and three species of *Nasa* were chosen, each representing either a different pollination mode and/or a different breeding system. We obtained data on selfing ability, seed weights, petal length (as proxy for floral size), anther thigmonasty, pollen and nectar, and the P/O ratios where calculated.

We specifically address the following questions:

1. How do the investigated species differ in their breeding systems?

2. Do all species have thigmonastic pollen presentation and do the rates of anther and pollen presentation differ between species?

3. What are the P/O ratios of some Loasaceae with different pollination modes and does the thigmonastic pollen presentation permit reduced P/O ratios?

4. Are there differences in the P/O ratios between the two large genera of Loasaceae with thigmonastic pollen presentation?

5. Is the (active and highly efficient) pollen collection by short tongued bees in *C.stenocarpa*, *C. arechavaletae* and *N. urens* offset by higher P/O ratios?

6. Is there a correlation between thigmonastic anther movement and pollination mode?

7. What type and amount of nectar, and how much sugar is offered by the flowers?

8. What is the overall correlation between the different pollination systems and plant resource investment in pollen and nectar?

6.2 Materials & Methods

Plant material and cultivation. Observations on the flowers and sampling of all species were made on cultivated plants in the greenhouses at the Institut für Biologie, Freie Universität Berlin. Seeds collected on field trips to Peru and Brazil were used to raise plants in the greenhouse [vouchers: *Caiophora stenocarpa*: M. Ackermann 758 (BSB, HUSA, USM) *Caiophora arechavaletae*: M. Weigend 9330 (B, F, M, MO); *Caiophora carduifolia*: M. Ackermann 762 (BSB, HUSA, USM); *Nasa urens*: M. Weigend & H. Förther 97/542 (BSB); *Nasa macrothyrsa*: M. Weigend et al. 7471 (B, HUT, M, USM); *Nasa ranunculifolia*; seed set-data: Henning & Brokamp 5 (B, USM), all other datasets: Henning & Schulz 25 (B, USM)]. Seeds were sown into standard seedling soil (Compo Sana®96). Seedlings were singled out into 5 cm clay pots as soon as the cotyledons were fully developed, and then repotted into successively larger clay pots (potting soil: 2 parts mature leaf compost, 1 part peat, fertilized with a mixed inorganic/organic fertilizer (Garten- and Gemüsedünger, ASB Grünland, H. Aurenz GmbH) and basalt powder (Neudorffs Urgesteinsmehl®100, W. Neudorff GmbH KG). Flowering took place from November to April, when the plants were kept in the greenhouses with artificial lighting (12h, high pressure sodium lamps: Philips SON-T AGRO® 104 400W) and temperatures of 20–25°C during the day and 10–15°C at night (closely resembling the temperature regimes in the natural habitats).

Self-pollination. Selfing ability of the species was investigated by marking individual flowers and then leaving them either unmanipulated, or hand-pollinating them with dehisced anthers from other flowers of the same plant (geitonogamous selfing, *C. stenocarpa*, *C. carduifolia*, *N. urens*, *N. macrothyrsa* & *N. ranunculifolia* – greenhouse experiments), or leaving them for open-pollination (*C. arechavaletae* – field experiments, see Schlindwein & Wittmann, 1997b). Subsequently fruit/seed set was recorded. In the greenhouse cultivation took place under pollinator exclusion (winter). Flowers of *C. arechavaletae* were bagged with gauze (Schlindwein & Wittmann, 1997b).

Stamen movement . Stamen movement was triggered by slightly bending all five nectar scales outwards with a dissecting needle and thus imitating a pollinator visit (*C. carduifolia*, n=10 flowers; control: n=5 all other species, n=20 flowers; control: n=10; control = autonomous movement without stimulation of the floral scale). Details of the methodology are provided in Weigend *et al.* (2010).

Seed weight. Thousand seed weights were calculated by counting out 100 seeds with a fine paint brush, weighing them with a Sartorius® R2000D lab balance and multiplying the results with 10 to extrapolate the weights of 1000 seeds (Weigend *et al*. 2004).

Nectar measurements. The entire amount of nectar present in each flower was extracted by inserting micro-capillaries into the floral scales (micro capillaries: 1- and 2-mL Microcaps; Drummond Scientific Co., Broomall, PA, USA; 5, 10 and 25-mL Duran Ringcaps®; Hirschmann Laborgeräte, Eberstadt, Germany). Nectar was harvested twice from each floral scale within 5 min to obtain the full amount of nectar. Brix measurements were then made with a handheld refractometer (neoLab-Handrefraktometer Universal; 10–80% Brix). Mean values and standard deviation were calculated. Data for *Nasa macrothyrsa* were taken from Weigend *et al*. (2010).

Pollen/Ovule ratios. The number of anthers per flower was counted in 10 fully developed flowers per species. Then the number of pollen grains per flower was determined by counting the number of grains per anther. Ten mature but still undehisced anthers of mid-anthetic flowers were carefully cut off and transferred into 1.5 ml Eppendorf tubes. After adding 50 µl glycerol and 50 µl distilled water to each tube, the tubes were carefully mixed (5 min), using a laboratory vortex (Heidolph®, Reax 1). Afterwards, the tubes were placed in an ultrasonic bath (Bandelin®, Sonorex RK 52) for another 20 minutes, to ensure that all pollen grains were released and evenly dispersed. Glycerol was added to increase viscosity of the suspension in order to ensure that the pollen grains stay evenly dispersed and do not start to sediment at the bottom of the tube. The tubes were vortexed again right before the subsample was removed for counting with a hemocytometer (Chamber-depth: 200µm, Type: Fuchs-Rosenthal, Marienfeld®). The number of particles per area can here be translated into a defined volume and then extrapolated for the whole sample (total volume in which the pollen of an anther was suspended). The grid consists of 16 squares of 1 mm^2 each. Five of these squares where chosen randomly before the first samples were transferred onto the hemocytometer. For each of the 10 samples all pollen grains within

these five squares where counted and the mean number was used to calculate the number of grains in the stock solution.
Ovules were counted by opening the ovary with a longitudinal cut using a razorblade. Each ovary contains three placentae with numerous ovules (Fig. 6.1 H). Depending on the number of ovules the content of all placentae or a single placenta was counted and the total number of ovules was then obtained by multiplication. In *Nasa macrothyrsa* (Fig. 6.1 H) ovaries and ovules where large enough to be counted using a stereo microscope (Wild®, Herbrügge). Digital imaging of isolated placentae was necessary for counting in species with large ovaries and high numbers of small ovules (e.g. *C. arechavaletae*, *N. ranunculifolia*, Fig. 6.1 L, M). The placentae were therefore dissected longitudinally and both parts where scanned on a flatbed scanner (Epson Perfection 1640 SU) at 9600 dpi and the magnified ovules where counted using Photoshop (Photoshop® CS3, Adobe Inc.). The P/O ratio was calculated as the number of pollen grains divided by the number of ovules per flower.

Statistics, graphs and figures. An overall comparison of the anthers per flower and pollen grains per anther and per flower presented thigmonastically and number of ovules per flower and pollen grains per anther for all species is given in the appendix. A test for normal distribution for each dataset was followed by the respective significance-tests (t-test if a normal distribution is given, Mann-Whitney-U-Test in case of a non-normal distribution of the data). Datasets were prepared using Microsoft Excel®, statistical analyses was performed with SPSS® for Windows®. Graphs (Fig. 6.2–6.5) were created using SigmaPlot® and graphically processed using Adobe® Illustrator® CS. Photographs for figure 6.1 were graphically enhanced and labelled using Adobe® Photoshop® CS and arranged using Adobe® InDesign® CS.

6.3 Results

Data on anther numbers per flower, pollen number per anther, flower size, nectar data and seed weights are summarized in Table 6.1. A statistical comparison of the differences of ovules per flower and pollen grains per anther throughout all species examined is provided in the appendix (supplementary data C1, Tab. 6.3). Floral morphology, flower size and the immature and dehisced stamens are illustrated in Fig. 6.1. Flowers are smallest in *Perditomorpha* pollinated *C. arechavaletae* (petals c. 13 mm, Fig. 6.1 A, L) and largest in hummingbird pollinated *N. ranunculifolia* (petals c. 36 mm, Fig. 6.1 F, M). Within genera, floral display follows a gradient from small flowers in species that are pollinated by short-tongued bees, over medium-sized flowers that are pollinated by long-tongued bees, to large flowers in ornithophilous taxa (Tab. 6.1; Fig. 6.1).

Pollination. No fruit set was observed in the absence of pollinators and without hand pollination in perennial *Caiophora stenocarpa*, *C. carduifolia*, *Nasa macrothyrsa* and *N. ranunculifolia*. Conversely, hand-pollination led to 48–100% fruit set in all taxa examined (Tab. 6.1, Fig. 6.1 J). Annual *C. arechavaletae* and *N. urens* showed high fruit set with all flowers developing into fruits in the absence of any flower visitors (Tab. 6.1).

Tab. 6.1: Summarized data (mean and standard deviation) on the pollinator, seed- and fruit set, petal length as a proxy for flower size, pollen and ovule numbers, the resulting P/O ratios, nectar data and seed weight of all species examined.

species	***Caiophora arechavaletae***	***Caiophora stenocarpa***	***Caiophora carduifolia***	***Nasa urens***	***Nasa macrothyrsa***	***Nasa ranunculifolia***
pollinator	short-tongued bees (*Perditomorpha pampeana*)	short-tongued bees (Colletids)	hummingbirds	short-tongued bees (colletids & megachilids)	long-tongued bees (*Neoxylocopa lachnea*)	hummingbirds
fruit set selfing in %	**100** (10/10)	**0** (0/10)	**0** (0/10)	**100** (10/10)	**0** (0/10)	**0** (0/10)
fruit set hand-pollinated in %	**100** (10/10)	**80** (8/10)	**100** (10/10)	**100** (10/10)	**85** (25/28)	**48** (16/33)
flower size (petal length, mm)	13.73 ± 1.1 (n = 11)	14.91± 1.3 (n = 10)	20.00 ± 1.26 (n = 11)	22.00 ± 0.77 (n = 11)	30.09 ± 2.12 (n = 11)	36.45 ± 2.16 (n = 11)
no. of anthers per flower	105.1 ± 4.61 (n = 10)	115.9 ± 9.67 (n = 10)	90.5 ± 3.63 (n = 10)	71 ± 5.71 (n = 40)	98 ± 10.14 (n = 42)	111.7 ± 22.08 (n = 6)
pollen grains per anther	16,945 ± 2581 (n = 20)	14,420 ± 5569 (n = 10)	11,920 ± 3387 (n = 10)	10,290 ± 3999 (n = 10)	11,590 ± 1640 (n = 10)	79,740 ± 9177 (n = 10)
ovules per flower	585 ± 132 (n = 10)	191 ± 27 (n = 10)	245 ± 33 (n = 6)	142 ± 15 (n = 10)	130 ± 33 (n = 10)	1247 ± 249 (n = 5)
app. pollen production per flower (rounded)	1.8×10^6	1.7×10^6	1.1×10^6	0.73×10^6	1.14×10^6	8.9×10^6
P/O ratio	**3044**	**8750**	**4409**	**5145**	**8743**	**7144**
nectar (µl/flower)	**0.43** ± 0.33 (n = 26)	**5.65** ± 2.38 (n = 53)	**59.97** ± 28.43 (n = 149)	**2.50** ± 2.10 (n = 63)	**75.13** ± 28.98 (n = 64)	**56.1** ± 23.97 (n = 24)
sugar concentration %	55.58 ± 8.36	32.66 ± 14.13	14.84 ± 4.50	55.0 ± 13.51	32.39 ± 10.61	31.63 ± 8.29
sugar amount (mg/flower)	0.23 ± 0.18	1.77 ± 1.04	8.45 ± 4.35	1.43 ± 1.31	24.04 ± 11.51	17.92 ± 9.05
thousand seed weight (g)	0.3115 (n = 3 x 100)	0.3297 (n = 3 x 100)	0.202 (n = 3 x 100)	0.188 (n = 3 x 100)	1.2946 (n = 3 x 100)	0.3146 (n = 3 x 100)

Tab. 6.2: Summary of the data (mean and standard deviation) obtained for stamen movement and pollen presentation in all species examined and a statistical comparison of the significance of the triggered movement and the amount and timing of the pollen presentation for each species.

		Caiophora arechavaletae	*C. stenocarpa*	*C. carduifolia*	*Nasa urens*	*N. macrothyrsa*	*N. ranunculifolia*
anthers presented autonomously	5 min	0.34 ± 0.68	0.14 ± 0.41	0.48 ± 0.82	0.125 ± 0.34	0.48 ± 0.46	0.38 ± 1.3
	30 min	1.84 ± 0.59	1.02 ± 0.49	1.12 ± 0.45	0,75 ± 0.71	2.44 ± 0.725	1.78 ± 0.83
anthers presented thigmonastically	5 min	1.86 ± 1.78	0.69 ± 0.42	0.44 ± 0.71	1.19 ± 1.29	1.46 ± 1.4	0.97 ± 1.47
	30 min	2.94 ± 0.75	1.58 ± 1.01	1.6 ± 0.5	2.39 ± 0.69	3.39 ± 0.8	3.28 ± 0.93
pollen presented autonomously	5 min	5,761 ± 11,658	2018 ± 5,834	5,722 ± 9,810	1,286 ± 3,509	5,563 ± 5331	30,446 ± 104,635
	30 min	31,179 ± 10,116	14,708 ± 7,164	13,350 ± 5,412	7,717 ± 6997	28,280 ± 8,400	142,096 ± 66,258
pollen presented thigmonastically	5 min	31,547 ± 30,179	13,843 ± 15,598	5,245 ± 8,404	12,214 ± 13,305	16,921 ± 16,176	77,565 ± 117,561
	30 min	49,818 ± 12,624	22,784 ± 6518	19,072 ± 6,008	24,593 ± 7,141	39,290 ± 9,204	261,692 ± 74,508
P-values: autonomous vs. thigmonastic stamen movement		**0.011**	**0.025**	0.567	**0.005**	**0.001**	**0.000**
Pollen presentation (thigmonastically vs. autonomously) accelerated by		**59.8%**	**54.9%**	**42.9%**	**319 %**	**38.9%**	**84.2%**
Percentage of pollen thigmonastically presented within first 5 min. after stimulus		**63.3%**	**60.8%**	**27.5%**	**49.7%**	**43.1%**	**29.6%**
Percentage increase in the amount of pollen presented after 5 min. thigmonastically versus autonomously		**541%**	**490%**	**90%**	**950%**	**304%**	**255%**
Proportion of overall pollen/flower presented per 30 min autonomously vs. thigmonastically (rounded)		**1.8/2.8%**	**0.88/1.36%**	**1.24/1.76%**	**1.05/3.37%**	**2.5/3.5%**	**1.59/2.94%**
Staminate phase in hours autonomously vs. thigmonastically (calculated from stamens/flower and anther movement)		**28.6/17.9 h**	**56.8/36.7 h**	**40.4/28.3 h**	**47.3/15.3 h**	**20.1/14.5 h**	**31.4/17.03 h**

Stamen movement and pollen presentation. Data for anther/pollen presentation per time are presented in Figures 6.2–6.5 and Table 6.2. A statistical comparison of the differences of anthers and pollen grains presented per unit of time across the species studied is presented in the appendix (supplementary data C2, Tab. 6.4). Stamen number per flower ranges from 71 in *N. urens* to 116 in *C. stenocarpa*. Interestingly, the number of anthers per flower shows an opposite trend within the two genera. In *Nasa*, the colletid-pollinated *N. urens* has the fewest stamens and ornithophilous *N. ranunculifolia* has the largest number of stamens whereas in *Caiophora*, the ornithophilous *C. carduifolia* has almost 30% less stamens than the colletid-pollinated *C. stenocarpa*. Pollen number per anther ranges from c. 10,000 (*N. urens*) over c. 17,000 (*C. arechavaletae*) to nearly 80,000 (*N. ranunculifolia*). Pollen number per flower shows a parallel trend (since anther numbers and number of pollen/anther are similar), but are relatively similar in five species (ca. 0.73–1.8 × 10^6), with only *N. ranunculifolia* having a much higher value (c. 9 x 10^6).
All six species show autonomous stamen movement (Tab. 6.2, Fig. 6.2–6.5). Autonomous stamen movement varies between 0.13 stamens/5 min and 0.75 stamens/30 min in *Nasa urens* to 0.48 stamens/5 min and 2.44 stamens/30 min in *Nasa macrothyrsa* (Tab. 6.2, Fig. 6.2). Manipulation of the floral scales led to a significantly accelerated stamen presentation in all species examined with the only exception of *C. carduifolia* (Tab. 6.2, Fig. 6.3). All taxa, irrespective of pollination syndrome, provide fresh pollen after a pollinator visit and (likely) nectar depletion, but timing differs between taxa. Anther presentation is most rapid in *C. arechavaletae* (1.86 stamens/5 min, 2.94 stamens/30 min) and slowest in *C. carduifolia* (0.44 stamens/5 min, 1.6 stamens/30 min) (Tab. 6.2, Fig. 6.3). Due to the difference in pollen number per anther, the overall amount of pollen grains thigmonastically presented in the flower varies and is highest in *Nasa ranunculifolia* (Tab. 6.2, Fig. 6.4; 5 min: 77,565 ± 117,560, 30 min: 261,692 ± 74,507) and lowest in *C. carduifolia* (Tab. 6.2, Fig. 6.5: 5 min: 5,245 ± 8,404, 30 min: 19,072 ± 6,008). An increase in the amount of stamens presented in stimulated flowers between 39% and 85% is found in five of the six species. In *N. urens* pollen presentation is even more dramatically increased (by c. 319%). The percentage of pollen presented within the first five minutes after the manipulation also varies. The insect-pollinated taxa rapidly present a large proportion of the pollen ranging from 43 to 50% (*Nasa*) and 61 to 63% (*Caiophora*) of the overall pollen presented per interval. In ornithophilous *C. carduifolia* and *N. ranunculifolia* only 27.5 respectively 29.6% of the pollen grains presented thigmonastically are available shortly after scale manipulation.

Pollen and ovule numbers and the P/O ratio. Overall, the P/O ratios vary between the six species studied (3,044–8,750). Within the genera, variability is slightly larger in species of *Caiophora* (3,044–8,750) than in those of *Nasa* (5,145–8,743). Ovule and pollen numbers also vary widely between the species examined. (Tab. 6.1). The number of pollen grains/flower is similar in *Caiophora* spp. (1.1–1.8 Mio) and in *N. macrothyrsa* (1.1 Mio), but much higher in *N. ranunculifolia* (8.9 Mio) and considerably lower in *N. urens* (0.73 Mio). Ovule numbers are lowest in *N. macrothyrsa* and *N. urens* (130 resp. 142), higher in all *Caiophora* spp. (191 to 585) and highest in *N. ranunculifolia* (1,247).

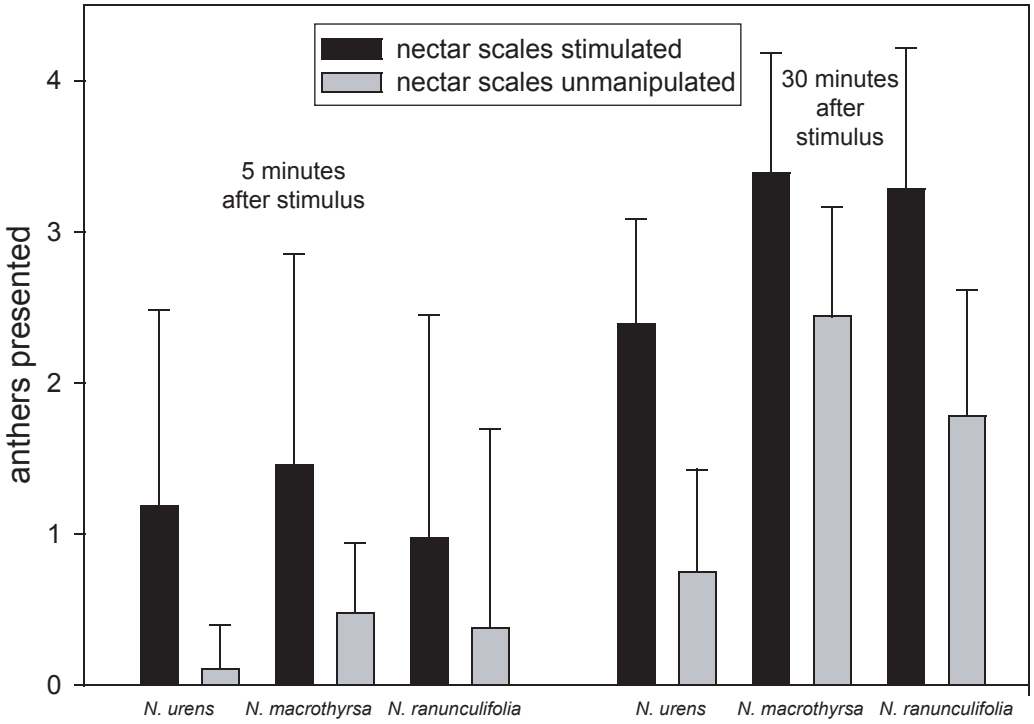

Figure 6.2: Number of anthers presented autonomously and thigmonastically in 5 min and 30 min intervals in *Nasa* spp..

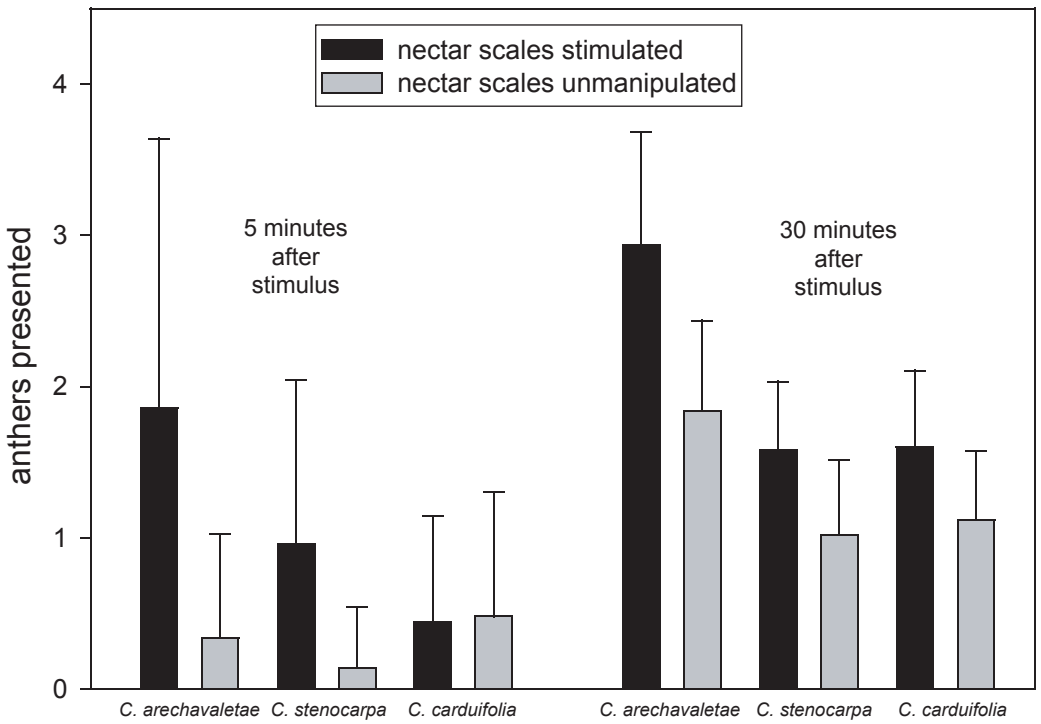

Figure 6.3: Number of anthers presented autonomously and thigmonastically in 5 min and 30 min intervals in *Caiophora* spp..

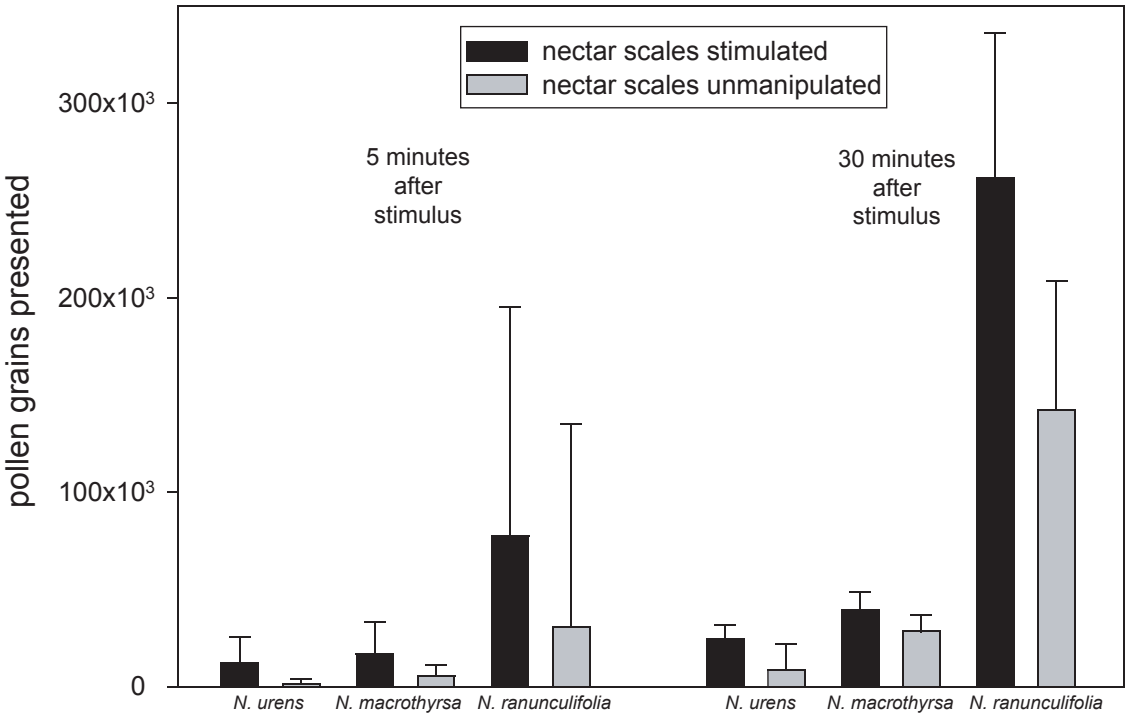

Figure 6.4: Total number of pollen grains presented in the center of the flowers autonomously and thigmonastically in *Nasa* spp. (calculated from anther numbers of Fig. 6.2 and number of pollen/anther of Tab. 6.1).

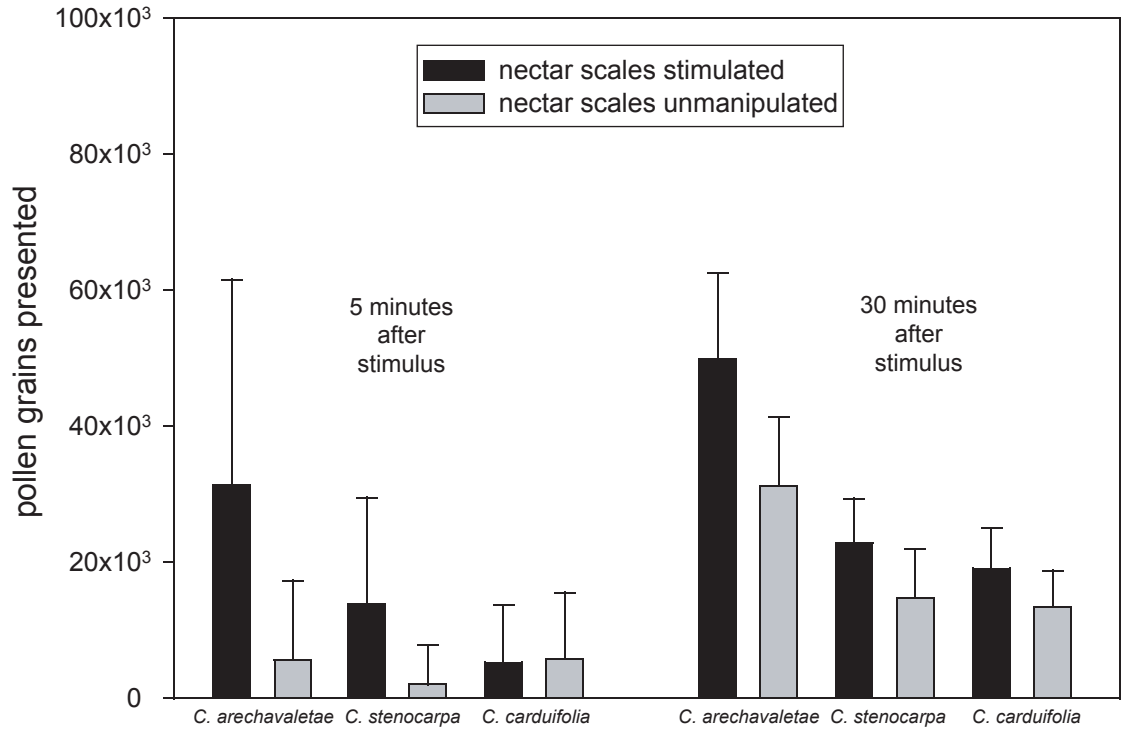

Figure 6.5: Total number of pollen grains presented in the center of the flowers autonomously and thigmonastically in *Caiophora* spp. (calculated from anther numbers of Fig. 6.3 and number of pollen/anther of Tab. 6.1).

Nectar. Small amounts of quite concentrated nectar are present in *C. arechavaletae* and *N. urens* (Tab. 6.1; 0.43 µl/flower 2.5 µl/flower and both 55% sugar, respectively). Dramatically higher amounts of more dilute nectar are found in *N. macrothyrsa* (75 µl/flower, 32% sugar) and *Nasa ranunculifolia* (56 µl/flower, 32% sugar). *Caiophora carduifolia* shows a similar amount of even more dilute nectar (60 µl/flower, 15% sugar) and *C. stenocarpa* shows a moderate nectar amount (5.65 µl/flower) with an intermediate sugar concentration (33% sugar). The overall sugar amount offered per flower is smallest in *C. arechavaletae* (0.23 mg), approx. 6–7 × as high in *Nasa urens* and *C. stenocarpa* (1.4 and 1.8 mg), almost 40 × as high in *C. carduifolia* (8 mg), 80 × as high in *N. ranunculifolia* (18 mg) and more than 100 × higher in *N. macrothyrsa* (Tab. 6.1; 24 mg).

6.4 Discussion

P/O ratios. Thigmonastic pollen presentation in small packages may represent a mechanism to increase male fitness (Weigend *et al.*, 2010), but the overall data set indicates that it does not make pollen transfer more efficient across pollination modes and mating systems: The complex floral behaviour of Loasaceae is here shown to be correlated with P/O ratios that are extraordinarily high in relation to the respective breeding systems. The P/O ratios of (facultatively xenogamous) *Caiophora stenocarpa* and *Nasa macrothyrsa* are higher than those documented for most obligately xenogamous plants in the literature (Cruden, 1977, 2000).

P/O ratios and breeding system. Neither anther number/flower, nor pollen number/anther, nor ovule number/flower increase parallel to flower size and all three characters appear to vary largely independently of flower size. Experimental data here presented show that the four perennial taxa *C. stenocarpa, C. carduifolia, Nasa macrothyrsa* and *N. ranunculifolia* are self compatible, but require pollinator activity for fruit set, confirming the correlation between breeding system and life history (Michalski & Durka, 2009). Annual *C. arechavaleta*e and *N. urens* are selfing, unless pollen is depleted before the carpellate phase. According to breeding system classifications the four perennial species would fall into the category "mixed mating" (Barrett *et al.*, 1996) or "facultatively xenogamous" (Cruden, 1977), whereas the annual species can be considered as "predominantly selfing" (Barrett *et al.*, 1996) or "facultatively autogamous" (Cruden, 1977). P/O ratios in the Loasaceae are thus extremely high for their respective breeding systems, which are generally characterized by much lower P/O ratios (facultatively autogamous – 168.5 ± 22.1; facultatively xenogamous – 796.6 ± 87.7; Cruden, 1977). P/O rates in Loasaceae lie well in, or even above the typical range of obligately xenogamous species (5859.2 ± 936.5, Cruden, 1977; 1,200–8,000, Cruden, 2000). Looking at the genera separately, the lowest P/O ratios are found in the two facultatively autogamous species (*C. arechalvaletae* and *N. urens*), as would be expected.

P/O ratios and pollination mode. The three species pollinated by short-tongued bees show wide variabilty in their P/O ratios, with low values in facultatively autogamous *N. urens* (5,145) and *C. arechavaleatae* (3,044) and very high values in facultatively xenogamous *C. arechavaletae* (8,750). Active pollen collection by short-tongued bees is thus apparently more than compensated for by facultative autogamy in the two annual species. The P/O ratios of short-tongued bee-pollinated *C. stenocarpa*, long-tongued bee pollinated *N.*

macrothyrsa and ornithophilous *N. ranunculifolia* are more similar to each other than those of congeneric *C. carduifolia* (hummingbird pollinated) and *C. stenocarpa* (short tongued bees), indicating that pollination mode has no, or at least no obvious effect on P/O ratios. The similarity of the P/O ratios of the species of *Nasa* pollinated by long-tongued bees respectively hummingbirds support findings of earlier works, indicating that the two pollinator groups are similar in pollen removal, loads and transfer (Castellanos *et al*. 2003). Castellanos *et al.* (2006) showed that long-tongued Bombus-pollinated *Penstemon* species have similar P/O ratios to hummingbird pollinated taxa. The data here presented indicate that this similarity may hold true for Loasaceae – although pollinated by other groups of long-tongued bees and hummingbirds. Studying the breeding system in the greenhouse may, however, represent an undue simplification: Observations in the natural habitat show that the foraging strategy of short-tongued, pollen collecting *Perditomorpha pampeana* on *C. arechavaletae* is efficient enough to keep the flowers continuously empty of pollen (Schlindwein & Wittmann, 1997) and may therefore enforce outcrossing in this facultatively autogamous species in nature. Pollen depletion by pollen-collecting short-tongued bees can also be assumed in *N. urens* and *C. stenocarpa*, but detailed observations in the natural habitat are still wanting. Conversely, pollen depletion is unlikely in the taxa pollinated by long-tongued bees and hummingbirds (i.e., *C. carduifolia, N. macrothyrsa* and *N. ranunculifolia*), which do not actively collect pollen. In summary, P/O ratios in the species investigated are correlated with life form and mating system, a correlation with pollination mode is not obvious.

Pollen presentation and pollination mode. Thigmonastic (triggered) stamen movement is higher than autonomous stamen movement in all species and differs significantly from the respective autonomous movement observed in all taxa investigated, with the only exception of *C. carduifolia*. The overall amount of pollen presented in the 30-min interval after the stimulus ranges between 19,000–50,000 and shows no obvious correlation to breeding system or pollination syndrome: Bird-pollinated *C. carduifolia* presents less than half as much pollen as colletid-pollinated *C. arechavaletae*, while ornithophilous *N. ranunculifolia* presents dramatically more pollen (c. 262,000) than any other species. Also, facultatively autogamous *C. arechavaletae* presents more than twice as much pollen as facultatively xenogamous *C. stenocarpa*. The situation is similarly complex with regards to the percentage of the pollen presented per unit of time of the overall pollen present in the flower (Tab. 2). The plants autonomously present 0.88 to 2.5% of the overall pollen per 30 min, respectively 1.36 to 3.5%/30 min when stimulated. Hence, in most taxa the thigmonastic stamen movement leads to an increase in anther/pollen presentation between 39 to 84%. However, *N. urens* shows a dramatical difference between autonomous presentation (1.05%/30 min) and thigmonastic presentation (3.37%/30 min), which means an increase in anther/pollen presentation by c. 320%. The rate of stamen presentation translates into the length of the staminate phase, i.e., the overall time of pollen presentation (Tab. 2). Assuming flower visits in 30-min intervals (roughly those found in Schlindwein & Wittmann, 1997 and Weigend *et al.*, 2010), reduces the duration of the staminate phase by approximately one third in most taxa (compared to non-visited flowers), but possibly more than two thirds in *N. urens*. Autonomous and thigmonastic pollen presentation thus independently permit the flowers to adjust both the absolute amount of pollen presented after a stimulus and the

percentage of overall pollen present per flower and overall duration of the staminate phase, exerting an extraordinary extent of control over pollen dispensation.

Overall pollen presentation after a stimulus shows no correlation to pollination mode. However, the speed of pollen presentation within the 30-Min interval correlates to pollination mode and with it the amount of pollen presented briefly after a previous visit compared to autonomous movement: Presentation is particularly rapid in taxa pollinated by pollen collecting short-tongued bees (*Caiophora stenocarpa* and *C. arechavaletae*, c. 5 × as much pollen after 5 Min., *Nasa urens* c. 10 ×), but less pronounced in taxa pollinated by long-tongued bees (*N. macrothyrsa*, c. 3 ×). Bird-pollinated *C. carduifolia* shows a (non-significant) negative effect and *N. ranunculifolia* a moderate increase (c. 2.5 × as much pollen). It has been shown that flower visits in some bee-pollinated taxa of Loasaceae occur at very high frequencies (*C. arechavaletae*: Schlindwein & Wittmann, 1997; *N. macrothyrsa*: Weigend *et al.*, 2010). Conversely, hummingbirds typically make more sporadic flower visits at longer intervals (Mayfield *et al.*, 2001; Castellanos *et al.*, 2003; Kay & Schemske, 2003). The different speeds of pollen presentation may thus correlate to the typical visitation frequencies of different pollinators and/or the additional role of pollen as floral rewards in taxa pollinated by short-tongued bees. Visitors, irrespective of the time lapse after a previous visit, will all find similar pollen loads in the center of the flower and will potentially obtain similar pollen loads.

The "cost" of different pollination modes and breeding systems. The amount of pollen per flower, the P/O ratios, nectar volume and concentration and the rates of autonomous and thigmonastic pollen presentation all differ remarkably between the species here investigated. The amount of nectar and its concentration are closely parallel to the corresponding pollinators and nectar per flower varies by a factor of c. 175 and the amount of nectar sugar/flower by a factor of c. 100 between the species here studied. Nectar offerings correlate closely to pollinator groups, with short-tongued bees beeing the "cheapest" pollinators and long-tongued bees and hummingbirds beeing much more expensive, as described in Ackermann and Weigend (2006). Ackermann and Weigend (2006) argue that nectar characters evolve rapidly and under little phylogenetic constraint. Conversely, pollen varies much less, with pollen/flower differing by a factor 12 and P /O ratios by a factor of 3 between the species here studied. P/O ratios of *Nasa* were on average slightly higher (5,145–8,743) than in *Caiophora* (3,044–8,750). Overall, *Nasa macrothyrsa*, pollinated by long-tongued bees, has by far the most "expensive" pollination mode (very high P/O ratio, highest amount of nectar sugar), followed by bird-pollinated *N. ranunculifolia* (second highest P/O ratio, second highest amount of sugar). *Nasa urens*, pollinated by short-tongued bees, has both much lower nectar sugar and lower P/O ratios than the other two species of *Nasa*. Short-tongued bee-pollinated *C. stenocarpa* has the highest P/O ratio among the species studied and this may here indeed reflect pollen loss through actively collecting colletid bees. Ornithophilous *C. carduifolia* is, in turn, the *Caiophora* species with the largest amount of nectar sugar, but has only an intermediate P/O ratio compared to the other two species (Tab. 1), whereas facultatively autogamous *Caiophora arechavaletae* has both the lowest P/O ratio and the by far lowest amount of nectar sugar among the species studied. Within the genera examined, facultatively autogamous, short-tongued bee-pollinated taxa have the by far most economical pollination mode. It is somewhat surprising that P/O ratios very

much less between taxa than nectar production and nectar sugar. This is likely due to the fact that selection acts directly upon nectar as the primary reward and a factor determining pollinator preferences and their flower constancy, whereas P/O ratios are subject to a complex suite of selective forces, which may not always have the same effect (Burd, 2011). It has also been argued, that P/O ratios may indeed be under a phylogenetic constraint, as previously shown for *Penstemon* (Castellanos *et al.*, 2006) and *Pedicularis* (Yang & Guo, 2007). However, a much larger and phylogenetically explicit data set on Loasaceae would be required to distinguish differences in selective pressures on the evolution of pollen and nectar characters from a possible phylogenetic constraint on the evolution of P/O ratios.
Pollen presentation theory argues that pollen release in plants may be adjusted to the frequency of flower visits in order to optimize male function (Lloyd & Yates, 1982; Harder & Thomson, 1989; Thomson, 2003; Castellanos *et al.*, 2006). The data here presented certainly provide one of the most astonishing examples in the plant kingdom: Depending on pollination mode flowers can accelerate pollen presentation 3–10 × in the first 5 min after a flower visit, ensuring the optimal use of the "hungry pollinators" (i.e., those finding the flowers empty of nectar) under high visitation rates.

7. Total control – pollen presentation and floral longevity in Loasaceae are modulated by light, temperature and visitation rates*

Abstract

Anther movements can be observed in several plant families and may be understood as a mechanism influencing pollen presentation and increasing outbreeding success of hermaphroditic flowers via optimized male function. In poly- and proterandrous Loasaceae a quite complex floral behaviour, centered on thigmonastic stamen presentation has evolved. Stamen movement in these flowers is triggered by flower visitors. Additionally, an autonomous stamen-movement occurs in the absence of flower visitors. So far little is known about the regulation of anther movements.

In this study we experimentally analyzed the factors regulating both autonomous and thigmonastic stamen movements in eight species from four genera of Loasaceae. Autonomous and thigmonastic stamen movements are found to be positively influenced by light and temperature and come to a virtual standstill in the dark and at low temperatures (12° C). Pollen presentation is thus discontinued during periods where pollinators are likely not active. Overall stamen presentation increases with increasing flower age. Contrary to expectation, there seems to be now geometrical correlation between the floral scale stimulated and the stamen fascicle reacting, indicating that the stimulus is transmitted over the receptacle and stamen maturation dictates which and how many stamens react. Thigmonastic stamen presentation is dramatically accelerated compared to autonomous movement (3–37 times), indicating that the rate of stamen maturation can be adjusted to different visitation schedules. Flowers can react relatively uniformly to stimulation down to stimulation intervals of 10–15 min., consistently presenting comparable numbers of stamens in the flower ca. 5 min. after the stimulus and can thus keep the amount of pollen presented more or less constant even under very high visitation frequencies of 4–6 visits/h. Thigmonastic pollen presentation dramatically reduces the overall duration of the staminate phase (to 1/3rd in *N. macrothyrsa*). Similarly, the carpellate phase is dramatically reduced after pollination, down to 1 d from 4 d. Overall flower longevity is thus reduced by more than 2/3rds under high visitation rates (< 3 d versus 10 d under visitor exclusion) and depleted and pollinated flowers are rapidly removed from the pool. Complex floral behaviour in Loasaceae thus permits a near-total control over pollen dispensation schedules and floral longevity of the individual flower by an extraordinary fine-tuning to both biotic and abiotic factors.

* Henning T, Weigend M. in prep. Total control - pollen presentation and floral longevity are modulated by light, temperature and visitation rates. To be submitted to *Plos One*.

7.1 Introduction

The lack of obvious movements is one of the most striking features differentiating plants from animals. Plant movements have therefore particularly fascinated scientists across the ages (Darwin, 1875; Braam, 2005). Some plant movements have been studied in detail, such as the trap mechanisms of *Dionaea muscipula* and *Aldrovanda vesiculosa* (Sibaoka, 1969), or the leaf movements of *Mimosa* (*Mimosa pudica*: Weintraub, 1952) and *Albizzia* (*Albizzia julibrissin*: Satter *et al.*, 1973). It has been reported that plant movements are influenced by temperature (in *Dionaea muscipula* and *Aldrovanda vesiculosa*: Sibaoka, 1969; *Mimosa pudica*: Weintraub, 1952; *Albizzia julibrissin*: Satter *et al.*, 1973) and light (solar tracking, Satter & Galston, 1981), or the lack thereof (nyctinasty, e.g. in *Espeletia schultzii*: Smith, 1974). Plant movements can have a range of functions, most frequently serving to protect the plants from damage, but sometimes functioning in trap mechanisms or in pollination ecology. In pollination ecology stamen movements are the most common type: Thigmonastic anther movements in *Berberis* (Berberidaceae) were amongst the first active floral movements to be reported in the scientific literature (Linnaeus, 1754), but anther movements have since also been reported from a range of taxa such as *Sparmannia* (Malvaceae, Scott 1903), *Portulaca* (Portulacaceae, Jaffe *et al.*, 1973), *Nigella* (Ranunculaceae, Weber, 1993) and *Opuntia* (Schlindwein & Wittmann, 1997a). In all these cases the movement of the anthers is triggered by a mechanical stimulus and usually leads to the simultaneous outward or inward movement of all stamens present, maximizing pollen deposition on visiting insects. In his review on "rapid plant movements", Sibaoka (1969) ascribes the thigmonastic response of several plant species (*Berberis* spp., *Sparmannia africana*, *Mahonia aequifolium*, *Helianthemum vulgaris*, *Portulaca grandiflora*) to a stimulation of the filaments by flower visitors.

Thigmonastic stamen movements generally concern all stamens of the androecium and are caused by stimulation of the stamen (filament) itself. The response is thus fairly simple and shows little plasticity. Exceptions are found in *Berberis*, where the number of stamens moved depends on the strength of the mechanical stimulus (Lebuhn & Anderson, 1994) and *Opuntia*, where the direction of the movement depends on the site of the stimulus (Grant *et al.*, 1979).

The South American Loasaceae subfam. Loasoideae (Fig. 7.1) show a much more complex type of floral behaviour. The flowers have complex staminodial complexes, so-called nectar scales, into which nectar is secreted. These staminodial complexes are flexible, and are bent outwards by the nectar harvesting flower visitors (Fig. 7.1 J). The polyandric androceum of these plants consists of numerous, initially reflexed stamen bundles of sequentially maturing anthers (Fig. 7.1 D, G) alternating with the staminodial complexes. Mature anthers present their pollen in the center of the flower by autonomously moving into an upright position (Fig. 7.1 B, G, K-L, Urban, 1886, 1892). Anther dehiscence occurs immediately before or during the stamen movement. As first reported by Schlindwein & Wittmann (1992) the manipulation of the floral scales in *Caiophora arechavaletae* (Urb.) Urb. & Gilg, leads to a direct reaction in the stamen bundles, causing the thigmonastic movement of individual stamens from a reflexed position into the center of the flower. These thigmonastic stamen movements have since been reported from a range of taxa in Loasaceae subfam. Loasoideae (genera *Blumenbachia*, *Caiophora*, *Loasa*, *Nasa* - Harter

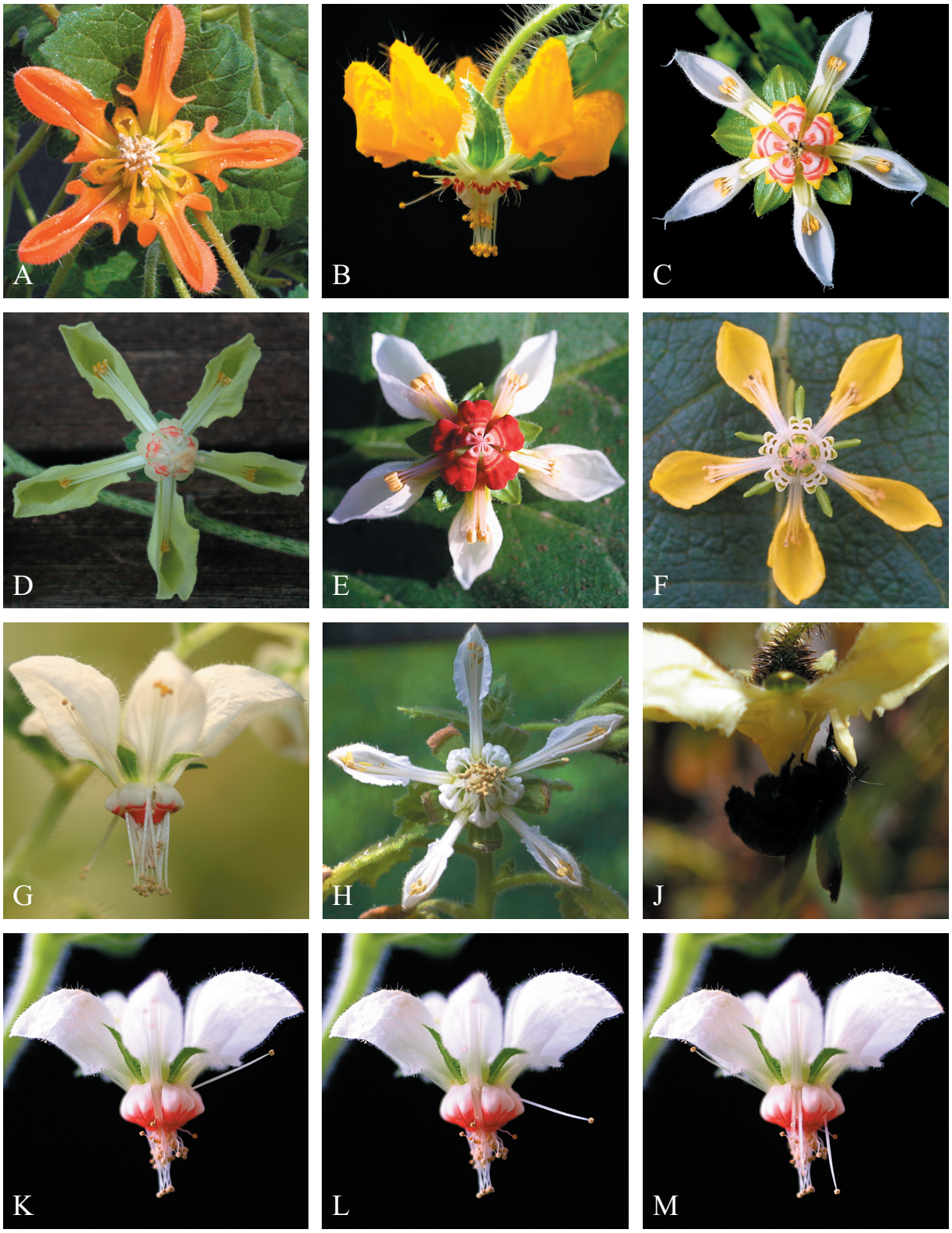

Fig. 7.1: A-H: Flowers of the species of Loasaceae subfam. Loasoideae used for this study. A. *Caiophora cirsiifolia*, B. *Loasa insons*, note the stamens on their way into the center of the flower, C. *Nasa dyeri* subsp. *australis*, D. *Nasa macrothyrsa*, E. *Nasa moroensis*, F. *Nasa urens*, G. *Nasa vargasii*, H. *Presliophytum heucheraefolium*, J. *Neoxylocopa lachnea* Moure, harvesting nectar on *Nasa macrothyrsa*, note the outwards bended nectar scale, K-L: sequence of the stamen movement in *Nasa vargasii*. A mature stamen leaves the boat-shaped petal (K), passes the gap between two nectar scales (L) and reaches the center of the flower to present its pollen (M).

et al., 1995; Schlindwein & Wittmann, 1992, 1997b; Schlindwein, 2000; Weigend *et al.*; 2010; Wittmann & Schlindwein, 1995). Unlike in other flowers, there is spatial separation between the place of manipulation (the floral scale) and the place of the reaction (the stamen bundle). It is unclear how the stimulus is communicated from the floral scale to the anthers that show the thigmonastic reaction and it is also unknown whether the stimulus from an individual staminodial complex is communicated to all five stamen bundles of the flower or affects only the two stamen bundles neighbouring the floral scale that has been manipulated.

The function of thigmonastic anther presentation in Loasaceae has been explained in the framework of the Pollen Presentation Theory (PPT - Lloyd & Yates, 1982; Harder & Thomson, 1989; Thomson, 2003; Castellanos *et al.*, 2006): PPT argues that pollen release in plants is adjusted to the frequency of flowers visits in order to optimize male function. A selection for increased male fitness in hermaphrodite flowers may favor a protracted period of pollen donation with pollen released in minor amounts by dispensing or packaging (Harder & Wilson, 1994). This probably increases the likelihood of siring outcrossed offspring. The rapid presentation of fresh pollen in the flower shortly after a flower visit and nectar depletion likely reduces self-pollen deposition and increases pollen export (Weigend *et al.*, 2010). Stamen movement in Loasaceae subfam. Loasoideae is present in several genera of the group and differs from stamen movements in other plant families: In Loasaceae, individual stamens in the polyandrous androecium are triggered by the manipulation of a separate floral organ (the floral scale) and this mechanism leads to numerous separate stamen movements across the staminate phase, i.e., in the course of several days. Recently, it has been shown that thigmonastic anther movement is present across pollination modes in Loasoideae in taxa pollinated by short-tongued bees, long-tongued bees and hummingbirds (Henning *et al.*, 2012, submitted - Chapter 6). Weigend *et al.* (2010) therefore detached the floral behaviour from the behaviour of individual pollinators and offer a general functional explanation: Rapid thigmonastic stamen presentation is advantageous, irrespective of pollinator species, ensuring the availability of pollen in flowers recently depleted of nectar and thus makes optimal use of the "hungry pollinators": Flies, bees and hummingbirds that visit flowers that have been recently visited and where nectar is depleted are more likely to move on to different plants (Biernaskie *et al.*, 2002; Cresswell, 1990; Johnson *et al.*, 2004; Jersákova & Johnson, 2006; Irwin & Brody, 1998) and thus are more likely to sire outcrossed offspring. Rapid, thigmonastic pollen presentation ensures that these valuable "hungry pollinators" are dusted with pollen. Theoretical works on pollen dispensing schedules (Harder & Wilson, 1994; LeBuhn & Holsinger, 1998) "predicted" such a phenomon as the "unlikely case in which the number of visits to be received is higly predictable and the individual plant possess the ability to adjust pollen-dispensing schedules accordingly". If so, they underscored: "plant fitness may increase substantially". Pollinator behaviour and especially visitation rates may vary throughout the time a flower is presenting pollen. Visitation frequency may change depending on weather conditions, daytime or competition among pollinators. It is currently unknown in how far thigmonastic and autonomous stamen movements are dependent on light or temperature: If autonomous stamen movement was independent of light and temperature, then a large number of mature stamens would be autonomously presented during the night. As pollinator activity follows a diurnal rhythm, the passive

presentation of pollen via the autonomous stamen movement as known for Loasaceae (Weigend *et al.*, 2010) would represent a "waste" of pollen during the night. In their natural habitat (mostly the Andes of Southern America), Loasaceae underlie a distinct nightime temperature drop. Pollinator activity is generally limited by thermal constraints, which in turn are (especially in the High Andes) closely correlated to solar radiation (Willmer, 1983). The dramatic fluctuations between day- and nighttime temperatures at high altitudes and the rapid sunset and sunrise close to the equator mainly determine pollinator activity. It would therefore be expected that these abiotic factors affect stamen movement. However, so far it is unclear to which degree flowers of Loasaceae are able to adjust pollen presentation to these different external factors.

Another interesting aspect is the possible effect of thigmonastic stamen presentation on the overall duration of the male phase and flower longevity. Flower longevity is a crucial aspect of pollination biology: Withdrawal of sucessfully pollinated flowers respectively flowers whose pollen load has been depleted or which have been successfully pollinated from the overall flower stock ensures the concentration of pollinator activity on those flowers still requiring their attention (Primack, 1985; Van Doorn, 1997; Webb & Littleton, 1997). Flower longevity reflects the ecological, genetical and physiological constraints a plant is subject to during flowering (Primack, 1985). Flower longevity is often considered as being mainly influenced by an either prolonged (not pollinated) or shortened (successfully pollinated) stigmatic phase. Ashman & Schoen (1996) revealed a correlation between the duration of floral attraction and pollinator frequency independent of successful pollination. In Loasoideae, these two factors are likely to be directly linked. Since pollinator frequency (visitation rates) varies (Weigend *et al.*, 2010) and the pollen presentation (staminate phase) within the anthesis is accelerated by the pollinator, it would be expected that the entire staminate phase can be shortened or extended accordingly. This in turn would directly affect flower longevity, since the female phase follows immediately upon the male phase.

There are thus a range of open questions in the context of (autonomous and thigmonastic) stamen movement in Loasaceae, which we want to address in this study:

1. Do light and temperature affect autonomous and thigmonastic stamen movement?

2. Can the stamen movement be adjusted to different visiting intervals?

3. Is the accelerated pollen presentation associated with an accelerated stamen maturation and the overall duration of the staminate phase thus influenced by the number of (simulated) flower visits?

4. Is the duration of the carpellate phase influenced by pollination?

5. Does the age of the individual flower (during the staminate phase) influence the number of stamens presented under constant visitation rates?

6. Is there a spatial relationship between the nectar scale manipulated and the stamen bundle from which an anther/anthers are presented?

7.2 Materials & Methods

Plant material and cultivation. Observations on the flowers were made on cultivated plants in the greenhouses at the Institut für Biologie, Freie Universität Berlin. Seeds collected on field trips to Peru had been used to raise plants of these species in the greenhouse [vouchers: *Caiophora cirsiifolia*: M. Weigend 7559 (BSB), Fig. 7.1 A; *Loasa insons* Weigend 8724 (BSB), Fig. 7.1 B; *Nasa dyeri* ssp. *australis*: N. Dostert 98/80 (M, USM), Fig. 7.1 C; *Nasa macrothyrsa*: M. Weigend *et al*. 7471 (B, M, USM, HUT), Fig. 7.1 D, J; *Nasa moroensis*: M. Weigend 8424 (BSB, HUT, USM) Fig. 7.1 E; *Nasa urens*: M. Weigend & H. Förther 97/542 (BSB) Fig. 7.1 F; *Nasa vargasii*: M. Weigend 5463 (B, HUT, M, USM) Fig. 7.1 G, K-L; *Presliophytum heucheraefolium*: M. Weigend 7691 (BSB, USM) Fig. 7.1 H]. Plants were raised and cultivated as described earlier. For detailed information see: Weigend *et al*. 2010.

Flower longevity and artificial pollination. Flowers were individually marked and the different phases of the anthesis were noted three times a day. Pollination was carried out by transferring pollen from entire, freshly deshisced anthers of flowers from different plants to the stigmatic surfaces.

Stamen movement. Stamen movement patterns were investigated for five species out of three different genera, namely: *Loasa insons*, *Nasa dyeri* ssp. *australis*, *Nasa macrothyrsa*, *Nasa urens* and *Presliophytum incanum*. Mid-anthetic flowers (e.g. Fig. 7.1 G) were individually marked and mature stamens that already had moved into the center of the flower were cut off one hour prior to the experiment.

Autonomous stamen movement. Flowers were prepared as described above and kept at different temperatures and light exposures to examine the characteristics and regulatory mechanisms of the autonomous stamen movement. Autonomous stamen movement overnight (13.5 hours) was investigated for 22°C (= day temperature in the greenhouse) and 12°C (expected night temperature in the field) respectively. Flowers were kept in the dark and autonomously presented stamens were counted the next morning before sunrise. The data for the autonomous movement during the day at 22°C were calculated from the data of flower longevity (under pollinator exclusion) made at the same time in our greenhouses. Therefore, the total number of stamens per flower was divided by the duration of the staminate phase minus the number of stamens that are expected to be autonomously moved at night (12°C, 13.5 h).

Thigmonastic stamen movement. Stamen movement was triggered by slightly bending all five nectar scales outwards with a dissecting needle and thus imitating a pollinator visit (cf. Fig. 7.1 J). Stamens were counted after they reached an upright position. In all experiments, the stimulus was given in five consecutive intervals irrespective of interval length. Only flowers in the middle staminate phase (see below, "flower age") were used in this experiment.

*Regular visitor frequency/30 minute interva*l. The timing of experimental visits to flowers should reflect the natural visitation rate (Harder & Wilson, 1994). Accordingly five consecutive 30 minute intervals between stimuli were chosen, based on field observations indicating an average interval between two visits to individual flowers of ca. 25 minutes (Weigend *et al.*, 2010). These experiments were carried out on all species.

Divergent visitor frequencies. Five additional stimulation intervals were chosen, imitating different visitor abundancies. For *Nasa macrothyrsa* three datasets (30-, 60-, 180-min. intervals) are compared to examine low visitation rates. For the 60-min. interval flowers were manipulated every hour for 10 consecutive hours. The experiment was carried out one day and the influence on the duration of the staminate phase was calculated from total stamen number. For the very low visitor frequency (180-min. interval) flowers were manipulated three times a day at 9.00 am, 12.am and 3 pm, respectively, over the entire staminate phase of the flowers. Three other species out of two different genera of Loasaceae were used to investigate the effect of high visitation rates (10-, 15-, 20-min. intervals). The latter three species [(*Loasa insons*, *Nasa dyeri* ssp. *australis* (*Nasa triphylla* – group Dostert & Weigend, 1999) and *Nasa urens* (*Nasa poissoniana* – group, Henning & Weigend, 2009b)] are all rather distantly related, entomophilous taxa. They share the same basic flower morphology and pollination system and the data here presented are thus representative for a large proportion of Loasaceae subfam. Loasoideae. The short intervals were tested to investigate to which degree stamen presentation can be accelerated under high visitation rates.

Location of the response relative to the stimulus. Five different species were examined in this respect, namely: *Nasa moroensis*, *N. macrothyrsa*, *N. vargasii*, *Caiophora cirsiifolia* and *Presliophytum heucheraefolium*. The flowers were prepared as described above. The position of the scales in comparison to the stamen-fascicles was noted on a flower diagram. A single nectar scale was chosen randomly and stimulated for a total of six times at 30-min. intervals. The number of moving stamens reacting from each fascicle was recorded.

Flower age. Flowers were prepared and manipulation was carried out as above with a 30 –min. interval between stimulations. Prior to the experiment, flowers were sorted into four categories (n=10 flowers each) relative to the stage of the staminate phase. 1. beginning - flowers with ≤ two stamens already presented; 2. early - 2–10 stamens presented, 3. middle > 10 stamens presented and > 10 stamens still reflexed into the petals; 4. late - ≤ 10 stamens still hidden in the petals.

Statistics. All statistics were done with R (version 2.13.0). To compare the stamen movement of flowers in different staminate phases, a one-factorial ANOVA followed by a Tukey HSD post-hoc test was conducted. Normal distribution and homogeneity of the data were tested by the Shapiro-Wilk Normality Test and the Bartlett Test of Homogeneity of Variances, respectively. In the case of stamen movement vs. light, temperature and stimulus intervals (dataset *Nasa macrothyrsa*, Tab.7.1 Fig. 7.2) and the datasets on the different visiting intervals (*Nasa urens*, *Loasa insons*, *Nasa dyeri* ssp. *australis* Tab. 7.2, Figs. 7.4-7.6),

the underlying assumptions of the ANOVA were not met due to unequal variances. Here, a fitted linear model using generalized least squares using the function "gls" of the package "nlme" (Pinheiro *et al.*, 2011), including the formula "weights = varIdent(1)" (Zuur *et al.*, 2009), was applied.

7.3 Results

Autonomous and thigmonastic stamen movement dependent on light and temperatur. Fig. 7.2 compares the autonomous and thigmonastic stamen presentation in flowers of *Nasa macrothyrsa* depending on temperature and light. Treatments and detailed results are summarized in Table 7.1. Flowers showed an autonomous stamen movement of 1.5 stamens/flower/h in the light at 22° C, but only of 0.8 stamens/flower/h in the dark at 22° C. In the dark and at 12°C, stamen movement almost comes to a standstill with 0.025 stamen/flower/h. Thigmonastic stamen movement was also higher (6.78 st./h) at 22° C in the light than in the dark at the same temperature (5.2 st./h). Autonomous and thigmonastic stamen movement are thus positively correlated to both light and temperature.

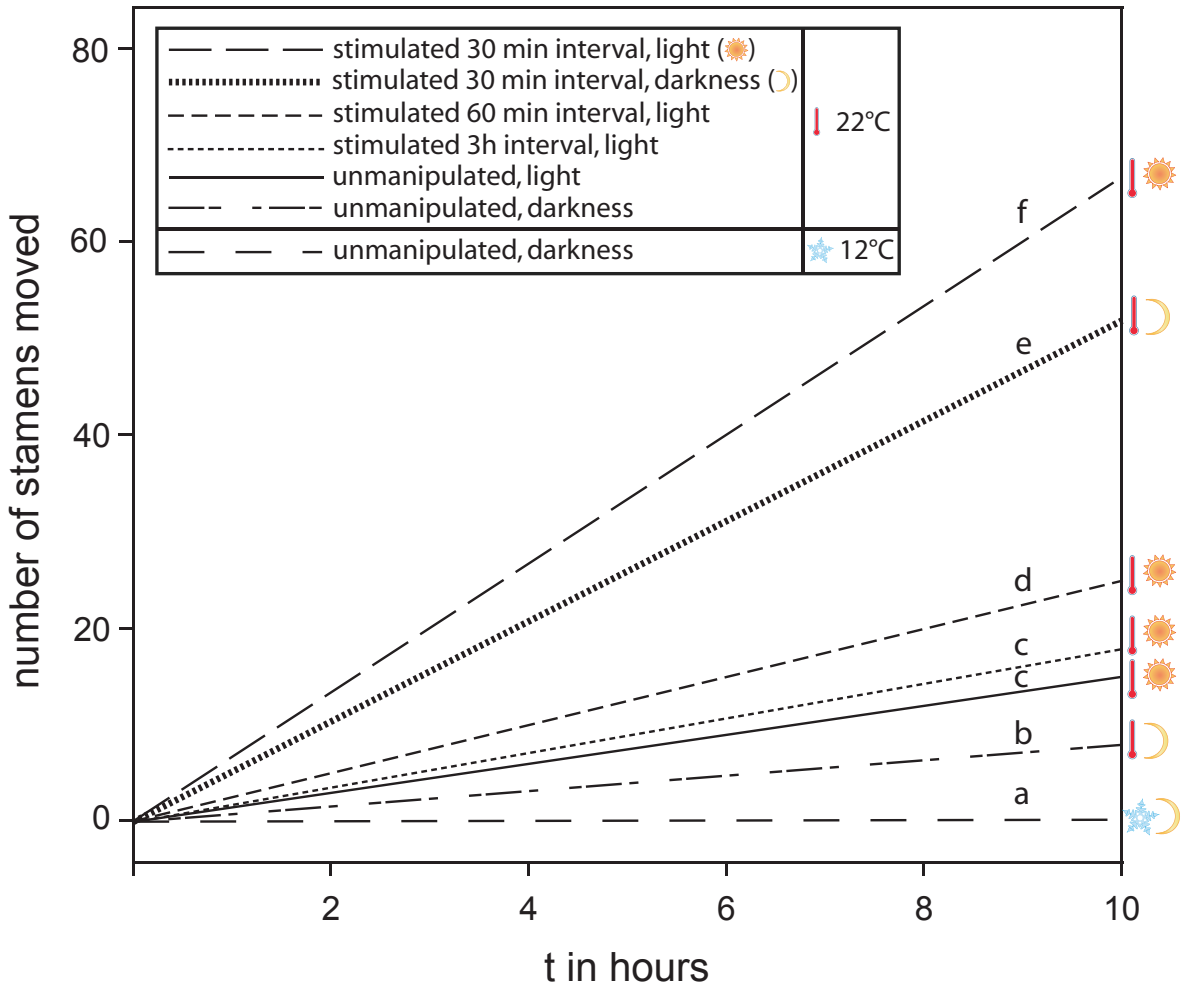

Fig. 7.2: Comparison of the actual rate of the stamen movement depending on different abiotic factors and visitation rates in *Nasa macrothyrsa*. Different letters indicate significant differences between the treatments in the number of stamens moved per unit time (gls model; $P < 0.05$).

Tab. 7.1: Stamen movement depending on stimulation and abiotic factors in *N. macrothyrsa*

nectarscales stimulated	duration of experiment in hours	stimulus interval in min	light	temperature in °C	n	average stamen-movement per h
yes	2.5	30	daylight	22	20	6.78 ± 1.58
yes	2.5	30	darkness	22	20	5.2 ± 1.27
yes	5	60	daylight	22	20	2.83 ± 0.24
yes	9	180	daylight	22	21	1.83 ± 0.13
no	2.5		daylight	22	42	1.54 ± 0.14
no	13.5		darkness	22	25	0.81 ± 0.31
no	13.5		darkness	12	26	0.025 ± 0.42

Visitation rates, thigmonastic stamen movement and the duration of the male phase. Three different scenarios were tested, imitating different visitation rates (Tab. 7.1, Fig. 7.2). Simulated flower visits at 3-h intervals only marginally increased stamen presentation to 1.83 ± 0.13 stamens/h compared to autonomous stamen presentation (1.542 ± 0.141, n = 42). Simulated flower visits at 1 h intervals nearly doubled stamen presentation compared to autonomous movement (to 2.83 ± 0,6 stamens/h). Visitation frequencies observed in the field are close to 30 min. (Weigend *et al*. 2010). Imitating these intervals leads to a presentation of 6,78 ± 1.59 stamens/h, i.e., more than four times the rate of autonomous stamen presentation. The flowers of *N. macrothyrsa* have ca. 100 stamens each (98 ± 10, n= 42) and the duration of the staminate phase depends on the number of stamens presented per unit time: Based on stamen presentation rates under different (simulated) flower visitation rates the duration of the staminate phase falls from ca. 6 d (6.255d ± 0.522, n = 55) in the absence of flower visitors to ca. 5 days (5.275d ± 0.419, n = 21) with three visits per day, to ca. 4 d (4.122 d, n = 21, calculated from the 60 min.-experiment conducted for 10 h) under hourly visitation. Under visitation frequencies of 30 min., i.e., close to those observed in the field, the staminate phase of the anthesis would be reduced to under 2 days (ca. 1.45 d, n = 20) (Fig. 7.3).

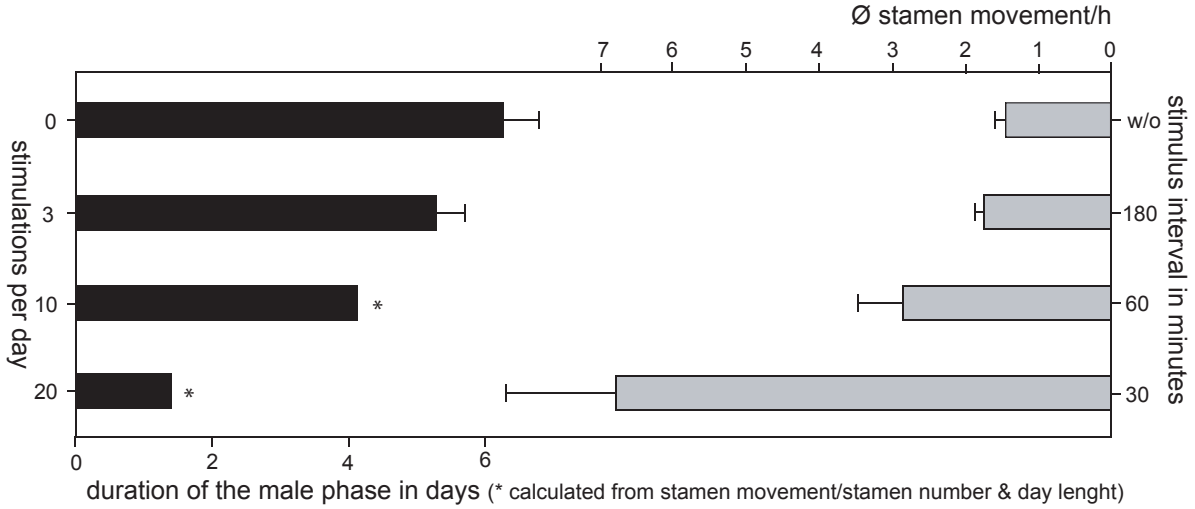

Fig. 7.3: Influence of different visitation rates (stimulus intervals) on the stamen movement rate and the corresponding length of the staminate phase in *Nasa macrothyrsa*.

Three species were studied with shorter visitation intervals of 10, 15, 20, 30, 60 min. They showed no clear statistically significant differences in the immediate response upon

Tab. 7.2: Average stamen movement values per flower at different stimulus intervals (bold: experimentally derived data, rest: calculated)

species	*Loasa insons*					*Nasa dyeri* ssp. *australis*					*Nasa urens*				
stimulus interval in minutes	10	15	20	30	60	10	15	20	30	60	10	15	20	30	60
stamens moved per hour	4.56	4.84	4.74	5.04	3.22	6.78	5.93	5.22	4.87	3.63	5.88	9.84	4.38	4.79	2.39
total amunt of stamens moved per stimulus	**0.76**	**1.21**	**1.58**	**2.52**	**3.22**	**1.13**	**1.48**	**1.74**	**2.44**	**3.63**	**0.98**	**2.46**	**1.46**	**2.39**	**2.39**
stimulus response (tot. –auton. movement)	0.49	0.8	1.03	1.7	1.58	0.55	0.61	0.57	0.69	0.13	0.96	2.42	1.41	2.32	2.24
response within first 5 minutes after stimulus	**0.48**	**0.43**	**0.71**	**1**	**0.81**	**0.61**	**0.87**	**0.93**	**1.02**	**0.71**	**0.9**	**2.28**	**0.98**	**1.19**	**1.19**
autonomous stamen movement	0.27	0.41	0.55	**0.82**	1.64	0.58	0.88	1.17	**1.75**	3.5	0.25	0.38	0.5	**0.75**	1.5

stimulation throughout, i.e., the number of stamens present 5 min. after stimulation is apparently independent of the visitation intervals in all three taxa studied (Tab. 7.2, Figs. 7.4-7.6). *Nasa urens* represents the only exception, with a significantly higher response when stimulated every 15 min (Fig. 7.4). As would be expected, the total amount of stamens moving per stimulus interval increases with interval length, with the exception of *N. urens*, which peaks at the 15 min. interval. The overall number of stamens presented per hour under different stimulation intervals is variable: In *L. insons* the response shows a slight (not significant) increase from 10 to 30 min., followed by a significant drop to the 60 min. interval (Fig. 7.5). In *N. dyeri* the shortest interval shows the strongest overall response (stamen movement per hour), which gradually falls with increasing interval

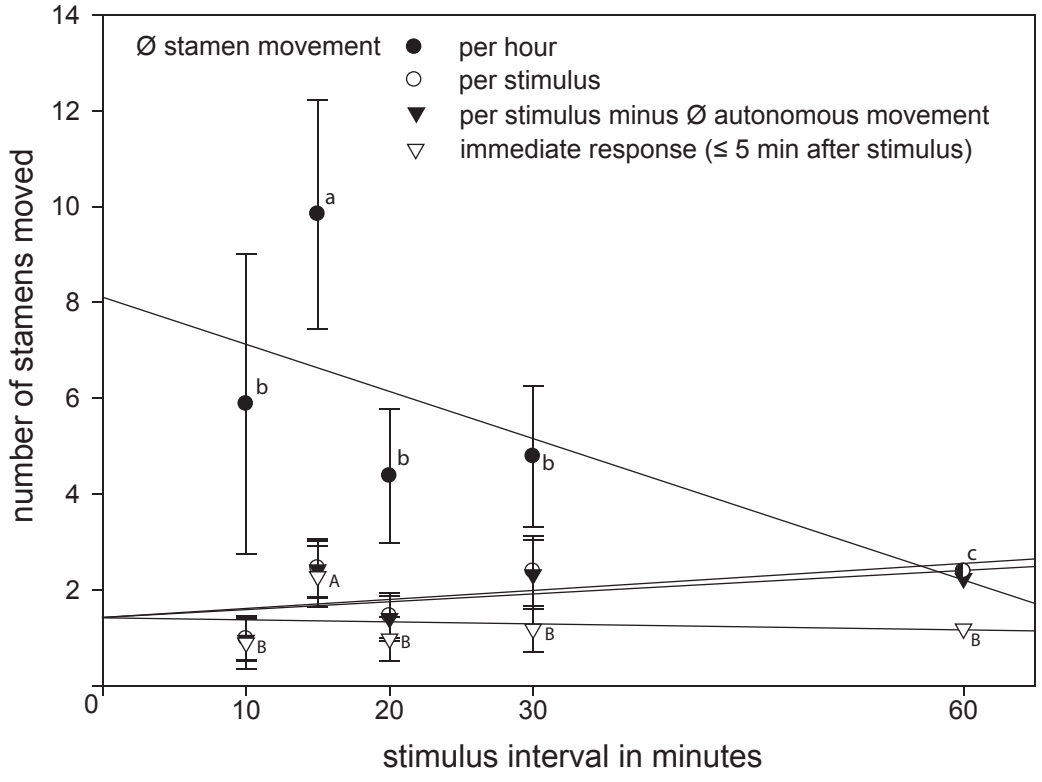

Fig. 7.4: Detailed stamen movement under different visitation rates (stimulus intervals) in *Nasa urens*. Different letters indicate significant differences between the amount of stamens moved (gls model; $P < 0.05$).

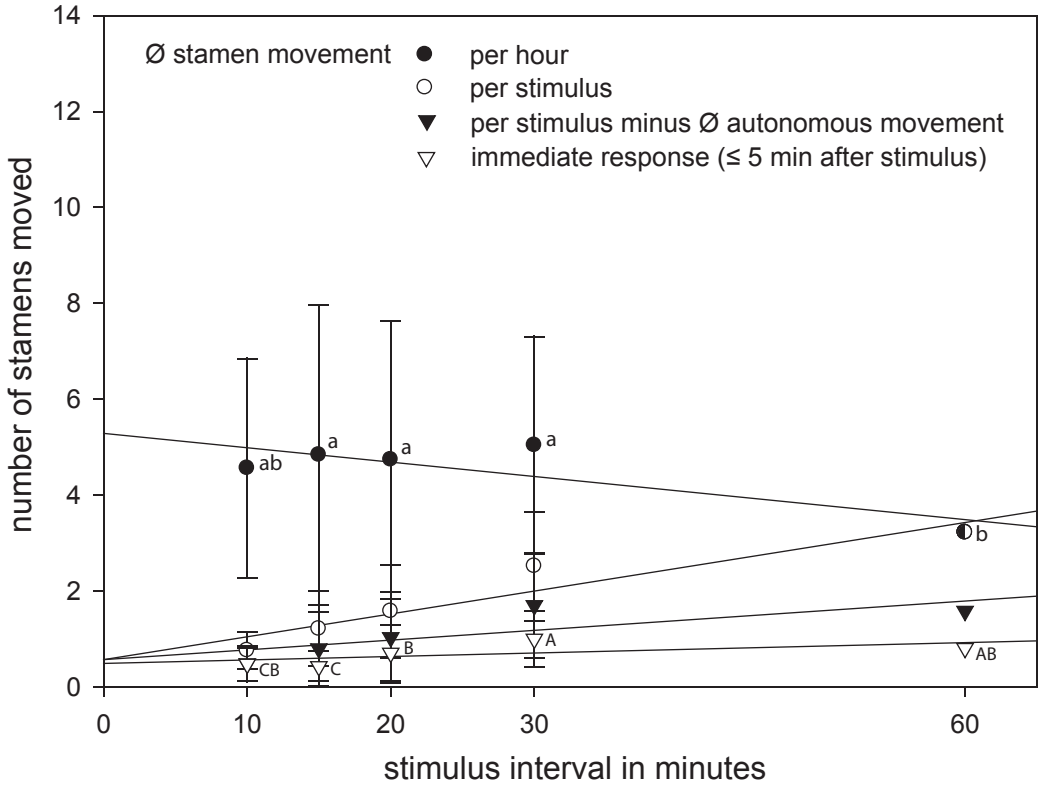

Fig. 7.5: Detailed stamen movement under different visitation rates (stimulus intervals) in *Loasa insons*. Different letters indicate significant differences between the amount of stamens moved (gls model; $P < 0.05$).

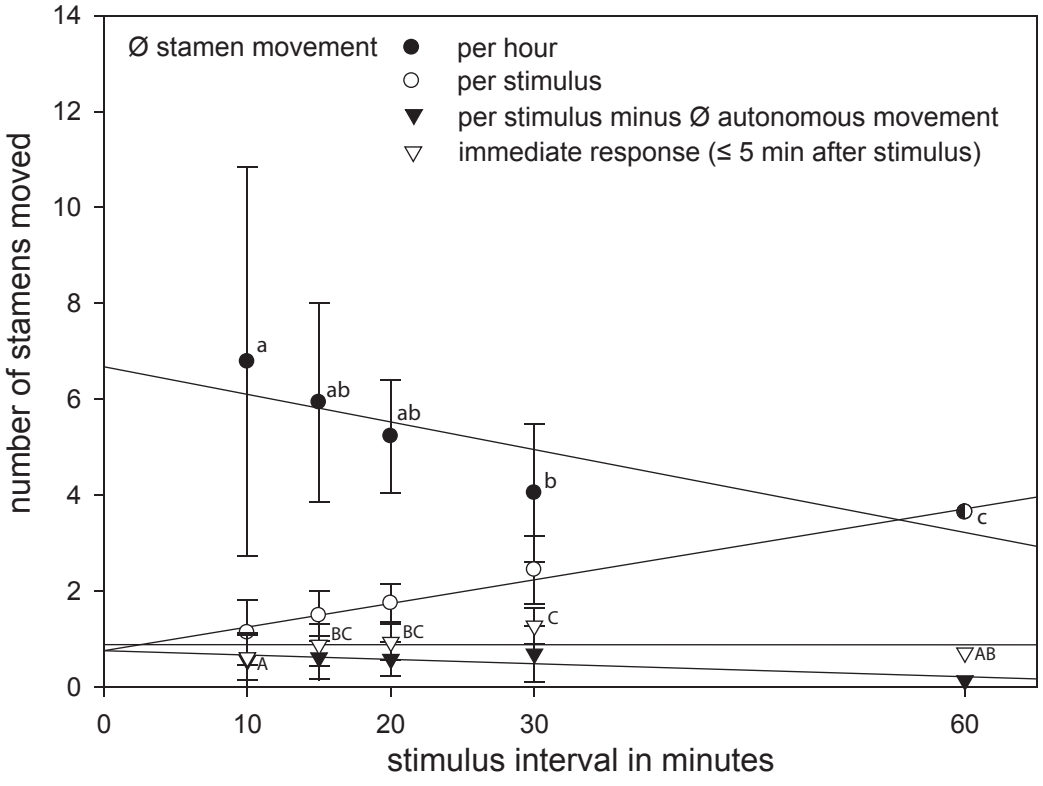

Fig. 7.6: Detailed stamen movement under different visitation rates (stimulus intervals) in *Nasa dyeri* subsp. *australis*. Different letters indicate significant differences between the amount of stamens moved (gls model; $P < 0.05$).

length (Fig. 7.6). In *N. urens* the 10 min. interval shows a strong response, the 15 min. interval the strongest response, which then falls moderately to 20 and 30 min. and again dramatically to 60 min (Fig. 7.4). A certain proportion of the stamen movements following a stimulus can be ascribed to the autonomous stamen movement that would normally occur in the corresponding time interval. Substracting autonomous movement from the overall movement leads to actual stamen movement caused by stimulation. In both *L. insons* and *N. urens* stimulation at intervals of 60 min. approximately doubles the overall number of stamens moving compared to the autonomous movement (Figs. 7.4, 7.5), in *N. dyeri* the stimulus makes no difference and stimulation every 60 min. has no effect on the overall number of stamens moving per unit time (Fig. 7.6). Conversely, shorter intervals dramatically increase stamen presentation per unit time – by a factor 17 (10 min., *N. dyeri*) respectively 37 (15 min. *N. urens*).

Thigmonastic stamen movement and flower age. The influence of the "age" of an individual flower on the thigmonastic stamen presentation was tested in *Nasa urens*. Fig. 7.7 and 7.8 show the results from the qualitative and quantitative side. The overall number of stamens "triggered" by a stimulus increases with flower age (Tab. 7.3, Figs. 7.7, 7.8): The youngest flowers moved by far the least, the oldest the highest number of stamen in response to a stimulus (Fig. 7.8). The speed of the response was more or less equal between flower ages, i.e., all flower ages show a rapid reaction within the first five minutes of the stimulus (Tab. 7.3, Fig. 7.7). This immediate response is followed by a drop of activity between 10 and 15 minutes after the stimulus. This drop is less pronounced in flowers that are at the very end of the staminate phase. Recently opened flowers show a rather consistent decrease in activity, reaching a minimum between 20 to 25 minutes after the stimulus. All but the youngest flowers show considerable additional stamen movement after the immediate response. Middle-staminate flowers show a second peak of stamen activity in the second half of the time after the stimulus. Flowers at the end of the staminate phase have an even course of stamen activity throughout the period of observation with a weaker drop after the immediate response and a uniformly high activity in the second half of interval. These data indicate that during the course of the staminate phase the rate of stamen maturation gradually increases.

Tab. 7.3: Thigmonastic stamen movement at different points of the staminate phase in *Nasa urens*.

stamens moved	status of the staminate phase			
	beginning	early	middle	late
Ø within the 1st 5 minutes	0.86	1.28	1.1	1.18
Ø per interval per flower	1.2	2	2.44	2.74
Ø in total (5 intervals) per flower	6	10	12.2	13.7

Pollination and duration of the carpellate phase. Under pollinator exclusion, the carpellate phase of *Nasa macrothyrsa* has a mean duration of > 4 days (4.1 d ± 0.7, n = 26). Under hand pollination flowers wilt rapidly and the corolla and androecium are shed. The mean duration of the carpellate phase is reduced to ca. 1 day (1.1 d ± 0.3, n= 27) after hand pollination. Thus, the carpellate phase is dramatically reduced under pollination and is terminated soon after a single successful pollination event.

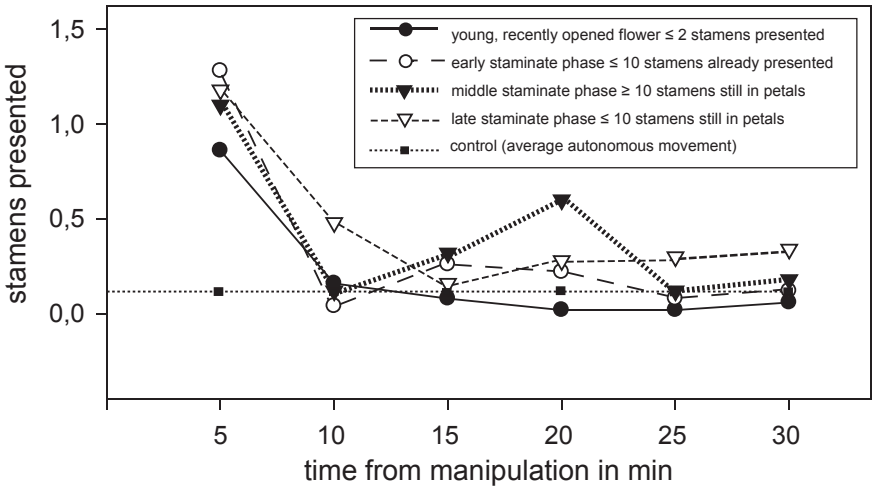

Fig. 7.7: Sequence of the stamen movement of *Nasa urens* at different stages of the age of an individual flower.

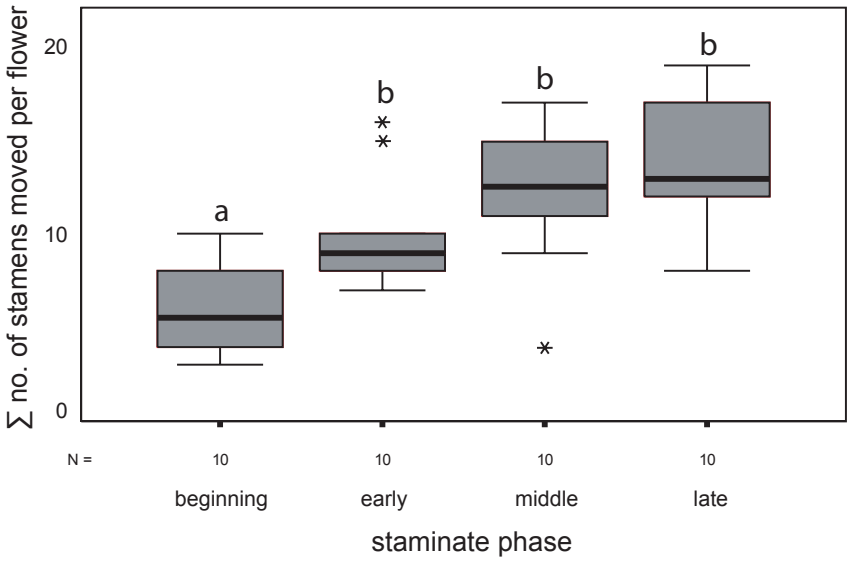

Fig. 7.8: Stem and leaf plot of data for the different flower ages incl. extremes (*) in *Nasa urens*. Different letters indicate significant differences between the amount of stamens moved (gls model; $P < 0.05$).

Spatial relationship between stimulus and thigmonastic response. Five species were examined in order to identify a possible spatial relationship between the trigger (i.e., the nectar scale manipulated) and the responding stamen(-s): *Nasa vargasii* (n = 51 flowers), *N. moroensis* (56), *N. macrothyrsa* (25), *Caiophora cirsiifolia* (23) and *Presliophytum heucheraefolium* (18). If all stamen fascicles reacted equally to the manipulation of a single nectar scale, irrespective of their spatial relationship to the scale, then it would be expected that 20 % of the overall thigmonastic stamens would originate from each of the five stamen bundles. Observed summary data were close to this expectation: In *N. vargasii* and *N. moroensis* 19 to 22 % of the responding stamens originated in the fascicles directly flanking the manipulated nectar scale (Fig. 7.9 A-C). In *Caiophora cirsiifolia* these fascicles showed a weaker response (17–18 %, Fig. 7.9 D). Conversely, in *P. heucheraefolium* these fascicles were the most active (25% both) (Fig. 7.9 E). However, while there are differences in the overall number of

Tab. 7.4: Sample sizes and *P*-values of the experiment on the stamen origin.

species	n	stamen total	*P*
Nasa vargasii	51	693	0.201
N. moroensis	56	573	0.244
N. macrothyrsa	25	281	0.947
Caiophora cirsiifolia	23	158	0.569
Presliophytum heucheraefolium	18	262	**0.011**

thigmonastic stamens from the different fascicles, a statistical comparison (Friedmann-Test) revealed no siginificant differences in all species of *Nasa* and *Caiophora* examinend (Tab. 7.4), i.e., the response of the thigmonastic stamens is independent of which nectar scale is stimulated. Only *Presliophytum* seems to have a spatialised stamen reaction ($P < 0.05$). A Wilcoxon-Test revealed that mainly those two fascicles neighbouring the manipulated scale differ significantly from the others. It has to be assumed, that these differences can only be found between the two neighbouring and two of the fascicles more distant to the stimulus. The activity of the third remote fascicle varies to a lesser extent. Thus there is no significant difference in the activity of this particular fascicle, compared to the fascicles flank to the scale manipulated. Furthermore, for *P. heucheraefolium* only 18 flowers were available and it cannot be excluded that this significance is an artefact of the smaller sample size.

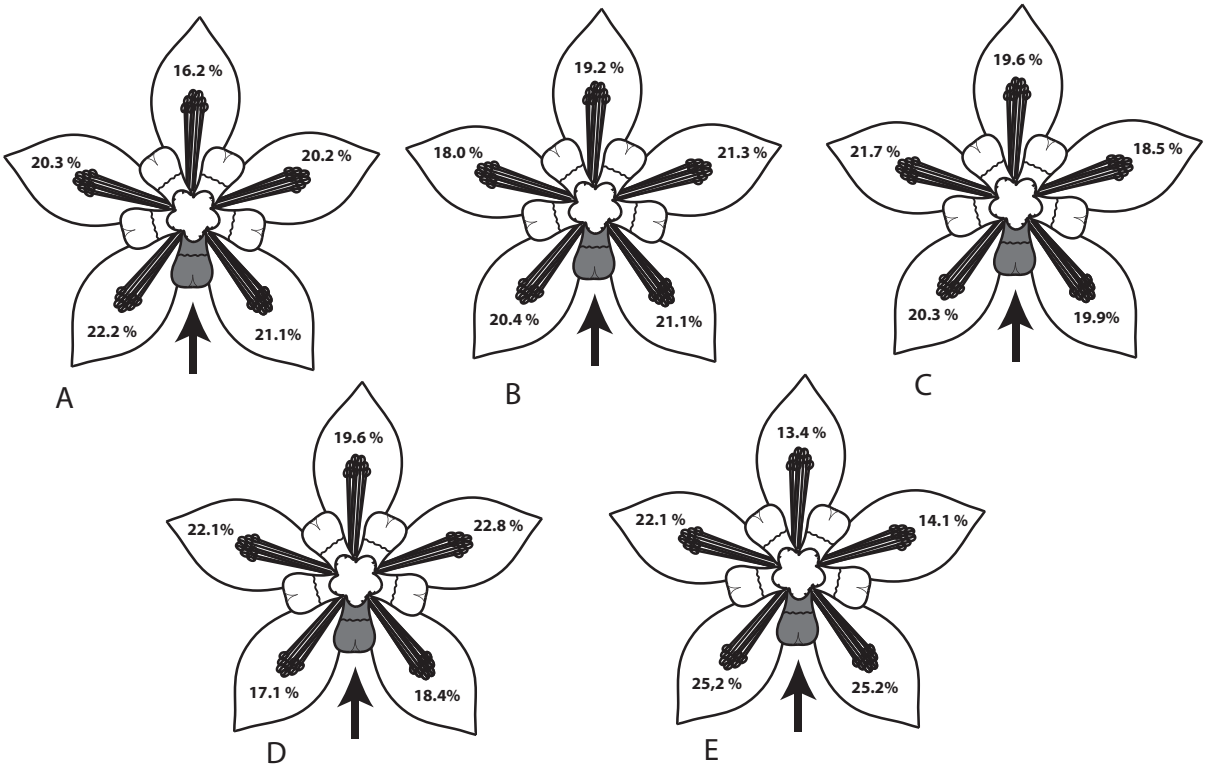

Fig. 7.9: Summary of the data on the stamen origin experimentally obtained. Grey filling/arrow = nectar scale stimulated. Percentage of stamen movement for each fascicle is given in the corresponding "petal". A. *Nasa vargasii*, B. *N. moroensis*, C. *N. macrothyrsa*, D. *Caiophora cirsiifolia*, E. *Presliophytum heucheraefolium*.

7.4 Discussion

The present study on pollen presentation in Loasaceae documents a near total control of the flower over pollen dispensation. Surprisingly, there appears to be no direct spatial correlation between the stamen bundle providing the thigmonastic stamen and the localization of the stimulus. The stimulus is apparently transmitted to all stamen fascicles via the receptacle, leading to a reaction in a determined number of stamens from all fascicles.
Pollen dispensation schedule is modulated by both biotic and abiotic factors: It comes to a virtual standstill in the dark and at low temperatures (here 12° C), so that in the absence of pollinators during the (Andean) nights nearly no pollen is presented. At higher temperatures (here 22° C) and daylight, pollen is gradually presented by autonomous stamen movement, but this stamen movement is slow, leading to a total duration of the staminate phase in *N. macrothyrsa* of ca. 6 days. Under flower visitation the anther presentation schedule can be dramatically accelerated, reducing the staminate phase to less than 2 d, i.e., less than a third, under flower visitation rates of 2 visits/h. Experiments with other species further show that an acceleration of pollen presentation is possible down to visitation intervals of 4 visits/h (in *N. urens*) respectively 6 visits/h (*N. dyeri*). In the field, visitation rates are unlikely to be equal throughout anthesis and differ for each individual flower: The data provided by Weigend *et al*. 2010 show a wide spread in visitation intervals. Thus, temperature, light (day/night) and visitation rates fluctuate widely during anthesis and for each individual flower. The direct response of thigmonastic stamen movement to both stimulation and external factors permits an extreme degree of short-term fine-tuning of the pollen presentation schedule and even of the overall duration of the staminate phase, approaching the ideal condition formulated by Harder & Wilson (1994). Stamen presentation per unit time can be dramatically accelerated (up to 37 x compared to autonomous movement) and the duration of the staminate phase can be dramatically reduced under very high visitation frequencies. Possibly the most striking result is, that the average number of mature stamens presented in the center of the flower after 5 min. is held virtually constant irrespective of visitation frequencies. Shortly after nectar and pollen depletion any visitor will therefore always find approximately the same amount of pollen (and no nectar) in the center of the flower. Pollen dispensation is thus remarkably even and the amount of pollen presented shortly after a previous visits is more or less equal, irrespective of visitation intervals. Withdrawal of sucessfully pollinated flowers from the overall flower stock (Primack, 1985, Van Doorn, 1997, Webb & Littleton, 1997) is taken care of very effectively, with flowers terminating their carpellate phase virtually immediately after pollination, whereas unpollinated flowers remain open for a much longer time. Under ideal conditions floral longevity in *N. macrothyrsa* can thus be reduced from over 10 d (>6.25 d staminate, >4.1 d carpellate) to less than 3 d (1.45 d staminate, 1.1 d carpellate), i.e., by more than 2/3rds. Direct observational data for *Nasa macrothyrsa* confirm this conclusion and it can even be extrapolated on shorter intervals. The effect may be even more pronounced in other species with an even more extreme acceleration of stamen presentation: In our experiments we found no saturation in intervals of 30 min. and shorter (at least in *N. dyeri* and in *L. insons*) – the total amount of anthers presented per hour either increased with shorter intervals, or was kept constant. This indicates that even under consistently high pollinator activity

(6 visits/h) stamen maturation and presentation can be accelerated sufficiently to ensure the immediate presentation of fresh pollen after a flower visit. The only major difference in stamen presentation is introduced by flower age, with the rate of stamen maturation apparently accelerated in older flowers and consequently overall pollen dispensation gradually increasing throughout the staminate phase. Nevertheless, differences in the amount of stamens moved upon a stimulus at different flower ages are minor compared to the differences caused by changes in visitation rates.

Although not specifically examined here, short pollen viability in general is thought to be linked to pollen allocation modes with a frequent release of small quantities of pollen and vice versa (Dafni & Firmage, 2000). In the species here studied anthers dehisce immediately before or during stamen movement, so that pollen presented is always fresh and viable. The immature anthers are reflexed and hidden in the petals, so that pollen collecting bees will usually not be able to deplete ("rob") the pollen of an individual flower in one visit.

Harder & Wilson (1994) mentioned the "unlikely case in which the number of visits to be received is higly predictable and the individual plant possesses the ability to adjust pollen-dispensing schedules accordingly" and argued that this would mean that "plant fitness may increase substantially". The data here presented on several species of Loasaceae provide an unexpectedly extreme example of such total control over pollen-dispensation in flowering plants.

8. Stamen movement and pollen packaging in Loasaceae subfam. Loasoideae*

Abstract

The stamen movement of Loasaceae subfam. Loasoideae has to be understood as a complex mechanism to increase outbreeding success by optimizing the male function and fitness. By moving mature anthers into the center of the flower, triggered by flower visitors at a previous visit, the pollen presentation is modified in terms of pollen packaging. Besides this thigmonastic movement, an autonomous stamen movement occurs to ensure the availability of fresh pollen after a period of pollinator absence. Previous studies revealed variations of the process of the stamen movement depending on the breeding system, pollination syndrome and systematic placement of the respective species of Loasaceae examined. The present study expands the investigations of this phenomenon onto a broader taxon sampling of as much species of Loasaceae subfam Loasoideae as possible. In total, 45 species from 10 genera have been analyzed experimentally. All taxa show an autonomous stamen movement that varies between 0.16 and 4.88 stamens per hour. The thigmonastic response onto a stimulus in a 30 min interval varies between 0.24 and 4.24 stamens per flower. Two species show an unchanged (*N. chenopodiifolia*) or diminished (*N. dillonii*) stamen activity after stimulation. In 12 species the increase of the number of stamens moved thigmonastically compared to the amount of stamens presented autonomously is not significant. Thigmonastic stamen movement in the remaining 33 taxa is significantly increased. Furthermore, 43 of the 45 species show a significant shift of the stamen movement activity towards the stimulus, regardless of the numerical comparison of thigmonastic vs. autonomous stamen movement. Hence, apart from two species, all taxa of Loasaceae subfam. Loasoideae show a significant reaction of stamen movement onto a stimulus of the flower as done by the pollinator, either numerically nor temporally. Only two species (*Huidobria fruticosa* and *Nasa chenopodiifolia*) show no clear, significant stimulus-response in both analyses. These two exceptions allow interpretations on the origin (*Huidobria fruticosa* representing the most basal genus in Loasaceae subfam. Loasoideae) and the eventual abandonment of this mechanism (*Nasa chenopodiifolia* as the only truly cleistogamous species known so far) within the whole subfamily.

8.1 Introduction

Pollen presentation. The successful deposition of viable pollen on a receptive stigma is limited by its preceded presentation in a flower and its effective delivery by a suitable pollen vector. This process and all related structures and matters are referred to as the male function of a flower. Besides several morphological traits such as size or colouration and orientation of particular sexual organs, the timing as an important option for the

*Henning T, Weigend M. in prep. Stamen movement and pollen packaging in Loasaceae subfam. Loasoideae. To be submitted to *BMC Evolutionary Biology*.

regulation of pollen release and dispersal has long been neglected. Pollen release is usually not a unique event and zoophilic flowers are predominantly visited several times by a potential pollinator. If so, the complete handover of all pollen to the first flower visitor would logically reduce the propability of a successful transfer to the stigma. Instead, the major selective force guiding floral evolution is to increase the proficiency of pollen donation (Loyd & Yates, 1982; Lloyd, 1884). This adjustment of pollen presentation to the quality and quantity of the pollinator has since been a central subject to pollination ecology (Harder & Thomson, 1989; Harder & Wilson, 1994; LeBuhn & Holsinger, 1998; Thomson, 2003) and all theoretical approaches in this context are compiled under the term "Pollen presentation theory" (PPT) (Percival, 1955).

If a zoophilic plant "makes use" of several pollinator visits, it has to decelerate pollen presentation and restrict pollen removal by an individual pollinator, by either packaging or dispensing the pollen. By packaging, the overall pollen production is divided into inflorescences, flowers, stamens or even anther sacs that open consecutively. By dispensing, the pollen is presented partially (e.g. poricidal anthers or gradually opening anthers), via secondary pollen presentation (e.g. Asteraceae, Campanulaceae, *Lupinus* L. – Fabaceae) or by active pollen dusting mechanisms (e.g. *Kalmia* L. – Ericaceae, Real & Rathcke, 1993; *Berberis* L. – Berberidaceae, LeBuhn & Anderson, 1994).

Which mode of pollen presentation is implemented and its detailed patterns is closely linked to the type of pollinator observed. Castellanos *et al*. (2003, 2006) convincingly demonstrated that hymenopteran-adapted species of *Penstemon* Mitch. (Scrophulariaceae) present their pollen more gradually than their ornithophilous (hummingbirds) relatives. These differences in pollen presentation could be explained by the divergent quality (grooming behaviour) and quantity (flower constancy and revisits) of hymenopterans and hummingbirds. Hymenopterans (esp. bees) often collect pollen actively (often with special grooming devices) and groom pollen grains off, especially after being heavily dusted (Harder & Thomson, 1989, Harder, 1990a, b). They often are, however, short distance trapliners that are more or less flower constant and shuttle frequently between populations of the same species, making repeated visit likely and a gradual pollen presentation useful. Conversely, hummingbirds neither actively collect pollen nor have special grooming devices, but are known to be unreliable in terms of flower constancy and revisits, since they tend to fly long distances during a foraging bout.

LeBuhn and Holsinger (1998) concluded that: "a plant should allocate pollen such that all pollinators that visit remove pollen". If pollinators are scarce, the deposition of as many pollen as possible at a single or a small series of visits may be favourable. If pollinators are abundant and repeated visits are likely, the release of small amounts of pollen by dispensing or packaging can be advantageous.

Ideally, the latter pollination system would require either a very constant frequency of revisits or a flexible mode of pollen presentation. Harder and Wilson (1994) called this the "unlikely case in which the number of visits to be received is higly predictable and the individual plant possess the ability to adjust pollen-dispensing schedules accordingly" by which "plant fitness may increase substantially".

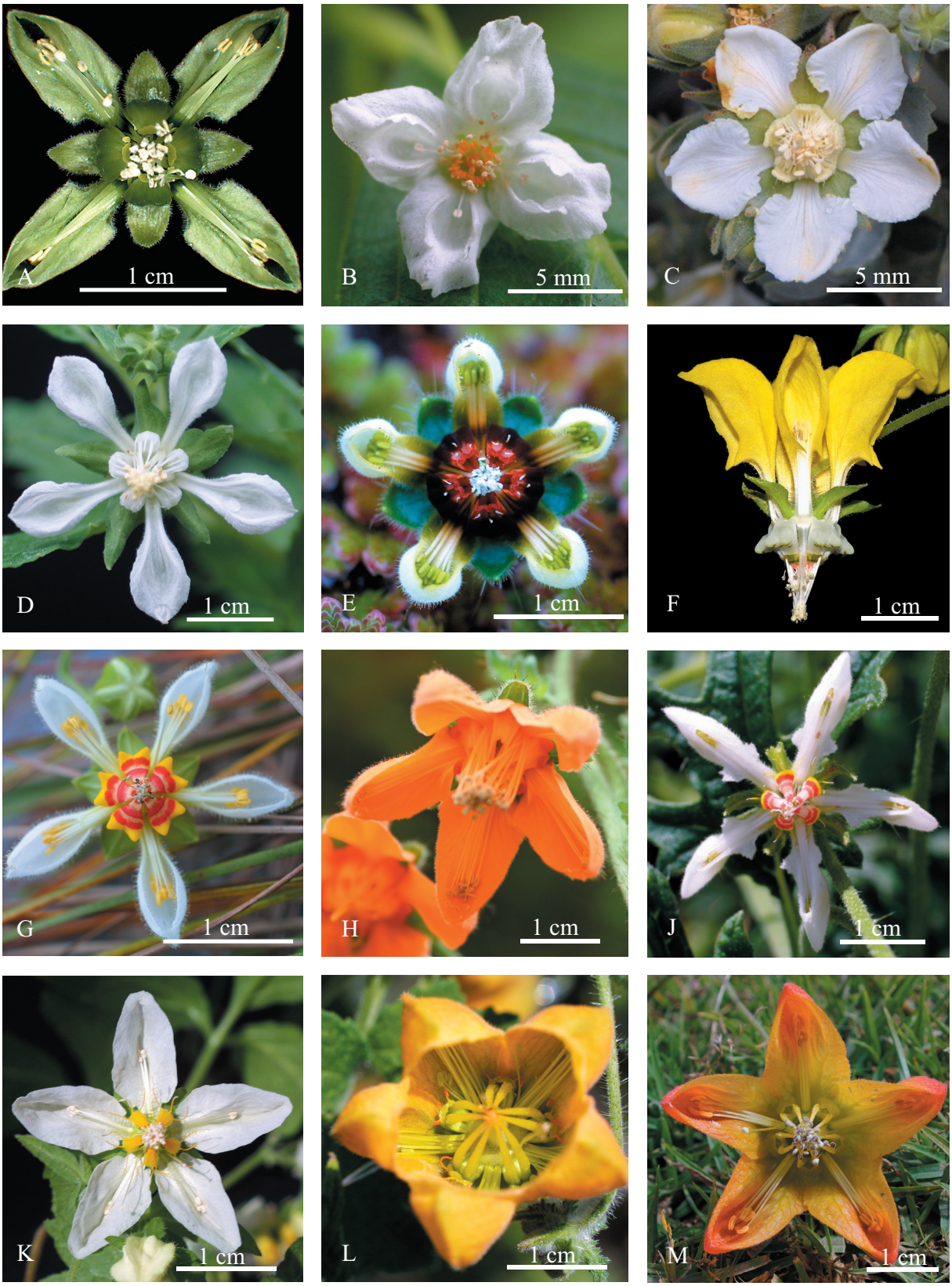

Fig. 8.1: Flowers of some of the species of Loasaceae subfam. Loasoideae used for this study. Klaprothieae: A. *Xylopodia klaprothioides*, B. *Plakothira parviflora*; „Lower Loaseae": C. *Huidobria fruticosa*; „Higher Loaseae": D. *Presliophytum incanum*, E. *Aosa rupestris*, F. *Nasa urens*, G. *Nasa triphylla* subsp. *triphylla*, H. *N. ranunculifolia* subsp. *ranunculifolia*; „South Andean Loasas": J. *Blumenbachia insignis*, K. *Loasa gayana*, L. *Caiophora canarinoides*, M. *Caiophora cirsiifolia*.

Stamen movements. Stamen movements are known for a long time (*Berberis* – Berberidaceae, Kölreuter, 1761; *Parietaria* L. – Urticaceae, Smith, 1788). Furthermore, the functional interrelation between these movements and flower visitors (Smith, 1788) or the process of pollination (*Nigella* L. – Ranunculaceae, Sprengel, 1793) has been noticed quite early. Several more or less spectacular cases of stamen movements have been reported from a variety of plant families. These movements are either singular movements driven by unrepeatable release of stored energy (e.g. *Ricinus* L. – Euphorbiaceae,Bianchini & Pacini, 1996; *Trophis* P.Browne – Moraceae, Bawa & Crisp, 1980; Simons, 1992; *Catasetum* Rich. ex Kunth - Orchidaceae, Romero & Nelson, 1986; *Cornus canadensis* L. - Cornaceae, Edwards *et al*., 2005) or can be repeatedly tripped by flower visitors (e.g. *Berberis* - Berberidaceae, LeBuhn & Anderson, 1994). Tripped, thigmonastic stamen movements are reported for several plant families: Aizoaceae, Berberidaceae, Cactaceae, Cistaceae, Malvaceae, Portulacaceae and Tiliaceae (Kabsch, 1861; Unger, 1863; Juel, 1906; Bünning, 1959; Guttenberg, 1971; Jaffe *et al*., 1977), but are often restricted to a genus or even a single species.

Loasaceae. The unique floral morphology of Loasaceae has repeatedly been subject of different studies (Brown & Kaul, 1981; Gilg, 1893, 1925; Hufford, 1990, 2003; Leins & Winhard, 1973; Payer, 1857; Urban, 1886, 1892; Urban & Gilg, 1900; Weigend, 1197, 2004a, 2004b; Weigend & Gottschling, 2006). Within polyandric Loasaceae, the subfamily Loasoideae has developed a unique flower morphology which led to the description of two exclusive subtypes of the so-called revolver flowers (Endress, 1994; Weigend 2004b, Weigend & Gottschling, 2006), namely tilt- and funnel-revolver flowers. Both types share the same overall morphology.

The plants have proterandrous flowers with 30–100(–240) stamens in five groups, with the stamens initially more or less enclosed in pockets formed by the petals (Fig. 8.1; Weigend & Gottschling, 2006). Individual anthers mature over several days and one-by-one move into the center of the flower, where they dehisce and present their pollen (e.g. Fig. 8.1 F, H). The five stamen groups alternate with five boat-shaped staminodial complexes. These staminodial complexes consist of an outer, deeply boat-shaped nectar scale (open at the apex and towards the inside of the flower) and two free staminodes closing the inside of the scale. Seen from above, the apical openings of these floral scales form a circle, like the holes in the drum of a revolver (Fig. 8.1 E). Nectar is secreted into the scales and the flower visitors have to probe each scale to harvest all nectar available. By doing so, a thigmonastic stamen movement is triggered, which could already be shown for some species of *Caiophora* C.Presl and *Blumenbachia* Schrad. (Schlindwein & Wittmann, 1995, 1997) and later on for additional species of *Caiophora* and *Nasa* Weigend (Weigend *et al*., 2010; Henning *et al*., 2012 - submitted). Two major types of revolver-flowers can be distinguished. Firstly, the so-called "tilt-revolver" flowers (Weigend & Gottschling, 2006), the plesiomorphic flower type of (mainly) entomophilous taxa. The floral scales of these flowers are flexible and the petals are more or less spreading. The stamens are hidden in the boat shaped petals and hence bended backwards by ≥ 90°. The pollinators (mostly short- and long-tongued bees) are driven to bend the flexible nectar scales backwards to harvest the nectar. While probing each nectar scale, they are ventrally dusted with pollen from the anthers presented in the center of the flower. The scales of these plants are often bicolorous, show individual

markings that guide the pollinators to the nectar and provide ridges and grooves at the scale surface to facilitate the landing and crawling of the insects.
A second (derived) floral type observed in Loasaceae subfam. Loasoideae has evolved several times independently and is strongly correlated with the repeated colonization of the High Andes (Weigend & Gottschling, 2006). With the exploitation of high altitudinal habitats, insects as efficient pollen vectors are no longer available. High Andean taxa of Loasoideae (as well as many other plant groups) have thus recruited hummingbirds as their main pollinators at altitudes above 3000m .a.s.l.. As an adaptation to ornithophily, the flowers underwent substantial changes in their morphology. The so-called "funnel-revolver" flowers (Weigend, 2004b; Weigend & Gottschling, 2006) are characterized by being notably larger, having a usually campanulate, robust corolla, which hides the now merely ≤ 90° backwards-bended stamens only suggestively. The nectar scales in these flowers are stiffly erect and fixed and lack a conspicuous colouration and handling structures [e.g. *Nasa ranunculifolia* (Kunth) Weigend subsp. *ranunculifolia* T.Henning, E.Rodr. &Weigend, Fig. 8.1 H].
Until very recently, thigmonasty within Loasaceae subfam. Loasoideae was thought to be restricted to entomophilous species with tilt-revolver flowers, since previous studies reported its loss in two species with a divergent pollination syndrome (ornithophily - *Caiophora hibiscifolia* (Griseb.) Urb. & Gilg Harter *et al*., 1995; rodent pollination - *C. coronata* (Gillies ex Arn.) Hook. & Arn., Cocucci & Sercic, 1998). Conversely, Henning *et al*. (2012 - submitted, Chapter 6) could reveal a thigmonastic stamen movement for *C. coronata* and a few other ornithophilous species of *Caiophora*. Additionally, thigmonasty could be proven for *Nasa ranunculifolia* with funnel-revolver flowers.

The following questions have been addressed in this study:

1. Do all species of Loasaceae subfam. Loasoideae examined show a movement of the stamens from inside the petals into the center of the flower in order to present their pollen?

2. Is this stamen movement also thigmonastic in all genera observed?

3. Is there a difference in the timing and quantity of the pollen presentation via this movement?

4. If so, are these differences linked to the phylogenetic position of the respective taxon?

5. And/or are the different movement patterns rather a result of the respective pollination syndrome of a single species?

8.2 Materials & Methods

Plant material. A total of 45 species from 10 genera of Loasaceae subfam. Loasoideae were investigated. A voucher list of all species examined including author citations and herbaria information is given in the appendix (Appendix D1). The data for *Huidobria fruticosa* were obtained on plants in the natural habitat. All other datasets were gained from cultivated plants. All species were raised from seeds collected in the wild, with the only exception of *Blumenbachia insignis* and *B. hieronymi*, which were obtained from cultivated material of unknown provenance from botanical gardens (Appendix D1). Plants were cultivated in the greenhouses at the Institut für Biologie, Freie Universität Berlin (2001 to 2010). Seeds were sown out into standard soil for seedlings (Einheitserde® Werkverband) and covered with plastic sheets until germination. Seedlings were pricked as soon as the cotyledons were fully developed into 5 cm clay pots filled with the same soil and then re-potted into successively larger clay pots (potting soil: 2 parts mature leaf compost, 1 part peat, fertilized with a mixed inorganic/organic fertilizer (Garten- and Gemüsedünger, ASB Grünland, H. Aurenz GmbH) and basalt powder (Neudorffs UrgesteinsMehl®, W. Neudorff GmbH KG). In winter (October–April) artificial light was used in the greenhouses (12h, high pressure sodium lamps: Philips SON-T AGRO® 400W). High Andean and south temperate taxa such as *Caiophora* (all taxa), *Nasa dillonii*, *N. macrothyrsa*, *N. ranunculifolia* ssp. *ranunculifolia*, *Loasa sclareifolia*, *L. insons* and *L. acerifolia* were cultivated with night-time temperatures of 5–15 °C and daytime temperatures of 20–25°C; all other species were grown at night-time temperatures of 18–20°C and daytime temperatures of 20–25°C. The following taxa were used for the investigations: *Aosa rupestris*, *Blumenbachia insignis*, *B. hieronymi*, *B. latifolia*, *Caiophora andina*, *C. canarinoides*, *C. arechavaletae*, *C. carduifolia*, *C. cirsiifolia* (four accessions), *C. coronata*, *C. stenocarpa*, *Huidobria fruticosa*, *Loasa acerifolia*, *L. gayana*, *L. insons*, *L. nitida*, *L. sclareifolia*, *L. tricolor*, *L. triloba*, *Nasa chenopodiifolia*, *N. dillonii*, *N. dyeri* ssp. *australis*, *N. macrothyrsa*, *N. moroensis* (two accessions), *N. olmosiana* (two accessions), *N. picta*, *N. poissoniana*, *N. ranunculifolia* ssp. *ranunculifolia*, *N. triphylla* ssp. *flavipes*, *N. triphylla* ssp. *triphylla* (two accessions), *N. urens*, *N. vargasii*, *N. weigendii*, *Plakothira parviflora*, *Presliophytum heucheraefolium*, *P. incanum*, *Scyphanthus elegans* (two accessions) and *Xylopodia klaprothioides*.

Thigmonastic stamen movement. The experimental treatment of the flowers depended to some degree on the number of plants (and hence flowers) in cultivation: inflorescence branches were cut off and placed into glass vials in the laboratory for the experiments. Previous experiments had shown that the different treatment did not affect stamen movement. In those cases, were only a few plants were available for the experiments, these were carried out directly on the living plants in the greenhouse. Field data for *Huidobria fruticosa* were obtained on living plants in the natural haitat, using the same procedure as described below.

In both cases flowers were individually marked and mature stamens which already had moved into the center of the flower, were cut off one hour prior to the first stimulation experiment. 10–35 flowers were used for the experiment with a control group of 5–22 flowers (Tab. 8.1). To receive robust, comparable data, five consecutive 30 minute intervals

between stimuli were chosen, based on field observations indicating an average interval between two visits of individual flowers of ca. 25 minutes (Weigend *et al.*, 2010), following the principle that the timing of experimental visits to flowers should reflect the natural visitation rate (Harder & Wilson, 1994).
For purposes of recording, the overall interval of 30 minutes was subdivided into portions of 5 minutes and the anthers moving in each of these sub-intervals of 5 minutes were pooled to ease data recording and analyses. Also, the stamen movement from the reflexed into the upright position takes ca. 1–3 minutes, so that a more precise recording seemed to be inadequate. The resulting six sub-intervals are labelled with A to F within tables and figures in the results to ease differentiation from the numerical values recorded.
Stamen movement was triggered by imitating a pollinator visit by slightly bending all five nectar scales outwards with a preparation needle. Stamen movement was recorded when the stamen moved out of the fascicle and left the petal. Anthers of the newly moved stamens were carefully cut off to prevent double counting. Stimulation of the nectar scales was repeated after 30 minutes for a total of five intervals. Each experiment contained an unmanipulated control group. The 30 minutes interval was chosen as a standard interval to generate the largest data set to compare the patterns of the movement troughout the whole subfamily.

Statistical analyses. The datasets were tested for significance by performing a Mann-Whitney-U-Test (Sokal & Rohlf ,1969). We tested the different amount of stamens moved in the manipulated flowers vs. the control group for statistical significance (results are listed in Tab. 8.1). This test reveals only a quantitative difference in the stamen movement per unit of time between non stimulated (autonomous movement) and stimulated flowers (putative thigmonastic movement). To reveal a qualitative change in the movement patterns (e.g. a shift of movement activity towards the stimulus) we additionally compared the mean values (average stamen movement) for the six subintervals (A–F) with each other. Therefore, a posteriori, a Friedmann-Test was made to reveal significant differences throughout the whole dataset (subintervals A–F) for each species. If a significant difference within the values of these subintervals has been detected, a Wilcoxon-Signed-Rank-Test (Sokal & Rohlf ,1969) followed, comparing each five-minute-interval (A–F) with each other. The resulting *P*-values may, together with the diagrams, help to identify significant shifts of stamen movement peaks apart from a simple numerical increase of the overall activity of a flower. The results are summarized in table 8.2, *P*-Value = asymptotic significance (2-tailed).
The diagrams for each taxon examined show the course of the average stamen activity per 30 minute interval, partitioned into the respective six subintervals of manipulated flowers and the respective control. In the interest of clarity, the standard deviation is added to the bars only in one direction. The datasets were prepared using Microsoft Excel®, statistical analyses were performed with SPSS® for Windows®. Graphs (Figs. 8.2-8.5) were created using SigmaPlot® and graphically processed using Adobe® Illustrator® CS. Photographs for figure 8.1 were graphically enhanced and labelled using Adobe® Photoshop® CS and arranged using Adobe® InDesign® CS.

Tab. 8.1: Summarized data for stamen movement in Loasoideae (stim. vs. auton.) * Ø movement of the control calculated.

tribe	species	voucher	n stimulated	n control	*P*	∑ stamens stimulated per flower/30 minutes	mean SD	∑ stamens control per flower/30 minutes	mean SD
Klaprothieae	*Plakothira parviflora*	Weigend s.n.	30	15	0.057	0.74	0.37	0.57	0.34
Klaprothieae	*Xylopodia klaprothioides*	Weigend 97/450	35	22	<0.01	1.58	0.56	0.72	0.42
Lower Loaseae	*Huidobria fruticosa*	Kern 6	20	10	0.656	1.76	1.09	1.7	0.79
Higher Loaseae	*Presliophytum heucheraefolium*	Weigend 7691	30	5	<0.01	2.67	0.79	0.28	0.19
Higher Loaseae	*Presliophytum incanum*	Weigend 97/12	10	5	<0.01	0.9	0.44	0.68	0.31
Higher Loaseae	*Aosa rupestris*	Weigend 7138	10	5	<0.01	0.66	0.3	0.08	0.06
Higher Loaseae	*Nasa chenopodiifolia*	Weigend 8433	20	10	0.637	0.24	0.19	0.24	0.19
Higher Loaseae	*Nasa dillonii*	Weigend 7556	20	10	0.726	0.76	0.36	0.82	0.42
Higher Loaseae	*Nasa dyeri* ssp. *australis*	Dostert 98/80	28	18	<0.05	2.43	0.6	1.75	0.56
Higher Loaseae	*Nasa macrothyrsa*	Weigend 7471	20	10	<0.01	3.39	0.79	2.44	0.72
Higher Loaseae	*Nasa moroensis*	Weigend 7694	20	10	<0.05	1.92	0.6	1.62	0.47
Higher Loaseae	*Nasa moroensis*	Weigend 8424	20	10	0.165	2.1	0.49	1.72	0.6
Higher Loaseae	*Nasa olmosiana*	Weigend 8541	20	10	<0.05	1.67	0.53	0.76	0.37
Higher Loaseae	*Nasa olmosiana*	Dostert 98/163	20	10	<0.01	1.76	0.54	1.1	0.46
Higher Loaseae	*Nasa picta*	Dostert 98/158	20	10	<0,01	0,96	0,38	0,3	0,19
Higher Loaseae	*Nasa poissoniana*	Weigend 8007	20	10	<0.01	1.88	0.49	0.88	0.38
Higher Loaseae	*Nasa ranunculifolia* ssp. *ranunculifolia*	Henning 06/05	22	11	<0.01	3.28	0.93	1.78	0.83
Higher Loaseae	*Nasa triphylla* ssp. *flavipes*	Dostert 98/203	20	10	0.141	1.51	0.46	1.08	0.41
Higher Loaseae	*Nasa triphylla* ssp. *triphylla*	Ackermann 602	20	10	<0.01	2.72	0.74	1.14	0.49
Higher Loaseae	*Nasa triphylla* ssp. *triphylla*	Weigend 9031	20	10	<0.01	2.46	0.59	1.16	0.46
Higher Loaseae	*Nasa urens*	Weigend 7327	30	20	<0.01	2.39	0.69	0.44	k.A.
Higher Loaseae	*Nasa vargasii*	Weigend 5463	20	10	<0.01	2.02	0.55	0.96	0.42
Higher Loaseae	*Nasa weigendii*	Weigend 7913	20	10	<0.01	2.92	0.73	1.78	0.53
Higher Loaseae	*Blumenbachia hieronymi*	Ackermann 601	10	5	<0.01	1.78	0.47	0.24	0.16
Higher Loaseae	*Blumenbachia insignis*	Weigend 7475	30	15	<0.01	3.98	0.95	1.13	0.47
Higher Loaseae	*Blumenbachia latifolia*	Weigend 9135	20	10	0.837	1.02	0.36	0.88	0.39
Higher Loaseae	*Loasa acerifolia*	Weigend 9142	20	10	<0.01	2.3	0.61	0.82	0.37
Higher Loaseae	*Loasa gayana*	Weigend 7057	10	5	<0.01	2.56	0.64	1.36	0.49
Higher Loaseae	*Loasa insons*	Weigend 8724	20	10	<0.01	2.52	0.68	0.82	0.39
Higher Loaseae	*Loasa nitida*	Weigend 7346	20	13	0.515	0.88	0.38	0.72	0.34
Higher Loaseae	*Loasa sclareifolia*	Weigend 8183	20	10	<0.01	2.22	0.63	0.74	0.41
Higher Loaseae	*Loasa tricolor*	Weigend 9010	21	10	0.07	0.89	0.30	0.6	0.27
Higher Loaseae	*Loasa triloba*	Weigend 9008	33	10	<0.01	1.21	0.38	0.58	0.30
Higher Loaseae	*Scyphanthus elegans*	Weigend 9032	20	10	0.549	2.19	0.77	2.02	0.75
Higher Loaseae	*Scyphanthus* cf. *elegans*	Gardner & Knees 8351	20	10	<0.01	4.24	1.12	0.66	0.43
Higher Loaseae	*Caiophora arechavaletae*	Weigend 9330	20	10	<0.01	2.94	0.75	1.84	0.60
Higher Loaseae	*Caiophora canarinoides*	Ackermann 395	10	5	<0.01	1.42	0.54	0.48	0.23
Higher Loaseae	*Caiophora carduifolia*	Ackermann & Kollehn 288	10	5	<0.01	1.6	0.5	1.12	0.45
Higher Loaseae	*Caiophora* cf. *andina*	Schlumpberger 663	20	10	<0.01	1.68	0.58	0.28	0.18
Higher Loaseae	*Caiophora cirsiifolia*	Henning 08/30	20	10	<0.01	1	0.4	0.3	0.24
Higher Loaseae	*Caiophora cirsiifolia*	Weigend 7559	10	5	0.152	1.06	0.37	0.76	0.32
Higher Loaseae	*Caiophora cirsiifolia*	Weigend 7697	10	5	<0.01	1.96	0.57	1	0.4
Higher Loaseae	*Caiophora cirsiifolia*	Weigend 9043	21	10	0.156	1.36	0.41	1.08	0.39
Higher Loaseae	*Caiophora coronata*	Weigend 9152	20	10	<0.01	1.96	0.73	0.4	0.33
Higher Loaseae	*Caiophora stenocarpa*	Ackermann 758	20	10	<0.01	1.58	0.45	1.02	0.50

8.3 Results

We examined the genera *Aosa* (1), *Blumenbachia* (3), *Caiophora* (10), *Huidobria* (1), *Loasa* (7), *Nasa* (17), *Plakothira* (1), *Presliophytum* (2), *Scyphanthus* (2) and *Xylopodia* (1) (in brackets: number of taxa/collections investigated, 45 in total). The appearance and patterns of the thigmonastic stamen movement are here presented for all genera of Loasoideae except *Chichicaste*.

All taxa investigated show a movement of mature stamens from the petals into the center of the flower. The duration of this movement depends on the angle in which the immature stamens were initially bended backwards. This in turn depends on the angle in which the petals are inserted on the receptacle. This angle varies depending on the flower shape from 30°–80° in bellshaped (campanulate) flowers (all *Caiophora* species, *Nasa dillonii*, *N. olmosiana* and *N. ranunculifolia* ssp. *ranunculifolia*) to ca. 90°–120° in taxa with spreading petals (*Aosa*, both *Blumenbachia*, all *Loasa* species, remaining *Nasa* species except *N. urens*, *Plakothira*, both *Presliophytum*, *Scyphanthus* and *Xylopodia*) up to 170° in fully developed flowers of *Nasa urens*, whose petals are bended back upwards. The majority of taxa show an angle of 90°–120°. In these taxa a movement from the petals into the center of the flower lasts ca. one minute on average (65 sec. +/- 48 sec., n=10, *N. poissoniana*).

Autonomous stamen movement. The autonomous stamen movement varies from 0.16 (*Aosa rupestris*) to 4.88 (*Nasa macrothyrsa*) stamens per hour. Anthers dehisce sequentially while the stamens are bended backwards and thus hidden into the boat-shaped petals. Autonomous stamen movement follows dehiscens of the anthers and the movement as such is indistinguishable from a triggered movement. After successful presentation of the pollen in the center of the flower, the stamens wilt and contract forming a bundle of dried-out stamens below the newly presented stamens (Fig. 8.1 F). Only in *Caiophora* stamens are not contracted and collectively bended backwards again (into the petals), subsequent to the staminate phase.

Thigmonastic stamen movement. The number of stamens moved after a stimulation of the nectar scale ranges from 0.24 (*Nasa chenopodiifolia*) to 4.24 (*Scyphanthus* cf. *elegans*) per 30 min. If not stimulated the plants performed an autonomous stamen movement that varies between 0.08 to 2.44 stamen per 30 min. The ratio of stamens moved in stimulated flowers and the control group varies from less than 1:1 (*Nasa dillonii*) to more than 9:1 (*Presliophytum heucheraefolium*). The vast majority of taxa shows a ratio of 1.5:1 to 4:1.

In 43 of the 45 species the number of stamens that moved after a stimulation of the nectar scale exceeds the number of autonomously moved stamens. In one case (*Nasa chenopodiifolia*) no difference in the amount of ambulated stamens could be ascertained between the triggered flowers and the control group. Only one species (*Nasa dillonii*) showed a diminished activity in stamen movement onto a stimulus compared with the control group. In 33 of the taxa investigated the amount of stamens moved after a stimulation of the nectar scale is significantly higher than in their control groups ($P<0.05$, Tab. 8.1). The movement in these taxa is therefore proven to be thigmonastic. In the remaining 11 taxa the quantity of the movement differs not significantly between stimulated flowers and the control group. However, an analysis of the quality of the movement shows, that the movement is thigmonastic as well. In 41 of the

Tab. 8.2: Statistical analyses of the temporal course of the stamen movement in Loasoideae. *P*-values are given in bold letters if datasets received no or not the highest support ($P<0.01$). Peak significance was analyzed, if a difference could be detected within the dataset a posteriori (Friedmann-test, if $P<0.05$, see M&M section). For each interval all other statistically deviating intervals are listed unidirectional (all = differs from all other intervals, "-" = no difference) beginning at the first interval (A). Differences apply in reverse as well (not listed repeatedly for reasons of clarity).

				peak significance interval					
		P	*P*	A	B	C	D	E	F
species	voucher	movement	interval difference	differs significantly from interval					
Plakothira parviflora	Weigend s.n.	**0,057**	<0,01	B,D,E,F	-	D	-	-	-
Xylopodia klaprothioides	Weigend 97/450	<0,001	**0,743**						
Huidobria fruticosa	T. Kern 6	**0.656**	**0,033**	D,F	-	-	E	F	-
Presliophytum heucheraefolium	Weigend 7691	<0,001	<0,01	B,F	E,F	E,F	E,F	F	-
Presliophytum incanum	Weigend 97/12	<0,01	**0,322**						
Aosa rupestris	Weigend 7138	<0,01	**0,055**						
Nasa chenopodiifolia	Weigend 8433	**0,637**	**0,226**						
Nasa dillonii	Weigend 7556	**0,726**	<0,001	all	C	F	-	-	-
Nasa dyeri ssp. australis	Dostert 98/80	<0,01	<0,001	all	C	F	-	-	-
Nasa macrothyrsa	Weigend 7471	<0,01	<0,001	all	all	-	-	-	-
Nasa moroensis	Weigend 7694	**0,024**	<0,001	all	E,F	E	-	-	-
Nasa moroensis	Weigend 8424	**0,165**	<0,001	all	all	-	-	-	-
Nasa olmosiana	Weigend 8541	<0,001	<0,001	all	-	-	-	-	-
Nasa olmosiana	Dostert 98/163	<0,01	<0,001	all	D,E,F	E,F	F	-	-
Nasa picta	Dostert 98/158	<0,001	**0,044**	E, F	-	F	F	-	-
Nasa poissoniana	Weigend 8007	<0,001	<0,001	all	all	D	C	-	-
Nasa ranunculifolia ssp. *ran.*	Henning 06/05	<0,001	<0,001	C,D,E,F	C,D,E,F	-	-	-	-
Nasa triphylla ssp. flavipes	Dostert 98/203	**0,141**	<0,001	all	D, E	E	-	-	-
Nasa triphylla ssp. triphylla	Weigend 9031	<0,001	<0,001	all	-	-	-	-	-
Nasa triphylla ssp. triphylla	Ackermann 602	<0,001	<0,001	all	-	-	-	-	-
Nasa urens	Weigend 7327	<0,001	<0,001	all	-	-	-	D	-
Nasa vargasii	Weigend 5463	<0,001	<0,001	all	-	-	-	-	-
Nasa weigendii	Weigend 7913	<0,01	<0,001	all	E,F	E	E	-	-
Blumenbachia hieronymi	Ackermann 601	<0,001	<0,001	all	-	F	-	-	-
Blumenbachia insignis	Weigend 7475	<0,001	<0,001	all	E,F	E	-	-	-
Blumenbachia latifolia	Weigend 9135	**0.837**	<0,001	all	D,E	-			
Loasa acerifolia	Weigend 9142	<0,001	<0,001	all	-	-	-	-	-
Loasa gayana	Weigend 7057	<0,01	**0,011**	C,D,E,F	C,D,E,F	-	-	-	-
Loasa insons	Weigend 8724	<0,001	<0,001	all	all	-	-	-	-
Loasa nitida	Weigend 7346	**0,515**	<0,001	B,C,D,E	C,D,E	-	-	-	-
Loasa sclareifolia	Weigend 8183	<0,001	<0,001	all	E	E,F	-	-	-
Loasa tricolor	Weigend 9010	**0.07**	<0,001	D,E,F	C,D,E,F	B,E,F	F	-	-
Loasa triloba	Weigend 9008	<0,01	<0,001	all	all	E, F	E,F	F	-
Scyphanthus elegans	Weigend 9032	**0,549**	<0,001	all	D,E	-	-	-	-
Scyphanthus cf. *elegans*	Gardner & Knees 8351	<0,01	<0,001	all	all	-	-	F	-
Caiophora arechavaletae	Weigend 9330	<0,01	<0,001	all	-	-	F	-	-
Caiophora canarinoides	Ackermann 395	<0,01	<0,001	C,D,E,F	D,E,F	-	-	-	-
Caiophora carduifolia	Ackermann & Kollehn 288	**0,04**	<0,01	D,E,F	D,E,F	D	-	-	-
Caiophora cf. *andina*	Schlumpberger 663	<0,001	<0,001	all	D,E,F	D	-	-	-
Caiophora cirsiifolia	Weigend 7559	**0,152**	<0,01	F	D	-	-	-	-
Caiophora cirsiifolia	Weigend 7697	<0,01	0,001	all	E	-	-	-	-
Caiophora cirsiifolia	Henning 08/30	<0,001	<0,001	all	-	-	-	-	-
Caiophora cirsiifolia	Weigend 9043	**0.156**	<0,001	B,D,E,F	all	D,E,F	F	F	-
Caiophora coronata	Weigend 9152	<0,001	**0,025**	D	D	D	-	-	-
Caiophora stenocarpa	Ackermann 758	<0,01	<0,001	all	all	-	-	-	-

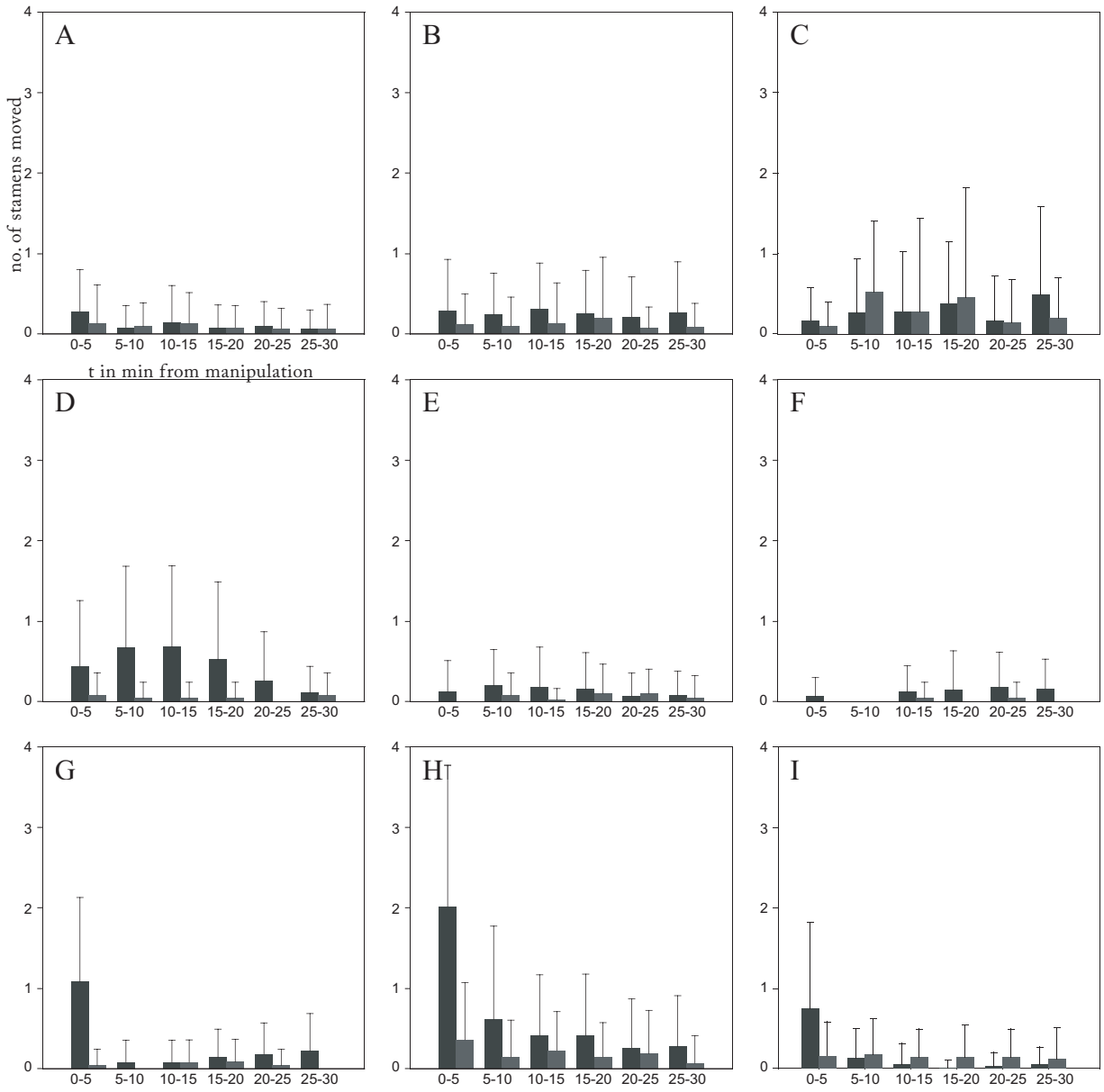

Fig. 8.2: Stamen movement in species of Loasaceae I. Black bars: movement after stimulation, grey bars: autonomous movement. A: *Plakothira parviflora*, B: *Xylopodia klaprothioides*, C: *Huidobria fruticosa*, D: *Presliophytum heucheraefolium*, E: *P. incanum*, F: *Aosa rupestris*. G: *Blumenbachia hieronymie*, H. *B. insignis*, I: *B. latifolia*

taxa examined a significant difference in the numerical distribution of the moved stamens (usually towards the first minutes after the stimulus) can be observed (Tab. 8.2). For example for *Scyphanthus elegans,* the quantitative analyses of the movement shows no significance at all (P = 0.549). But as the diagram of the movement indicates, a clear shift of stamen movement activity towards the first minutes after flower-stimulation can be observed. The same is true for *Nasa dillonii* with an even diminished stamen activity in stimulated flowers in terms of quantity (P = 0.726), but a significant movement peak following the stimulus. Hence, looking at the quantity and the quality of the movement, 43 of the 45 taxa show a highly significant response onto the stimulus by either an increase in the amount of stamens that move compared to the control group or a distinct peak in movement activity, which is absent in the control group and usually follows the stimulus immediately. Only two species reveal no or only a statistically weak supported response onto a stimulus at all. Namely *Nasa chenopodiifolia* and *Huidobria fruticosa*.

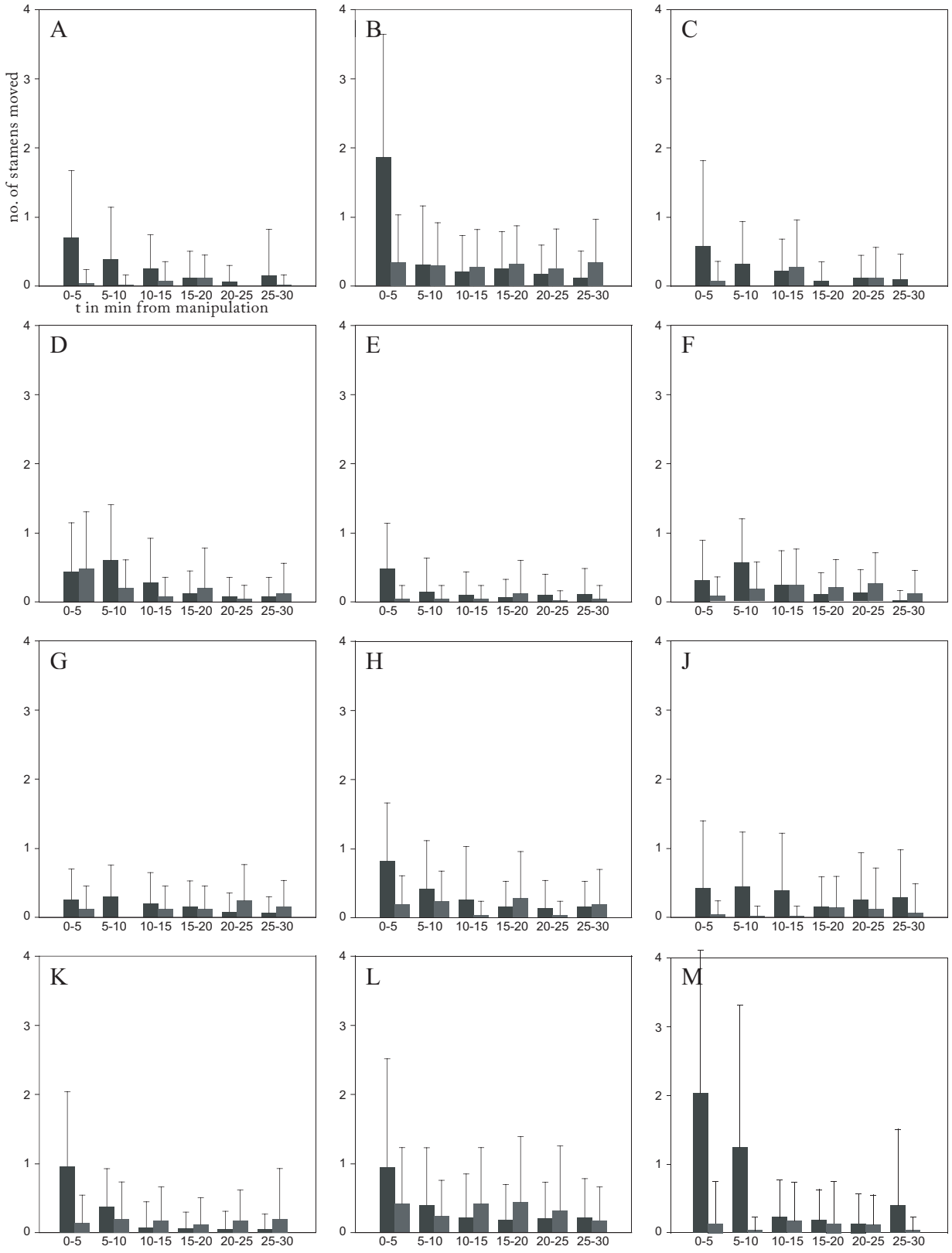

Fig. 8.3: Stamen movement in species of Loasaceae II. Black bars: movement after stimulation, grey bars: autonomous movement. A: *Caiophora* cf. *andina*, B: *C. arechavaletae*, C: *C. canarinoides*, D: *C. carduifolia*, E-H: *C. cirsiifolia*, E: Henning 08/30, F: Weigend 9043, G: Weigend 7559, H: Weigend 7697, J: *C. coronata*, K: *C. stenocarpa*, L-M: *Scyphanthus elegans*, L. Weigend 9032, M. *S.* cf. *elegans*, Gardner & Knees 8351.

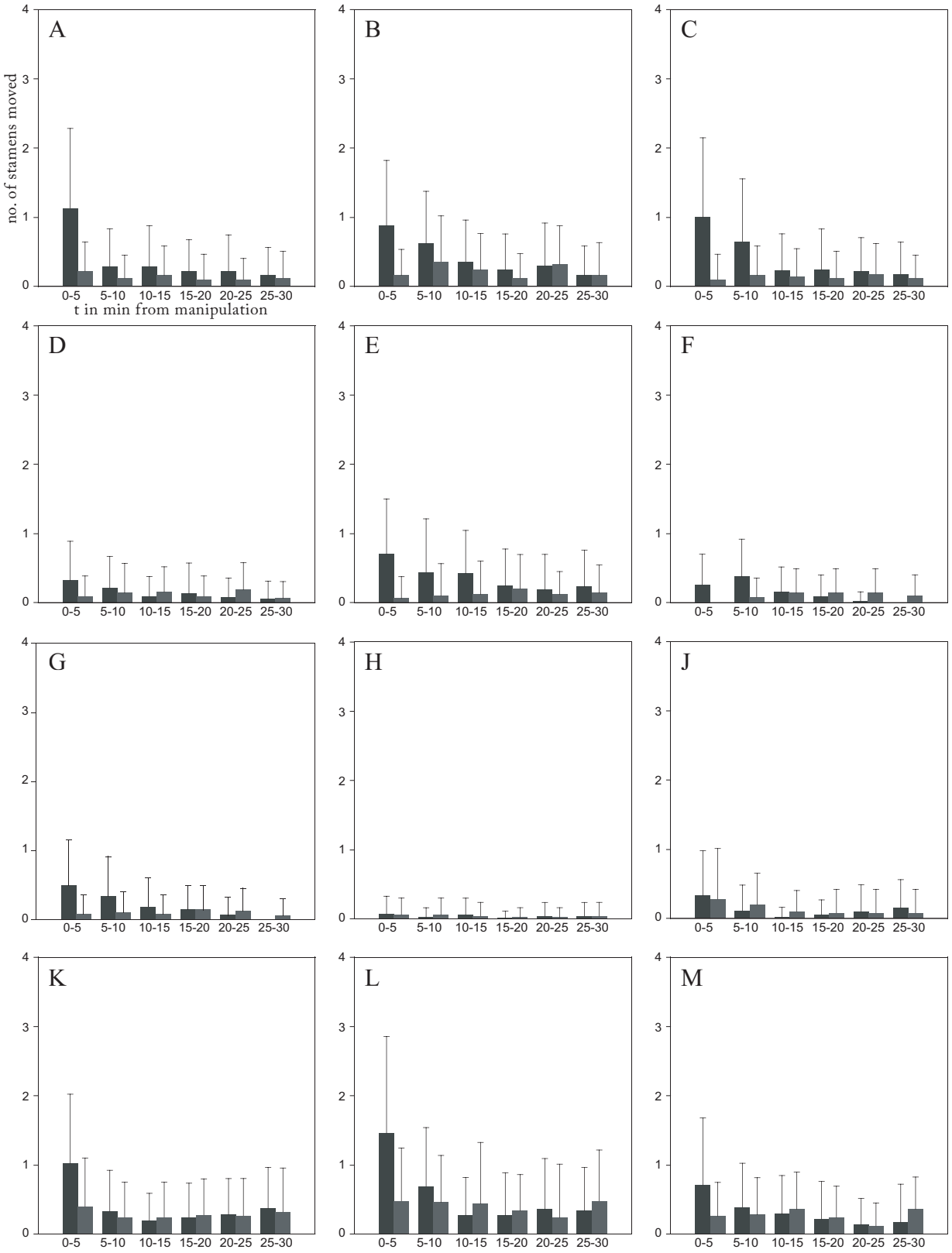

Fig. 8.4: Stamen movement in species of Loasaceae III. Black bars: movement after stimulation, grey bars: autonomous movement. A: *Loasa acerifolia*, B: *L. gayana*, C: *L. insons*, D: *L. nitida*, E: *L. sclareifolia*, F: *L. tricolor*, G: *L. triloba*, H: *Nasa chenopodiifolia*, J: *N. dillonii*, K: *N. dyeri* ssp. *australis*, L: *N. macrothyrsa*, M: *N. moroensis* (Weigend 7694).

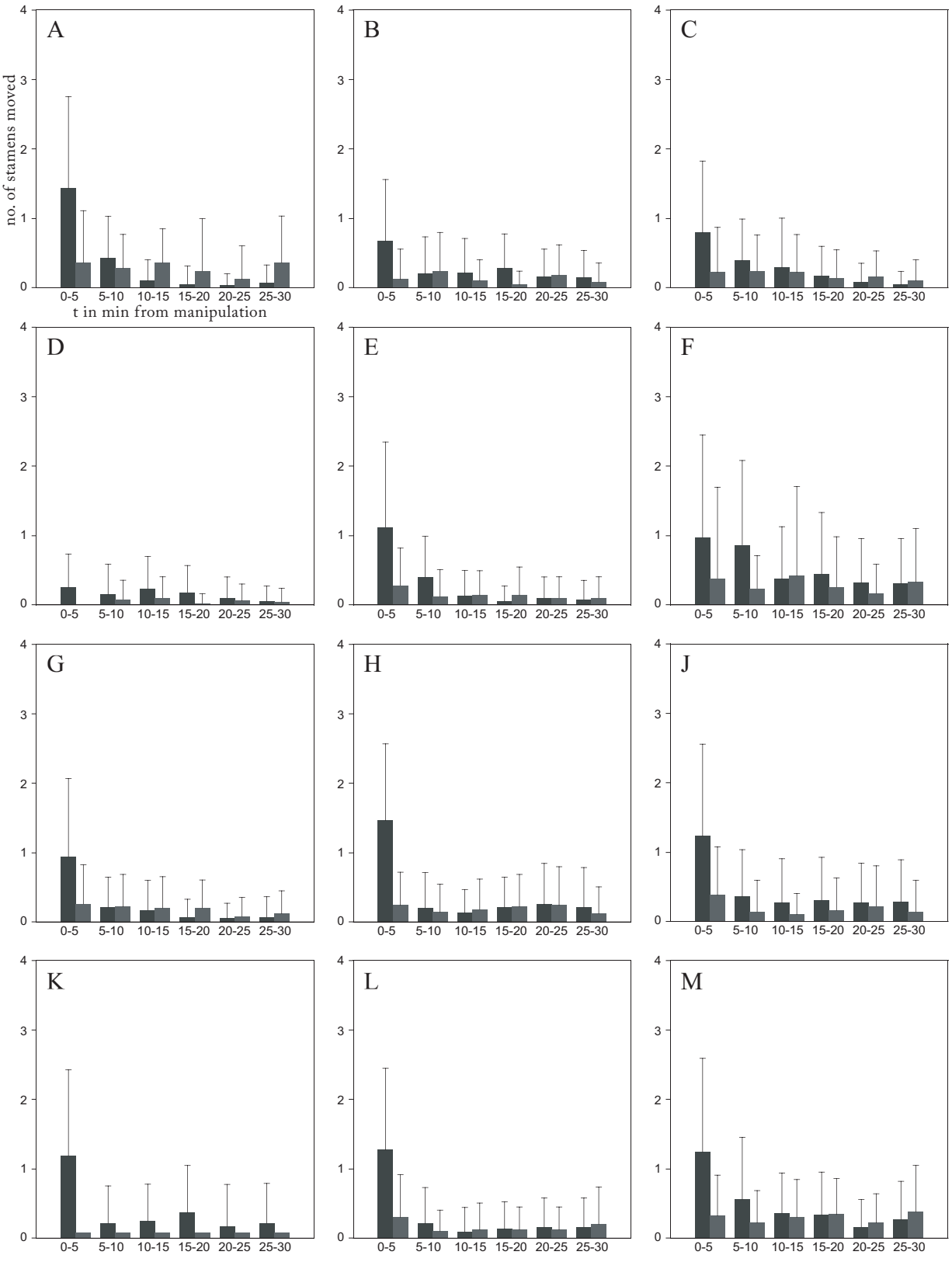

Fig. 8.5: Stamen movement in species of Loasaceae IV. Black bars: movement after stimulation, grey bars: autonomous movement. A: *Nasa moroensis* (Weigend 8424), B-C: *N. olmosiana*, B: Weigend 8541, C: Dostert 98/163, D: *N. picta*, E: *N. poissoniana*, F: *N. ranunculifolia* ssp. *ranunculifolia*, G-J: *N. triphylla*, G: ssp. *flavipes* (Dostert 98/203), H: ssp. *triphylla* (Weigend 9031), J: ssp. *triphylla* (Ackermann 602), K: *N. urens*, L: *N. vargasii*, M: *N. weigendii*.

Maximum response to a stimulus can usually be observed in the first five minutes after scale manipulation. This pattern could be observed in 35 of the 45 taxa investigated. The remaining taxa showed either a delayed peak in the stimulus-reponse or no at all. In six cases maximum stamen movement was observed in the second interval (5-10min) and in two cases in the third (10–15 min). In taxa with an immediate response within the first five minutes after manipulation, the peak usually surpasses the value of the other five intervals considerably. The maximum stamen movement per five minute-interval is 0.93 in average in these taxa. Taxa in which the maximum stimulus-response is delayed usually have a more vague peak, that in average reaches only a stamen movement of 0.38 stamens per five minute-interval.

Roughly four types of stamen-movement-patterns can be identified:

1. Stamen movement shows no significant difference in stimulated flowers compared to the control, neither in the overall amount of stamens moved nor in the temporal distribution of this movement after the stimulus. This type is found only in *Huidobria fruticosa* (Fig. 8.2 C), a species of Loasoideae with a basal phylogenetic placement. These plants are obviously not able to modulate their pollen presentation in accordance to pollinator activity and visitation rates. Pollen presentation is here passively staggered and the plants benefit only from the elongated male phase and the thereby increased male fitness (likelyhood of successful pollen transfer).

2. Stamen movement is only faintly to moderately increased and a significant peak is only shallow or absent. This pattern can be observed in both species of the tribe Klaprothieae, namely *Plakothira parviflora* (Fig. 8.2 A) and *Xylopodia klaprothioides* (Fig. 8.2 B). Futhermore in *Aosa rupestris*, one collection of *Caiophora cirsiifolia* (Fig. 8.3 G), *C. coronata* (Fig. 8.2 J), *Nasa chenopodiifolia* (Fig. 8.4 H), *N. picta* (Fig. 8.5 D) and *N. dillonii* (Fig. 8.4 J).

3. Stamen movement is delayed and a movement peak can be detected in the second or third interval after scale manipulation. This type of movement sequence can be found in both *Presliophytum* (Fig. 8.2 E,D) species examined. These findings and data of observed flower visitors indicate a divergent pollination syndrome in this genus.

4. Stamen movement activity is increased substantially after manipulation of the floral scale. The amount of stamens that are presented in the center of the flower is increased by 50–400%, compared to the control group. Stamen activity shows a significant peak that is restricted to the first and/or the second interval directly after manipulation. Hence, taxa performing this type of stimulus response, show a fast, intensive reaction to a flower visitor. Pollen is reliably presented with a short delay, indicating an adaptation to pollinator-visits. This type can be observed in all higher Loaseae which consist mainly of entomophilous and ornithophilous taxa (see Figs. 8.2-8.5).

8.4 Discussion

A stamen movement from inside the petals into the center of the flower is found in all taxa investigated. Experiments show that this movement is thigmonastic in all but one species, since it can be triggered by a mechanical stimulus of the receptacle. In *Huidobria fruticosa*, the stamen movement seems to be an autonomous movement which can neither be accelerated nor influenced over time by a stimulus. In all other species, either a significant increase in the number of stamens per unit-time or a shift of stamen movement activity towards the stimulus can be observed (Tab. 8.1, 8.2). This stimulus is either mediated via a deflection of the hinged nectar scales or received directly in a previously unknown way.
Referring to pollen-presentation-theory, the stamen movement of Loasaceae means an extreme case of pollen packaging. Most pollen-packaging strategies are usually related to several flowers or inflorescences that open consecutively. Anther related packaging modules usually have tight restrictions in terms of the number of packaging units. Secondary polyandry by dedoublement in Loasaceae allowed the increase in the number of these packages (see appendix D2, Tab.8.3). Additionally, more or less hooded petals are found in all genera investigated. Male fitness benefits substantially from both. The package-size is very constant and by keeping immature anthers closed and bended backwards into the petals, pollen viability is not affected as it might be in whole-flower-packaging-strategies or dispensing mechanisms. A sample calculation shows the potential of this packaging mode. *Scyphanthus elegans* (one accession), for example, has up to 242 stamen per flower on average. Experimentally, the presentation of 4.24 stamens can be triggered in intervals of 30 minutes. That means that, if the flower is visited in this frequency (which is indicated by field observations, e.g. Schlindwein & Wittmann 1997, Weigend *et al*. 2010), almost 60 pollen loads will be successfully positioned on a flower visitor. Pollen wastage is reduced substantially by presenting fresh pollen from recently opened stamens subsequent to a pollinator visit. Investigations on other Loasaceae indicate that the visiting intervals might be even shorter and the plants can accelerate pollen presentation accordingly (see "Total control" - Chapter 7). That would increase the number of pollen packages again dramatically, suggesting the potential of this mechanism in terms of pollen dispensing.
Within the staminate phase, stamen movement itself and the amount of stamens provided for this mechanism is depending on the number and the timing of visits that are received. The species of higher Loasoideae with a thigmonastic stamen movement obviously possess the ability to adjust pollen-dispensing schedules to the actual visitation rate of pollinators. In a recently published study on floral rewards and visitation rates in *Nasa macrothyrsa* (Weigend *et al*., 2010), first evidence for a high flower constancy and a predictable timing of revisits of the specialized pollinators (Carpenter bees, *Neoxylocopa* spec.) has been presented. Hence, plant-pollinator interaction in higher Loasaceae is the „unlikely case" described by Harder & Wilson (1994). Pollen presentation is closely linked to flower visits and can be accelerated or decelerated, depending on the actual visitation rate of the pollinator.

Stamen movement and phylogeny. Interestingly, the data on stamen movement reflects the phylogenetic relationships within Loasaceae subfam. Loasoideae. Within Loasoideae, the

tribes Kissenieae (*Kissenia capensis*, monotypic, Old World, not included in this analysis), Klaprothieae and Loaseae are recognized. The Klaprothieae (*Klaprothia*, *Plakothira* and *Xylopodia*) share some plesiomorphic characters (see Weigend, 1997; Weigend *et al*., 2004; Hufford *et al*., 2005) and have repeatedly been retrieved as monophyletic. Although not sufficiently resolved, the Loaseae have informally been subdivided into the Lower and Higher Loaseae. The Lower Loaseae comprise some genera, namely *Huidobria*, *Chichicaste*, *Aosa* and *Presliophytum* (incl. *Loasa* ser. *Malesherbioideae*), with a basal placement outside the Klaprothieae, either as part of or as sister to the rest of the Loaseae (Weigend *et al*., 2004). All other taxa belong to the Higher Loaseae (Weigend, 1997; Weigend *et al*., 2004).

Whereas the stamen movement patterns of the "higher Loaseae" observed are all very similar, the genera belonging to the basally placed Klaprothieae and Lower Loaseae share some interesting patterns that likely reflect the gradual evolution of the stamen movement. A stepwise development, from an autonomous movement without a recognizable reaction onto a respective stimulus (*Huidobria fruticosa*), to a significant, numerical increase in stamen movement (*Aosa rupestris*, *Xylopodia klaprothioides*), to a shift of this numerical increase that results in a delayed peak after the respective stimulus (*Plakothira parviflora*, *Presliophytum* spp.) can be observed. The logical further development, especially when pollinated by traplining insects and thus likely receiving a quick re-visit soon after the previous visit (as described for *N. macrothyrsa* – Weigend *et al*., 2010), is that the stamen movement follows the stimulus immediately. This movement pattern is typically found in all higher Loaseae examined, with the exception of *C. coronata* and *N. chenopodiifolia*, two species with a divergent pollination syndrome (see below).

Stamen movement and pollination syndrome. Within widespread Loasaceae several pollination syndroms have developed. Entomophily is the plesiomorphic pollination syndrome and can be found throughout the whole family and its putative sistergroup Hydrangeaceae. Bees are the most important flower visitors and are reported to be pollinators for the most taxa investigated (*Aosa*, *Blumenbachia*, most species of *Caiophora*, *Huidobria*, *Loasa*, most species of *Nasa* esp. those of *Nasa* "ser. *Saccatae"*, *Scyphanthus*, *Xylopodia*, Weigend & Gottschling, 2006, Weigend *et al*., 2010; Henning *et al*., 2012 - submitted). *Plakothira* (Klaprothieae) is, however, pollinated by flies and *Presliophytum* is predominantly visited by butterflies. Within *Nasa* and *Caiophora* multiple transitions to ornithophily related to the repeated colonization of High Andean habitats can be observed (as described above). Finally, for *Caiophora coronata*, Cocucci & Sercic (1998) could present strong evidence for rodent pollination.

Pollen packaging strategies are usually connected with pollination by hymenopterans, since they are known to be reliable flower visitors. They often show flower constancy and perform traplining, making a repeated visit likely and a repeated pollen release useful in terms of pollen presentation theory. Since pollen presentation is closely linked to pollinator behaviour, making use of a new guild of pollinators should influence the patterns of pollen presentation directly.

Bees. Angiosperm diversity is closely linked to the coevolution with bees, which are the most important pollinators world-wide (Endress, 1994). This is also true for the vast majority of species of higher Loasoideae, which are pollinated by more or less specialized bees. Thigmonasty likely originated in taxa with a close plant-pollinator interaction with specialized bees that strongly depend on the nectar and pollen and plants that require a reliable pollinator. This scenario, as described by Schlindwein and Wittmann (1992, 1997), is typical for Loasoideae and is supposed to be the "basic type" of pollination mode in the group. The response to a flower visit is quick in plants pollinated by bees, as described earlier by greenhouse as well as field experiments (Schlindwein & Wittmann, 1997, Weigend *et al*., 2010). The movement usually peaks directly (within the first five minutes) after the stimulation of the floral scale and then flattens off continuously until the next stimulation (re-visit). On average, one stamen is presented shortly after a previous vist. This ensures that fresh pollen is available after a short time, independent of the exact timing of the revisit of the (predominantly) traplining bee. This is the typical course and it can be observed in all (putative) bee pollinated taxa examined.

Hummingbirds. As described above, several lineages within Loasaceae subfam. Loasoideae have independently colonized High Andean habitats. In the course of this altitudinal exploitation a transition to ornithophily in *Nasa* and *Caiophora* took place. Besides a different nectar quantity and quality and colouration (Weigend & Gottschling, 2006; Ackermann & Weigend, 2006), three profound changes in flower morphology can be observed in taxa with such a pollination syndrome. Firstly, the now rather campanulate or bellshaped corolla consists of less hooded petals. Hence, immature stamens are not completely hidden as in e.g. bee-pollinated taxa. But since hummingbirds only feed on nectar, no need is given to hide the pollen stock. Secondly, all flower organs are more robust compared to insect pollinated flowers. The now fixed, stiffly erect nectar scales are rigid and cannot function as a sensor to mediate the stimulus of a visitor and elicite the stamen movement. Thirdly, the filaments of successful presented stamens are not wilting and contracting as observed in all entomophilous taxa. Hence, with ongoing anthesis, a brush-like bundle of stamens will accumulate in the center of the flower, enlarging the surface from which pollen is emitted and increasing the likelyhood of successful pollen deposition on a hummingbirds forehead. These complex morphological changes reflect what is expected for a typical hummingbird flower in terms of flower morphology. And since visitation rates of hummingbirds are known to vary dramatically, hummingbird flowers are usually expected to present large amounts (if not all) of pollen at a time. Furthermore, pollen packaging and a thigmonastic stamen movement have so far not been reported for bird-pollinated plants.
We therefore expected a complete loss of the thigmonastic nature of the movement as described for *Caiophora hibiscifolia* (Griseb.) Urb & Gilg) by Harter *et al.* (1995). Conversely, all ornithophilous taxa examined (*Nasa ranunculifolia*, *N. dillonii*, *N. olmosiana, Caiophora andina*, *C. carduifolia*, *C. canarinoides*) showed a significant response to a stimulus of the flower by imitating a hummingbird beak. Although pollen packaging is imprecise by only adding fresh pollen to the „brush" of stamens already presented, it is adjustable to the actual visitation rates of the pollinator. Instead of presenting all pollen simultaneously or visitation-rate-independent via an autonomous stamen movement, the replenishment of

the pollen „brush“ with fresh pollen takes place primarily after a successful flower visit. This is, in a way, the tradeoff between maximum pollen viability and the uncertain timing of the next visit.
What can be observed in ornithophilous Loasaceae seems to be an advancement of the “typical” hummingbird flower. There might be no need for pollen packaging to be successfully pollinated by a hummingbird. But the advantages of pollen packaging and a triggered pollen presentation in polyandrous flowers still seem to improve outbreeding success dramatically, if invented a priori. The contribution of a thigmonastic stamen movement to the successful colonization of High Andean habitats is underlined by the fact that this transition has taken place at least two times independently (Weigend & Gottschling, 2006), always combined with a switch from entomophily to ornithophily. For some of these lineages (*Nasa* Ser. *Grandiflorae* and *Caiophora*), the retention of thigmonasty could be proven here.

Flies. Plakothira parviflora is visited by flies (M. Weigend, *pers. comm.*). So far, no data on the behaviour of these flies is available. It can only be speculated whether these flies are trapliners and revisit individual flowers or not, which would be essential to discuss the stamen movement performance of *Plakothira parviflora*. A small peak of (overall insignificant) stamen movement can be detected within the first five minutes after stimulation of the flowers. It can be assumed that a flower visit at least leads to the rapid presentation of fresh pollen and hence the advantages of a triggered stamen movement may also hold true for dipteran-pollinated taxa.

Butterflies. Presliophytum spp. are predominantly visited by butterflies. The plants are medium sized shrubs that occur loosely spreaded in their natural habitat. They produce many flowers simultaneously (> 100 in old plants) and flower over several weeks. The stamen movement in this genus shows a unique sequence by being delayed compared to the other taxa investigated. A correlation between this type of pollinators and loosely dispersed, profusely flowering plants is conceivable. A visiting butterfly is harvesting nectar from several flowers of each plant, thus spending several minutes on the same plant. The data presented here are too weak for far reaching conclusions. Nevertheless, one could assume that the likelyhood to deposit fresh pollen on a second or returning pollinator may be considerably increased by presenting it with a short delay.

Mammals. As impressively described by Cocucci and Sercic (1998), *Caiophora coronata* is pollinated by small rodents (Gray leaf-eared Mouse, *Graomys griseoflavus*, fam. Muridae). The stamen movement is consequent to a visit evenly increased with only minor differences in the activity over the time. It can only be speculated that this conspicuous movement pattern in someway reflects a particularity in the behaviour of *Graomys griseoflavus* as a pollinator. But it is worth mentioning that a divergent pattern of stamen movement is linked to an (as known so far) unique pollination system in this High Andean lineage.

Cleistogamy. Another species with a divergent stamen movement pattern is *Nasa chenopodiifolia*. It has by far the weakest total stamen movement rate and shows no reaction onto a stimulus at all (Tab. 8.1). In the case of *N. chenopodiifolia* this makes

absolute sense, since it is a weedy species which is completely self compatible (Henning & Weigend, 2009b). It has a reduced number of stamens (Appendix D2, Tab. 8.3) and its degraded flowers usually do not completely open, if at all. Fruitset is, nevertheless, high and cleistogamy can hence be assumed for these plants. Although repeatedly monitored in the field by both authors, no flower visitor could so far be observed. An accelerated stamen presentation and any kind of a timed movement obviously doesn´t make sense in this context.

Summary. The spatial presentation of pollen via a thigmonastic stamen movement in higher Loasoideae is advantageous, irrespective of the type of pollinator. The data here presented indicate that almost all clades of Loasaceae subfam. Loasoideae possess the ability to adjust their pollen presentation in some way to either actual visitation rates (if re-visits in more or less regular intervals are part of the pollinators behaviour, e.g. entomophilous taxa) or at least multiple visits during the flowering of a single flower (if repeated visits are likely but unreliable, e.g. ornithophilous taxa). The only exception is *Huidobria*, which only performes an autonomous movement and hence a gradual pollen presentation without any specific response to the pollinator and its visitation rates. The almost completely resolved alpha-taxonomy of the subfamily and the well known flower morphology allows us to predict thigmonasty for the species of all other genera of this group known so far. It has hence to be understood as a key-invention that has been followed by a rapid radiation in Andean South America. Loasaceae subfam. Loasoideae are nowadays found in a variety of habitats, from the coastal deserts of western South America to the Puna and Paramo habitats in the High Andes. The outstanding habitational and morphological diversity of the group is at least partially a result of the unique flower morphology and the mechanism and timing of the stamen movement.

9. Conclusions

9.1 Systematics of *Nasa*

Since the establishment of *Nasa* Weigend (Weigend, 1997b; Weigend *et al.*, 2006) as part of the division of *Loasa* Adans. sensu Urban & Gilg (1900), the number of constituent taxa has increased steadily. It now comprises c. 140 taxa and thus more than 1/3rd of all taxa recognized in Loasaceae. This is mainly a result of an ongoing comprehensive taxonomic revision of the whole family by Weigend and colleagues within the last two decades. This in turn is founded on a continuing collecting effort and a successful cooperation with our South American colleagues. With an increasing accessibility of the local collections and own field work, the extent of infrageneric groups and relationships within *Nasa* (and other genera of Loasaceae) became identifiable. Two large species groups remained unrevised until very recently. Additionally, material of new taxa, which obviously belong to species-groups revised earlier or remain unplaced so far, became available and required formal taxonomic treatments.

9.1.1 The *Nasa poissoniana* group

The *Nasa poissoniana* (Urb. & Gilg) Weigend group (Chapter 2) has its main center of diversity in the inner Andean valleys of southern Peru. Its members have long been assigned to the large *Nasa* ser. *Saccatae* Urb. & Gilg, a paraphyletic assemblage defined by Urban & Gilg (1900). The series was originally founded on the basis of the superficially similar flower morphology and growth habit of a large set of species of *Nasa* (*Loasa* Adans.). Molecular data (Weigend *et al.*, 2004; Weigend & Gottschling, 2006) repeatedly revealed the paraphyly of this assemblage and the inapplicability of the plesiomorphic morphological characters, which were the basis of its delimitation in the first place. Two other species groups [*Nasa triphylla* (Juss.) Weigend group (Dostert & Weigend, 1999) and *Nasa stuebeliana* (Urb. & Gilg) Weigend group (Weigend & Rodríguez, 2003)] could already be identified on the basis of morphological synapomorphies and had been revised prior to the molecular studies, which confirmed their monophyly. With the *Nasa poissoniana* group, a third monophyletic group could repeatedly be retrieved by molecular data (Weigend *et al.*, 2004; Weigend & Gottschling, 2006), although it lacks clear morphological coherence separating its members from the remaining taxa of the former series *Saccatae*.
The members of the group share only few morphological characters that are, however, plesiomorphic in *Nasa* and represent no unique set of character states in terms of autapomorphies. The plants are ephemeral or annual, usually branch strongly from the base and the terminal shoots passing into flower in short sequence. They have the tilt-revolver flowers, typical for ser. *Saccatae*, with white [(*N. chenopodiifolia* (Desr.) Weigend, *N. ferruginea* (Urb. & Gilg) Weigend, *N. vargasii* (J.F.Macbr.) Weigend], yellowish-white [*N. poissoniana*, *N. raimondii* (Standl. & F.A.Barkley) Weigend] to yellow [*N. urens* (Jacq.) Weigend] petals. The floral scales are mostly yellow with one or several red or pink transversal and/or vertical calli on the back (*N. chenopodiifolia*, *N. ferruginea*,

N. poissoniana, *N. raimondii*, *N. weigendii* E.Rodr.). The floral scales of *N. vargasii* are, however, red and white and *N. urens* has a unique scale-colouration with white scales with yellowish to greyish-green markings (transverse bands). Some species have two distinct horn-like humps on their nectar scales (*N. poissoniana*, *N. urens* and *N. vargasii*). But even this character is frequently found in other, distantly related species of *Nasa* [e.g. *N. humboldtiana* (Urb. & Gilg) Weigend – Henning & Weigend, 2009; *N. bicornuta* (Weigend) Weigend – Weigend, 1996a] and of little sytematic value. The leaf shapes found in the group also differ widely and reveal no consistent trends. The leaves are poorly (*N. raimondii*) to irregularly (three- or fivelobed) lobed (*N. poissoniana, N. ferruginea*) to deeply, subpalmately lobed (*N. vargasii*). The lobes are irregularly dentate (*N. raimondii*) to distantly serrate (*N. vargasii*). The leaves of *N. weigendii* are, however, deeply lobed to truly trifoliolate and *N. urens* has deeply bipinnatifid leaves.

N. raimondii was originally placed in the *N. stuebeliana* group (Weigend & Rodríguez, 2003) based on the presence of amplexicaul bracts as typical for species of this group. Due to molecular findings, its affiliation to the *N. poissoniana* group has been retrieved by single and combined marker analyses (ITS1, trnL-ITS1 - Weigend & Gottschling, 2006). It is now tranferred to the *N. poissoniana* group and extends its morphological variation by a character actually typical for the members of the *N. stuebeliana* group. Both groups in their present extent are well supported by molecular data and clearly represent monophyletic entities. Their morphological circumscription remains, however, difficult and documents the ongoing, rapid diversification of *Nasa* in Andean South America and an exceptionally high degree of morphological variability even between closely allied taxa.

The *N. poissoniana* group now comprises eight taxa in seven species. Four out of the seven species can be found in the inner Andean valleys of southern Peru, where they usually are widespread over several provinces and Departments (namely: *Nasa poissoniana* ssp. *poissoniana*, *N. ferruginea, N. raimondii* and *N. vargasii*). *Nasa poissoniana* and *N. vargasii* show a disjunct distribution with the existence of isolated populations in northern Peru. Whereas the collections of *N. vargasii* are similar, the northern specimens of *N. poissoniana* are separated as subsp. *glandulifera* T.Henning & Weigend due to consistent morphological differences.

The only narrowly endemic species of the group is *Nasa weigendii* from the Department La Libertad. It is the only species of the group from the Amotape-Huancabamba Zone, which is the main center of diversity for most other groups of *Nasa*. With its main center of diversity in the southern Peruvian Andes, the *Nasa poissoniana* group proves the exception to the rule in the biogeography of *Nasa*. This exceptional position is underlined by the ecology and distribution of two other species belonging to the group: *Nasa chenopodiifolia* and *Nasa urens*. The first is a morphologically variable species and virtually the only species of *Nasa* that grows as a weed. It is furthermore the only species known so far from which cleistogamous flowers are reported (Chapter 8) and its distribution is likely a result of seed dispersal by agricultural land use. The second widespread species, *Nasa urens,* is an important floristic element of the Lomas vegetation of the Peruvian and Chilean coastal deserts. This is exceptional in *Nasa* and morphologically underlined by a unique leaf morphology with bipinnatifid leaves and a striking flower colouration with yellow petals and green-white nectar scales.

9.1.2 Two new species of *Nasa*

With *Nasa sanchezii* T.Henning & Weigend and *Nasa urubambensis* T.Henning & Weigend, two species have been described as new to science, which are only distantly related (Chapter 3). The placement of *Nasa sanchezii* within *Nasa* remains unclear at present and requires future molecular approaches. It can morphologically not be assigned to any of the groups from the former series *Saccatae*, although sufficient herbarium material is available. Conversely, *Nasa urubambensis* is known only from two collections from the Department Cuzco in southern Peru. The specimens came to my knowledge shortly after the revision of the *Nasa poissoniana* group had been published. Although the group is morphologically difficult to describe (Chapter 2), its core members (*N. poissoniana*, *N. vargasii*, *N. ferruginea* – Chapter 2) share a few morphological (flower morphology, growth habit) and distributional (inner Andean valleys of southern Peru) similarities and *Nasa urubambensis* clearly corresponds to this group. Chapter 3 is an example for a number of smaller taxonomic works that have been published within the last years (e.g. Henning & Weigend, 2009a; Henning *et al.*, 2009). These works are necessary in order to achieve a fully resolved α-taxonomy, which is the basis for future studies regarding phylogeny, biogeography or pollination ecology as described in Chapters 5 to 8.

9.1.3 The *Nasa ranunculifolia* group

Unlike Urban and Gilg´s (1900) *Nasa* ser. *Saccatae*, the series *Grandiflorae* Urb. & Gilg is a monophyletic group of (sub-)shrubs and rhizomatous herbs, which could repeatedly be retrieved by molecular data (Weigend *et al.*, 2004; Weigend & Gottschling, 2006). As the name indicates, the group comprises species of *Nasa* from Ecuador and Peru with rather large flowers (Weigend, 2000b). Furthermore, within ser. *Grandiflorae* some of the tallest and most profusely flowering species of the whole genus can be found. All species of *Grandiflorae* described earlier (Weigend *et al.*, 1998; Weigend & Rodríguez, 2002; Rodríguez & Weigend, 1999; Rodríguez *et al.*, 2003) are well defined species that can clearly be distinguished both, morphologically and by means of their distribution. With ongoing collecting effort in the Peruvian Andes, several new collections could be made, yielding numerous new taxa. Among many new species and a large set of obviously closely allied taxa that showed clear geographic vicariance but (especially in herbarium specimens) a rather gradual variance in morphology from one location to the other. Some taxa described already by Urban & Gilg [*Nasa* (*Loasa*) *macrantha* (Urb. & Gilg) Weigend, *N. cymbopetala* (Urb. & Gilg) Weigend & *N. macrorrhiza* (Urb. & Gilg) Weigend] fall into this morphological range and are now reduced to subspecies under *N. ranunculifolia* (Kunth) Weigend, which was the first species described already by Kunth (Humboldt *et al.*, 1806) and thus is the eponymous species of the group. Furthermore, with *N. tulipadiaboli* T.Henning, E.Rodr. & Weigend and *N. basilica* T.Henning, E.Rodr. & Weigend, two new species are described and *N. rugosa* (Killip) Weigend is redefined and subdivided into four subspecies on the basis of recent collections. All members of the *N. ranunculifolia* group presented here clearly represent a monophylum of High Andean, ornithophilous taxa of *Nasa* (Weigend & Gottschling, 2006).

Two basic types of growth habit can be found in the group. Tall, monocarpic, usually biennial herbs [*N. magnifica* (Urb. & Gilg) Weigend, *N. profundilobata* (Werderm.) Weigend, *N. tulipadiaboli* and *N. basilica*] and smaller, perennial, rhizomatous herbs (*N. ranunculifolia* and *N. rugosa*). The former form a massive (to 1m in diameter) leaf rosette in the first year and a tall, much branched flowering shoot in the second year. The base of the shoot may be decumbent and thickened and serves as a storage organ, but a distinct rhizome is never developed. The latter two species form a smaller leaf rosette in the first year and usually (with the exception of *N. ranunculifolia* subsp. *bolivariensis* T.Henning, E.Rodr. & Weigend) form more or less extensive colonies by branching above (rhizomatous branches) or below (underground runners) the ground. These plants show a varying ability to resprout from their underground organs after periods of drougth, but have generally to be designated as perennial taxa.

The group is defined on the basis of a variable, but clearly coherent floral and vegetative morphology. The leaves are long-petiolate with a deeply serrate to lobulate, often subpalmately lobed, rarely pinnatisect lamina. The lamina is ovate to circular, rarely flabellate in outline. The flowers are the typical funnel-revolver flowers described by Weigend (2004b) with orange or (rarely) yellow petals that are half spreading to erect, leading to a starshaped to campanulate corolla. The floral scales conform those typical for ser. *Grandiflorae* with two well-developed (sometimes incurved - some subsp. of *N. ranunculifolia*) nectar sacs and two distinct, erect, apical wings. The floral morphology, nevertheless, differs in colouration and shape. The scales can be unicoloured (pale orange in *N. rugosa* subsp. *llaqtacochaensis* T.Henning, E.Rodr. & Weigend; pale yellow to greenish white in *N. tulipadiaboli*) or bicolorous (white with red-orange nectar sacs – *N. magnifica*; pale orange with darker nectar sacs – *N. basilica* and all subspecies of *N. ranunculifolia*). Most members of the group, especially the tall biennial species, are profusely flowering from multiply branched inflorescences and have pendulous flowers. Conversely, *N. rugosa* has only one (*N. rugosa* subsp. *pygmaea* T.Henning, E.Rodr. & Weigend) to few flowers. These are held horizontally or are even facing upwards, which can be suspected in *N. rugosa* subsp. *rugosa* from the single specimen of the type collection.

Unlike the members of the *Nasa poissoniana* group, the vast majority of taxa from the *N. ranunculifolia* group are found in the Amotape-Huancabamba Zone (Berry, 1982; Young & Reynel, 1997) in North Peru and adjacent Ecuador. This area is a known hotspot of biodiversity (Luteyn & Churchill, 2000; Weigend, 2002b, 2004c; DeWitt Smith & Baum, 2006; Keating, 2008) with an extraordinary percentage of endemisms (Valencia *et al.*, 2000; Lozano *et al.*, 2002). This is true for Loasaceae (Weigend, 2004b; Weigend *et al.*, 2005, 2010; Rodríguez, 2009) but has also been shown for numerous other Andean plant groups (*Lysipomia* Kunth, Campanulaceae, Ayers, 1999; Asteraceae-Liabeae H.Rob. & Brettell, Soejima *et al.*, 2008), including northern temperate groups, such as *Urtica* L. (Urticaceae), *Ribes* L. (Grossulariaceae; Weigend *et al.*, 2005) and *Lithospermum* L. (Boraginaceae; Weigend *et al.*, 2010) and typical neotropical-montane taxa, such as *Calceolaria* L. (Calceolariaceae; Molau, 1988; Andersson, 2006), *Erythroxylum* P.Browne (Erythroxylaceae; Jara-Muñoz; 2011), *Fuchsia* L. (Onagraceae; Berry, 1982), *Iochroma* Benth. (Solanaceae; DeWitt Smith & Baum, 2006), *Solanum* L. (Knapp, 2002; Stern *et al.*, 2008), *Macrocarpaea* Gilg (Gentianaceae; Grant & Weaver, 2003), *Ourisia* Comm. ex. Juss. (Scrophulariaceae; Meudt & Simpson, 2006), *Passiflora* L. (Passifloraceae; Skrabal *et al.*, 2001) and *Polylepis* Riuz & Pav. (Rosaceae; Simpson, 1986).

It can furthermore be noted that members of the *N. ranunculifolia* group are predominantly dependent on moister habitats, which are found in the northern Andes and especially on the eastern slopes towards Amazonia, where the new species *N. basilica* and *N. tulipadiaboli* and the new subspecies of redefined *N. rugosa* come from. In the drier central Andes only rhizomatous and shrubby subspecies of *N. ranunculifolia* occur, which are able to survive a lengthy dry period and resprout from their underground organs. *Nasa magnifica* is the most widespread taxon of the group and is distributed from the Amotape-Hunacabamba Zone in the north down to the Department Moquegua in the south.
The two large groups revised show opposite distribution patterns. The (often widespread) members of the *N. poissoniana* group are predominantly found in the inner Andean valleys of southern Peru. Besides that, few widespread and disjunct taxa occur, only one species is found in the Amotape-Huancabamba Zone. Conversely, the taxa of the *N. ranunculifolia* group show a rather northerly distribution with a rich diversity of (often narrowly endemic) taxa in the Amotape-Huancabamba Zone. Only a few, more widespread species are found in the drier Central Andes and only one species is widespread throughout almost the entire Peruvian Andes. The centers of diversity of both groups are thus on opposite extremes of the Peruvian Andes. Nevertheless, representatives of both groups have successfully exploited distant habitats and both groups have conquered large parts of the High Andes. One via the moister eastern slopes (*N. ranunculifolia* group) and one via the drier western slopes and dry valleys of the Andes (*N. poissoniana* group).

9.2 Taxonomic status of *Nasa*

The α-taxonomy of *Nasa* is now largely resolved. Copious herbarium material from all herbaria accessible has been evaluated and there are only some highly fragmented specimens left whose placement is uncertain. *Nasa* now comprises of c. 100 species and c. 40 subspecies (Fig. 9.1).
The revisions of the two species groups and descriptions of numerous new species and subspecies in *Nasa* (Henning & Weigend, 2009a, 2009b, 2011; Henning *et al*., 2009, 2011; - Chapters 2-4) extended the limits of the genus and revealed remaining collection-gaps in the Peruvian Andes. With the continuing agricultural exploitation of the High Andes and especially the construction of new roads towards and across the eastern slopes, new collections still have to be expected for some habitats, which are so far isolated from human access. This is particularly true for the *N. ranunculifolia* group, whose Andean radiation mainly took place in the moister, northern and eastern parts of the Peruvian Andes. Especially the *Nasa rugosa* complex in its present extent, with the isolated type collection in the Department Huánuco and three new subspecies from close-by habitats way up north, justify the expectation of additional taxa in the intervening area. The eastern slopes of the Peruvian Andes are the least known and poorly collected region of the whole country. Virtually every collecting trip in the Departments of Amazonas, San Martín, Huánuco, Pasco and the eastern parts of La Libertad yielded new material and in the case of the *N. ranunculifolia* group often new taxa. The discovery of *N. tulipadiaboli* and recent collections of *N. basilica* from San Martín indicate that spectacular new collections even of tall, monocarpic members of the group can be expected from areas unaccessible so far.

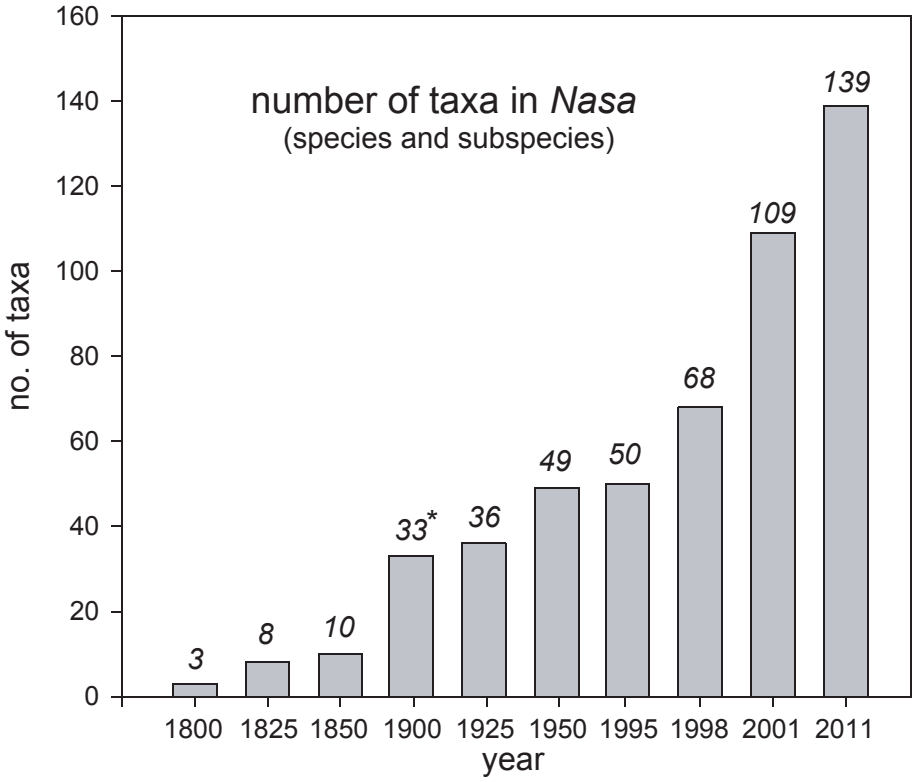

Fig. 9.1: Number of taxa recognized in *Nasa* (* impact of the "*Monographia Loasacearum*" by Urban & Gilg, 1900)

Conversely, the Central Andes and the western slopes nowadays can be reached with relative ease and their flora is fairly well documented. Nevertheless, the occurrence of narrowly endemic species in some isolated forest fragments cannot be ruled out. Especially High Andean and cloud forest species may still await their discovery in the leftovers of the cloud forest belt, which is highly fragmented and degraded by human settlements (e.g. *N. contumazensis* Weigend & E.Rodr. – Weigend & Rodríguez, 2003; *N. usquiliensis* Weigend, T.Henning & C.Schneid. – Weigend *et al.*, 2003; *N. sanagoranensis* T.Henning, Weigend & A.Cano – Henning *et al.*, 2009). In this case the *N. poissoniana* group could also be affected, since its distributional range covers large parts of the drier western slopes.

The Peruvian Andes are clearly the center of diversity of *Nasa* with a considerable concentration of taxon density in the Amotape-Huancabamba Zone. The southernmost outpost of the genus is represented by *N. urens* in the Lomas formations of northern Chile (Fig. 2.1). *N. chenopodiifolia* (Fig. 2.1) and *N. magnifica* (Fig. 4.5) reach the Department of Moquegua in southern Peru and *N. ferruginea* is reported from northern Bolivia close to the Peruvian border (Fig. 2.1). Species diversity thins out north of Peru with a descending number of taxa towards Colombia (Weigend, 2001a) and a few isolated outliers in Central America (*N. panamensis* Weigend – Weigend, 2002a). Due to drug traffiking and guerilla activities, botanical exploration of large parts of the Colombian Andes has long been virtually impossible. With a decrease of these activities, collecting trips may become possible in the near future and new records and likely new taxa may come to our knowledge.

However, with the studies here presented the bulk of the taxa of *Nasa* is likely formally described.

9.3 Floral ecology of Loasaceae subfam. Loasoideae

The Chapters 5 to 8 are focussed on the complex and fascinating pollination biology of the Loasaceae. Although the floral morphology was already analyzed in detail by Urban (1886, 1892) and Urban & Gilg (1900), the mechanisms and their significance in terms of the interaction with flower visitors remained hidden for a long time. That the plants perform a stamen movement had been reported repeatedly (Brown & Kaul, 1981; Leins & Winhard, 1973), but neither with an evaluation of its influence on reproductive biology nor in comparison with other aspects of the overall flower morphology.

First reports on the ecological context of stamen movement and nectar supply were provided by Schlindwein and colleagues (Harter *et al.*, 1995; Schlindwein & Wittmann, 1992, 1997b; Wittmann & Schlindwein, 1995; Schlindwein, 2000). They discovered that the stamen movement is thigmonastic, since it can (besides an autonomous movement) be triggered by nectar and pollen collecting bees. They provided some interesting case studies on a few melittophilous species of *Caiophora*, *Blumenbachia* and *Loasa* from southern South America (Brazil, Argentina and Chile). Their approach strongly focussed on the behaviour and reproductive strategy of the pollinating Colletid bees. They provided no satisfying explanation of this complex phenomenon regarding the cost-benefit ratio for the plants in the context of male fitness and outbreeding success. Pollen presentation via a thigmonastic stamen movement is best explained in the framework of the pollen presentation theory (Percival, 1955, Thomson *et al.*, 2000). Earlier works emphasized the increase in the proficiency of pollen donation as a major selective force guiding floral evolution (Lloyd & Yates, 1982; Lloyd, 1984). Pollen presentation theory unites numerous approaches that have outlined the importance of floral traits that determine the pollen pickup by pollinators and increase male fitness by adjusting pollen presentation to the quality and quantity of the pollinator (Harder & Thomson, 1989; Harder & Wilson, 1994, 1998; LeBuhn & Holsinger, 1998; Thomson, 2003). This can be ensured by, for example, sequential maturation of anthers of individual flowers ('packaging') or by providing the anther only with a small opening, releasing pollen in small portions ('dispensing'; Lloyd & Yates, 1982; Harder & Thomson, 1989; Thomson *et al.*, 2000). Pollen packaging mechanisms that use a stamen movement are reported from a number of plant families: Aizoaceae, Berberidaceae, Cactaceae, Cistaceae, Malvaceae, Portulacaceae and Tiliaceae (Kabsch, 1861; Unger, 1863; Juel, 1906; Bünning, 1959; Guttenberg, 1971; Jaffe *et al.*, 1977; LeBuhn & Anderson, 1994), although they are often isolated phenomena in the respective plant group. None of these examples has a complex flower morphology comparable to that of Loasaceae subfam. Loasoideae (e.g. nectar scales, boat-shaped petals that hide immature stamens) and the number of packages (i.e. anthers) is by far smaller. For species of Loasaceae subfam. Loasoideae average stamen numbers of 34 (*Plakothira* J.Florence) to 242 (*Scyphanthus* D.Don.) can be reported (Appendix D2, Tab. 8.3), with the vast majority of taxa having between 80 to 140 stamens and hence packaging units to be successively allocated. The mere number of packages and the fact that the complex flower morphology and a stamen movement, which is putatively thigmonastic, occurs throughout a relatively large subfamily, distinguishes Loasoideae from the other examples reported so far.

Since the first mention of thigmonasty by Schlindwein & Wittmann (1992) the number of species recognized in the subfamily has tripled (e.g. *Nasa*, Fig. 9.1) and especially the High Andes could be shown to be the hotspot of diversity for this group of plants (Chapters 2-4). The reported variability in terms of both, morphology and habitats successfully occupied by Loasaceae, thereby increased dramatically. The presentation of pollen via a triggered stamen movement and concomitant floral functions could not longer be seen as an unusual adaptation of a few Loasaceae to specialized Colletids. It is rather a complex floral trait that obviously improves the reproductive success of a whole plant group independent of a certain habitat or a single group of pollinators. Therefore a functional approach focussing on the detailed mechanisms, the consequences (costs and benefits) and the detailed occurrence of this "floral behaviour" in Loasaceae subfam. Loasoideae has been adopted (see Chapters 5-8).

9.3.1 Pollination in Loasaceae subfam. Loasoideae: complexity, schedule and costs

The exertion of influence on a successful pollen transfer and hence pollination in Loasaceae is based on two things: pollen presentation via stamen movements and nectar offered. Nectar is the main floral reward and it was shown that it is replenished and passively accumulates in the floral scales if pollinators are absent (Chapter 5). Nectar replenishment is quite common in angiosperms (Aizen & Basilio, 1998; Valtueña *et al*., 2007) and is known to be adjustable to actual visitation rates (Vickery & Sutherland, 1994; Castellanos *et al*., 2002; Hernández-Conrique *et al*., 2007). Pollen presentation generally reflects the overall pollination syndrome implemented (e.g. ornithophily, entomophily, etc.). In entomophilous plants it has a dual purpose, depending on the needs and respective behaviour of the flower visiting insect. Besides its sexual function it is an important floral reward, too, since it frequently serves as a food source for the pollinator itself and its brood (Proctor & Yeo, 1972; Faegri & van der Pijl, 1979) and is therefore often actively collected (Percival, 1955; Schlindwein & Wittmann, 2000).

For *Nasa macrothyrsa* (Urb. & Gilg) Weigend, *Neoxylocopa lachnea* (Apidae; Moure, 1951) was reported as the principle flower visitor and likely pollinator during field studies in North Peru in 2008 (Chapter 5). More than 50% of all flower visits received account to *Neoxylocopa*, followed by introduced honey bees (*Apis mellifera* L.) and hummingbirds. It was shown that *Neoxylocopa* is the only flower visitor which is successfully dusted with pollen due to its physique and its behaviour on the flower. The bees have to probe each of the five nectar scales in order to harvest the nectar, which is replenished constantly. In the process, they are dusted with pollen from anthers that already present their pollen in the center of the flower. Additionally, they cause the thigmonastic stamen movement by bending the floral scales outwards whilst harvesting nectar. *Neoxylocopa* is a trapliner and it was shown that 2/3rds of the flowers received a revisit within 10 minutes. The bees are then rewarded with little, less concentrated nectar and are on average dusted with the pollen of 1-2 stamens that have meanwhile moved and are presenting their pollen in the center of the flower. Competition between the pollinators forces the bees to head for as many flowers as possible. The thigmonastic stamen movement in *Nasa macrothyrsa* will provide a small package of pollen almost immediately after a previous visit. Conversely,

nectar is offered in increasing amounts, depending on the time lag to the previous visit. The deposition of small pollen packages by an accelerated pollen presentation via a thigmonastic stamen movement is thus ensured, whereas the amount of nectar received varies depending on the visitation interval. If no or only small amounts of nectar are available, the bees are likely motivated to move on to more distant flowers and hence outcrossing is promoted (Cresswell, 1990; Biernaskie *et al.*, 2002; Johnson *et al.*, 2004; Jersákova & Johnson, 2006).

This case study revealed mainly three things: Firstly, the pollination system as described by Schlindwein & Wittmann (1997b) is not an exclusive, extreme case of specialization between Colletids and Loasaceae, since *Neoxylocopa* belongs to a different group of Hymenopterans (Apidae). Secondly, with the observation of *Apis* and hummingbirds as nectar robbers it can be assumed that "unspecialized" and in the case of *N. macrothyrsa* ineffective flower visitors are not successfully excluded. This indicates that the possibility to recruit different pollinator taxa is implicit in this system. Specialization is not necessarily as mutual as initially described for *Caiophora arechavaletae* (Urb.) Urb. and *Perditomorpha pampeana* Urban, 1995 (Schlindwein & Wittmann, 1997b) and is likely an asymmetrical specialization on the part of the pollinator (Vázquez & Aizen, 2004). That signifies, thirdly, that "a rapid pollen presentation will likely increase male fitness, irrespective of the degree of pollinator specialization and irrespective of whether the flower visitors collect pollen, or nectar, or both" (Henning *et al.*, 2012, Chapter 6). This assumption is passively supported by the report of up to six different flower visitors (three for *C. arechavaletae*) in nine species of Loasaceae by Schlindwein & Wittmann (2000) without any explanatory approach.

These findings are supported by a broader sampling of species of Loasaceae subfam. Loasoideae conducted subsequently (Chapter 6). An additional set of 5 species of Loasaceae (3 each from the genera *Nasa* - incl. *N. macrothyrsa* - and *Caiophora*) was analyzed. The selection criteria for the sampling were pollination mode and breeding system. One facultatively autogamous, annual and two facultatively xenogamous, perennial species from each genus were chosen and one ornithophilous species from each genus was included. The results underline the previous findings that an accelerated pollen presentation via thigmonastic stamen movement in the sense of pollen packaging is advantageous, irrespective of the type of pollinator. It is furthermore largely indepedent of the breeding system and seems to increase outbreeding success, whether facultative selfing leads to fruit set by means of a backup mechanism or not.

The comparison of several varieties of the "floral behaviour" of Loasaceae subfam. Loasoideae, in terms of breeding system and pollinator taxon recruited, and how they are realized in detail revealed one remarkable result: It is obviously successful, particularly complex, but anything but cheap! Pollen to ovule ratios (P/O ratios) are an indicator commonly used to describe the efficiency (cost/benefit ratio) of a particular breeding system (Cruden, 1977, 2000; Cruden & Jensen, 1979; Aedo *et al.*, 2005, 2007). Loasaceae show extraordinarily high P/O ratios (c. 3000-8750) compared to those generally valid for plants with a similar breeding system (McDade, 1985; Gallardo *et al.*, 1994; Lopez *et al.*, 1999; Wyatt *et al.*, 2000; Jürgens *et al.*, 2002; Wang *et al.*, 2004), independent of the minor differences that could be determined between the species studied.

In general, the overall pollination system found in a certain species of Loasaceae subfam. Loasoideae is reflected by a modification of floral traits as follows:

> Nectar amount and nectar composition reflect the requirements of the corresponding pollinator and is adapted quickly.
>
> The amount of pollen produced by a flower is influenced mainly by anther number and size and rather depends on the overall flower size (e.g. *N. ranunculifolia*).
>
> The speed of pollen presentation reflects the type of pollinator observed and is slowest in ornithophilous taxa.
>
> The P/O ratios vary less, rather reflect the overall breeding system and are likely under a phylogenetic constraint.

This means that the efficiency in terms of the ressources that have to be invested by these plants is generally high compared to plants with similar pollination systems. The "investment costs" are primarily caused by the great number of pollen grains per flower. Compared with other (facultative) xenogamous plant species the P/O ratios revealed are high, although Loasaceae have a comparably high number of ovules (Tab 6.1, Chapter 6). The "typical range" of P/O ratios for plants with this breeding system was based on a comprehensive evaluation of pollen and anther numbers troughout a wide range of angiosperm families (Cruden, 1977, 2000). Most of these plants have one (e.g. *Senecio* L. – Asteraceae, P/O ratio: c.70-240, Lawrence, 1985), few (e.g. *Caulophyllum* Michx. – Berberidaceae, P/O ratio: c. 2100-5300, two ovules, Hannan & Prucher, 1996) to a moderate number of ovules (e.g. *Vaccinium* L. – Ericaceae, P/O ratio: c. 780-1700, 21-97 ovules, Jaquemart, 1997). Hence, pollen grain number in these flowers varies between 70 (*Senecio*) and 2650 (*Caulophyllum*). A comparable "expensive" floral investment is only reported from *Solanum* L. (Solanaceae) with P/O ratios between c. 750-24000 and ovule numbers of 20-370. Nevertheless, the highest number of pollen grains reported in this study is c. 1 x 10^6 (Mione & Anderson, 1992). Loasaceae in general show P/O ratios that are high, but still in the typical range of the respective breeding system (e.g. *Nasa ranunculifolia*: P/O: 7144). In order to assess the energetic effort invested by the plants one has to look at both factors of this ratio separately. Ovule and pollen development is costly and flowers of Loasaceae have plenty of both (e.g. *Nasa ranunculifolia*: ovules 1247, ø pollen grains per flower c. 8,9 x 10^6, Chapter 6).
The adaptation of the pollen dispensation to the respective pollinator is fine-tuned by the adjustment of nectar quality and quantity and the timing of pollen presentation. Nectar replenishment, stamen movement and overall flower longevity in Loasaceae, as illustrated in Chapters 4-8, require additional energetic efforts. Although these patterns vary remarkably between the species studied, the observed increase in complexity and specialization is not paralleled by a decrease in the overall energetic investment employed by the plants.

9.3.2 Thigmonastic stamen movement: regulation, patterns and presence

Nectar production and composition related to pollinators observed in Loasaceae subfam. Loasoideae has already been comprehensively studied (Ackermann & Weigend, 2006). To which degree the plants exert influence on the pollinator effectiveness by modulation of the detailed pollen presentation is experimentally examined in Chapters 7 & 8. Passive and active mechanisms could be determined that regulate the stamen movement (and hence pollen presentation) in accordance to the actual pollinator availability and the frequency of their flower visits. Stamen movement is controlled by a multi-channel system that reduces pollen wastage and is targeted on the dispensation of as much pollen packages as possible on as much different flower visitors as possible. The autonomous and thigmonastic stamen movements are positively correlated to both, light and temperature. Low temperatures and darkness negatively affect the ability of the flowers to perform a stamen movement. These abiotic factors have the strongest effect in combination and autonomous stamen movement comes to a virtual standstill in the dark at low temperatures (in *Nasa macrothyrsa* – see Chapter 7, Tab. 7.1, Fig. 7.2). Autonomous and thigmonastic stamen movement rates are dramatically increased at higher temperatures and daylight. Hence, the fundamental conditions for the rough adjustment of pollen presentation are distinct differences in light intensity and ambient temperature as found in the natural habitat as a result of the alternation of day and night. If daylight and the respective temperature is given, the rate of pollen presentation exhibits a minimal value in absence of any flower visitor by autonomously presented stamens. The value of this stamen movement is c. twice as high as the autonomous stamen movement in darkness and more than 60 times as high as in the dark at low temperatures (Tab. 7.1, Fig. 7.2). If ideal conditions for both, stamen movement and pollinator activity are given (daylight, 22°C), *Nasa macrothyrsa* is able to perform an accelerated pollen presentation via a thigmonastic stamen movement to an astonishing rate. This is done with a high degree of fine-tuning onto the actual visitation rates of their pollinator. The pollinators (Carpenter bees, *Neoxylocopa lachnea* Moure, 1951) trigger a stamen movement by bending the nectar scales outwards whilst harvesting nectar as described earlier (9.3.1). There appears to be no direct spatial correlation between the stamen bundle providing the thigmonastic stamen and the localization of the stimulus. The stimulus is apparently transmitted to all stamen fascicles via the receptacle, leading to a reaction in a determined number of stamens from all fascicles (Chapter 7, Tab. 7.4, Fig. 7.9). Depending on the visitation frequency, the stamen movement rate can be more than quadrupled compared to the autonomous stamen movement (1.54 stamens/h). Longer intervals thereby lead to fewer stamens presented (e.g 180 min = 1.83 stamens/h) than shorter intervals (30 min = 6.78 stamen/h). These findings are supported by data of three other species of Loasaceae, which were tested with shorter visiting intervals. The data indicate that even shorter intervals down to 10 minutes between two visits can further accelerate the pollen presentation in some species. Furthermore, only minor differences in the possible stamen movement rate throughout the staminate phase could be revealed (cf. *Nasa urens*, Chapter 7, Fig. 7.7, 7.8). The deceleration and acceleration of stamen movement thereby influences the overall floral longevity notably. For *N. macrothyrsa,* a reduction of the duration of the staminate phase from 6 days down to 2 days can be demonstrated. The carpellate phase varies between c. 1 day and c. 4 days depending on a successful pollination

(Chapter 7, Fig. 7.3). Hence, the temporal condensation of the staminate phase by 2/3rds can be extrapolated to the whole anthesis of a single flower that varies between c. 10 days if pollinators are scarce and c. 3 days under ideal conditions in terms of pollinator abundancy and successful pollination. Pollen presentation via stamen movement (autonomous and thigmonastic) is passively influenced primarily by light and temperature and to a lesser extent by the individual age of a flower. The thigmonastic stamen movement can actively be adjusted to the actual abundance and the visitation frequency. Thereby not only the actual pollen presentation rate, but also the overall flower longevity varies which is furthermore determined by the length of the carpellate phase. The duration of the carpellate phase results from the lapse of time between the moment when the stigmatic area becomes receptive and the successful pollination, which leads to a rapid decay (< 1 day) of the flowers.

These are the general mechanisms of the process of stamen movement in Loasaceae subfam. Loasoideae and they should be generally valid for constituent taxa that are capable of performing a thigmonastic stamen movement.

That this capability results from a stepwise development and shows some intermediate stages could be shown for Loasoideae by comparing its characteristics for 45 taxa from 10 genera (out of 13) (Chapter 8). Taxa from two of the three tribes recognized in Loasoideae (Weigend, 1997; Weigend *et al*., 2004; Hufford *et al*., 2005) could be analyzed, only the monotypic *Kissenia capensis* Endl. (Kissenieae, South Africa) was not successfully brought into cultivation. Tribe delimitation of Loasoideae still is only unsatisfactorily understood (Fig. 9.2) and only the monophyly of Loasoideae and Klaprothieae could be retrieved by molecular data repeatedly (Weigend *et al*., 2004; Hufford *et al*., 2003, 2005). The placement of *Kissenia* remains doubtful and it is likely a member of the Klaprothieae (Weigend *et al*., 2004, Fig. 9.2). Furthermore, Loasoideae have informally been subdivided into the “Lower" and "Higher” Loaseae. The “Lower Loaseae” originally comprised some genera, namely *Huidobria* Gay, *Chichicaste* Weigend and *Presliophytum* (Urb. & Gilg) Weigend (incl. *Loasa* Adans. ser. *Malesherbioideae*), with a basal placement outside the Klaprothieae either as part of or as sister to the rest of the Loaseae (Weigend *et al*., 2004). The term “Lower Loaseae” was introduced by Weigend (1997) to compile taxa that lack coloured floral scales and thigmonastic stamens. Weigend *et al*. (2004) already noticed the presence of coloured nectar scales in *Loasa malesherbioides* Phil. and the observation of thigmonastic stamens in *Presliophytum* spp. and suggested their inclusion into the “Higher Loaseae” (Fig. 9.2). All other genera (*Aosa* Weigend, *Blumenbachia* Schrad., *Caiophora* C. Presl., *Loasa* (excl. *L*. ser. *Malesherbioideae*), *Nasa* and *Scyphanthus* D.Don) belong to the “Higher Loaseae”, a clade well supported by morphological and molecular data (Weigend, 1997; Weigend *et al*. 2004).

Hence, there is uncertainty regarding the basal nodes of Loasoideae, the placement of the two tribes and their constituent genera. A thigmonastic stamen movement is now shown for all genera examined with the exception of *Huidobria* (two species, Chile, Grau, 1997). *Huidobria fruticosa* Phil. is the only species included in the analyses (Chapter 8) that shows neither a significant increase in the amount of stamens moved after a stimulus of the floral scales nor a reaction in terms of a significant shift of the overall movement observed towards the stimulus (Tab. 8.1, 8.2, Fig. 8.2 C). *Huidobria* obviously represents an intermediate developmental stage by being able to move its stamens and present its pollen successively but not thigmonastically triggered by a flower visitor. Throughout all other

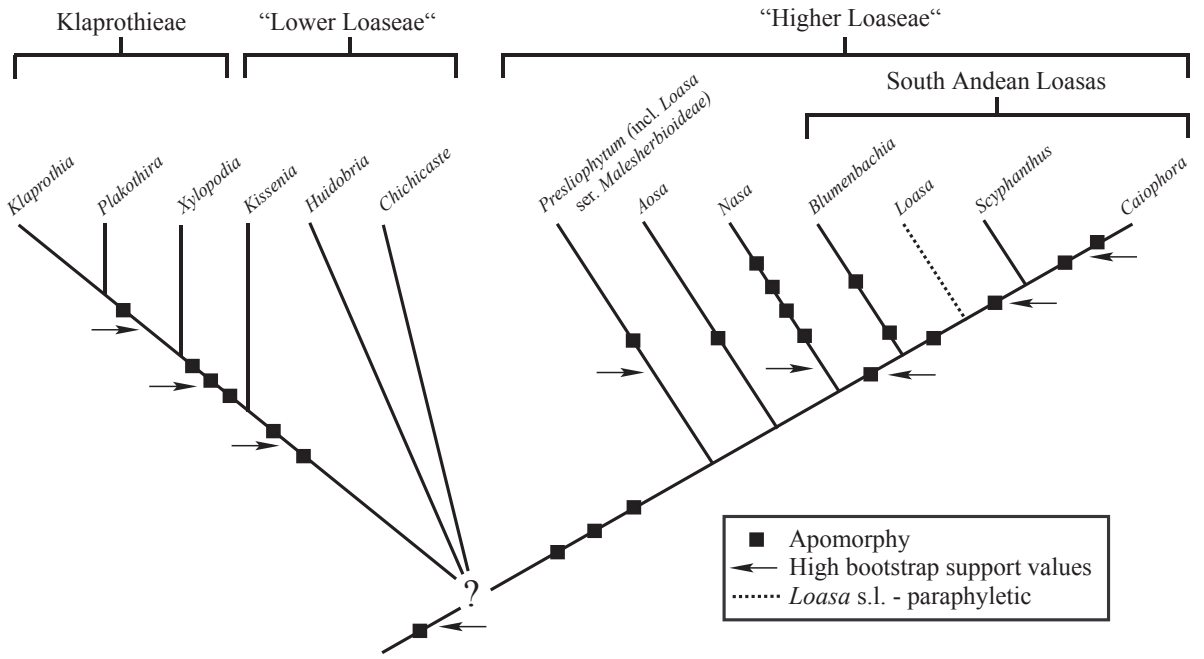

Fig. 9.2: Annotated cladogram of Loasaceae subfam Loasoideae, simplified and modified from Weigend *et al.*, (2004). The placement of Klaprothieae and "Lower Loaseae" is uncertain. Note the lack of support (by both morphological and molecular data) for *Huidobria* and *Chichicaste*.

genera examined thigmonasty occurs either by a significant increase of the movement following a stimulus or by a significant shift of overall movement activity towards this stimulus. The overall movement patterns observed are informally assigned to four different types that could roughly be identified (Chapter 8, Tabs. 8.1, 8.2, Figs. 8.2-8.5). *Huidobria* represents type one, where no thigmonasty was provable. Type two is characterized by a moderate, numerical increase in stamen movement activity that peaks only shallowly or not at all. This movement type is found in the two species of Klaprothieae included (*Plakothira parviflora* J.Florence and *Xylopodia klaprothioides* Weigend), *Aosa rupestris* (Gardner) Weigend ("Lower Loaseae") and five species of *Nasa* and *Caiophora*. The third type is so far exclusively found in *Presliophytum* spp. and is characterized by a delayed peak of the otherwise highly significant thigmonastic stamen movement. Type four is the most widespread type identified and typical for the remaining taxa of "Higher Loaseae" from the genera *Blumenbachia*, *Caiophora*, *Loasa*, *Nasa* and *Scyphanthus*. These plants show a strong response to a stimulus in terms of both, a significant increase in the amount of stamens presented and a significant shift of this movement towards the stimulus, graphically represented by a dinstinct peak in the movement curve.

The four basic types observed reflect not only the phylogenetics of Loasaceae. They also revealed particularities of single lineages in stamen movement that likely reflect pollination syndrome and breeding system. Although one standard experiment allows no far reaching conclusions and the complexity of the plant-pollinator interaction should be analyzed in detail for any species separately, some consistent patterns are striking. For example, both species of *Presliophytum* are characterized by a similar movement schedule that is likely linked to their pollination system. These plants are profusely flowering shrubs that are (among others) predominantly visited by butterflies, which have so far not been reported as pollinators of any other Loasaceae. Both species perform a similar thigmonastic movement and this is likely an adaptation to their pollinator, exceptional for Loasaceae.

Fig. 9.3: Flowers of two species with conspicuous stamen movement patterns. A: Flower of *Nasa chenopodiifolia* (Weigend 8433, cultivated plant), note the degenerated petals with irregular shapes and sizes and the reduced number of stamens. These plants develop predominantly imperfect flowers that are often truly cleistogamous and set seed without spreading the degenerated petals. B: Geoflorous flower of *Caiophora coronata* (Weigend 9152, cultivated plant) which is principally visited by small rodents (Photograph kindly provided by M. Ackermann).

Furthermore, and at first glance surprising, type two unites taxa from two rather distantly related lineages. Taking breeding system and pollination syndrome into account, it can be assumed that signals from phylogeny and floral ecology overlap here and independently cause similar overall movement patterns. On the one hand, type two comprises three genera with a basal placement either in the Klaprothieae (*Plakothira* and *Xylopodia*) or at the base of the "Higher Loaseae" (*Aosa rupestris*) and their movement patterns likely reveal a continued gradual development beginning with patterns observed in *Huidobria*. On the other hand, some taxa fall into this category that can clearly be assigned to the "Higher Loaseae" but show a divergent stamen movement. Two of these taxa differ from their closest relatives by being either selfing (to truly cleistogamous - *Nasa chenopodiifolia*, Henning & Weigend, 2009b) or rodent-pollinated [*Caiophora coronata* (Gillies ex Arn.) Hook. & Arn., Cocucci & Sercic, 1998]. These two reproductive traits are so far known to be unique in Loasaceae and likely responsible for the divergent patterns observed. In the case of *N. chenopodiifolia* (Fig. 9.3 A) the loss of thigmonasty is in accordance with "Baker´s rule" (Baker, 1965), which predicts generalization of pollination modes (or, as in *N. chenopodiifolia*, a breeding system that does not require pollinators at all) in colonizing, weedy species. It is obvious that a repeated thigmonastic pollen presentation is redundant in a completely selfing species. The loss of thigmonasty in *C. coronata* (Fig. 9.3 B), as claimed by Cocucci & Sercic (1998) is hereby disproved. The increase in stamen movement activity after a stimulus is highly significant, although it shows an unusual course. The plants are predominantly visited at night by small, nocturnal rodents (*Graomys grisoflavus*, Muridae, Cocucci & Sércic, 1998). Fielddata would be clearly desirable to compare the findings of the regulativ mechanisms (Chapter 7) and species-dependent movement patterns (Chapter 8) with observations on the behaviour of *Graomys* and its effictiveness in terms of a successful pollination. For two of the other three taxa [one accession of *C. cirsiifolia* C. Presl. and *N. picta* (Hook.F.) Weigend] no additional data regarding the detailed pollination ecology is available and it would be highly speculative discussing the patterns observed in a broader context. The third species, *Nasa dillonii* Weigend (*Nasa* ser. *Alatae*) represents one of three

ornithophilous lineages included in this analysis (Weigend & Gottschling, 2006). Whereas thigmonasty was observed for the other two lineages included [*N. ranunculifolia* (= *Nasa* ser. *Grandiflorae*, see Chapters 4, 6 & 8) and two species of *Caiophora* (*C. carduifolia* C. Presl. & *C. canarinoides* (Lenné & K.Koch) Urb. & Gilg)], *N. dillonii* likely shows a beginning regression of thigmonasty as already alleged for ornithophilous *C. hibiscifolia* (Griseb.) Urb. & Gilg and rodent-pollinated *C. coronata* (Harter *et al*., 1995; Cocucci & Sercic, 1998). In *N. dillonii*, thigmonasty can only be detected by the significant cumulative movement directly after a stimulus and the resulting peak, since a stimulation of the floral scale leads to no significant numerical increase in overall stamen movement. Nevertheless, *N. dillonii* shows a thigmonastic movement of mature stamens into the center of the flower triggered by a pollinator.

Thigmonasty seems to be a competitive advantage for the vast majority of Loasaceae examined so far, irrespective of pollination syndrome and breeding system (Chapters 5 & 6) and is now documented for 43 species from 10 genera.

9.4 Synopsis: a key invention and the conquest of Andean South America

The data here presented provide an overview of the presence and patterns of the thigmonastic stamen movement generally observed throughout Loasaceae subfam. Loasoideae. Since the taxonomic focus of this work targets towards *Nasa* (Chapters 2-4), the availability of plant material for cultivation strongly correlates with collections obtained by own field trips. The studies on floral ecology (Chapters 5-8) thus also focus on the large genus *Nasa*, but have been extended to as much taxa as possible from all genera available. The broad taxon sampling allows some comparative conclusions of the evolution of the complex pollination systems in Loasaceae (Chapter 8).

The work here presented provides strong evidence that the multifaceted reproductive ecology and taxonomic diversity of Loasaceae is centered around their complex flower morphology and floral behaviour. This assumption is supported by the fact that the complexity of all characters involved and the taxonomic diversity increase parallel in the phylogeny of the family towards the derived clade of Loasoideae. Seven relatively species-poor, often monotypic genera (except *Mentzelia* – c. 80 spp., a widespread, ecologically divergent lineage, Weigend, 2007) in the other three subfamilies recognized (Petalonychoideae, Gronovioideae and Mentzelioideae) show no stamen movement. Conversely, Loasaceae subfam. Loasoideae comprises 13 genera, amongst three of the largest and most diverse (*Nasa* – c. 140 spp. and ssp., *Caiophora* c. 50 spp., *Loasa* c. 35 spp.), and contains more than 70% of the c. 350 taxa of Loasaceae. Within Loasoideae, species diversity increases with complexity of floral morphology and behaviour. *Nasa,* for example, shows a tremendous diversity in various characters. This is true for overall morphology and distribution (Chapters 2-4) as well as for particular floral traits (Chapters 5-8). Morphological diversity and biogeography of *Nasa* is reflected in its taxonomic extent, which is now largely understood by the resolved α-taxonomy. Among other traits (e.g. flower morphology – Weigend & Gottschling, 2006; nectar – Ackermann & Weigend, 2006) the floral behaviour and the diverse patterns of stamen movement illustrated here again underline the causal link between the complex floral ecology and the noticeable diversity observed in *Nasa*. The

genus is subject to an ongoing radiation in Andean South America. Its floral behaviour is advantageous in spite of the type of pollinator recruited and is obviously efficient in terms of a successful pollination and outbreeding success. But it is also flexible enough as to enable the plants to make use of different pollinators in different habitats and quikly adapt to their requirements. These evolutionary transitions likely favour floral radiation and speciation as generally believed (Hodges, 1997) and also described for other tropical plants (e.g. *Disa* – Orchidaceae, Johnson *et al.*, 1998; *Dalechampia* – Euphorbiaceae, Armbruster, 1993). The innovation of a thigmonastic stamen movement in Loasoideae is a key invention and has promoted their adaptive radiation. This is particularily evident for *Nasa*, whose radiation mainly took place in the High Andes and its fragmented habitats. The high number of endemic species (Henning *et al.*, 2009; Weigend, 2002b, 2004b,c; Weigend *et al.*, 1998, 2005; Weigend & Rodríguez, 2001, 2002, 2003) and the inevitable description of several new taxa at subspecies level (Dostert & Weigend, 1999; Henning & Weigend, 2009a; Henning *et al.*, 2011 - Chapters 2 & 4) reveal these rapid adaptive divergences in floral ecology in allopatric populations.

On the basis of the studies presented above, further work is clearly encouraged, addressing unresolved questions regarding the evolution of Loasaceae. Well-founded phylogenies of both, the major clades of Loasaceae and subfam. Loasoideae in general and single genera in particular, using latest molecular approaches can now be sensibly accomplished based on, and substantiated with, the findings of taxonomy and ecology. Especially the evolution of *Nasa* and its floral biology is not satisfactorily understood in terms of a resolved phylogeny. Comprehensive molecular datasets using different markers (ITS, *trn*S-G, *trn*L-F, *psb*A-*trn*H, *rps*16) are in progress but show inconsistent topologies and contain dubious groupings of taxa. This is partly due to a lack of high-quality samples of species difficult to obtain, but crucial for a reliable identification of clades and evolutionary trends. These efforts will be continued in the near future and will also be based on the knowledge of taxonomy and floral ecology compiled here. The identification of evolutionary trends in the reproductive biology of Loasoideae may also enable us to clarify the composition and placement of the major clades of Loasaceae as indicated by the divergent patterns in floral function of *Huidobria fruticosa* illustrated above. Loasaceae offer the opportunity to combine various datasets that cover their diversity in morphology, distribution and ecology with molecular findings and vice versa. This will lead to a comprehensive understanding of the evolution of the family and the significance of floral ecology and behaviour which would be difficult to achieve by a single approach. Most importantly, this multidisciplinary approach will reach beyond generic limits and cover a broad range of morphological character states as well as distributional and ecological patterns. It will therefore provide a valuable contribution to similar interdependend studies at species level (e.g. *Salvia* L. - Lamiaceae, Claßen-Bockhoff *et al.*, 2003, 2004; Claßen-Bockhoff & Wester, 2007; Walker & Sytsma, 2007, Walker *et al.*, 2004; *Penstemon* Mitch. - Scrophulariaceae, Datwyler & Wolfe, 2004; Wilson *et al.*, 2004; Wolfe *et al.*, 2006).

Loasoideae and especially *Nasa* succeeded by "The conquest of South America" (Weigend, 1997). Their complex floral behaviour illustrated here was both, the source and the driving force for the adaptive radiation and the colonization of various habitats in (Andean) South America.

Zusammenfassung

Systematik und Blütenökologie von Nasa (Loasaceae subfam. Loasoideae) und verwandter Arten

Nasa Weigend ist die größte Gattung innerhalb der größtenteils neotropischen Loasaceae (Cornales). Ihre Verbreitung erstreckt sich vor allem über die Anden Südamerikas mit einigen isolierten Arten die bis nach Zentralamerika reichen. Im Zentrum dieser Arbeit stehen zwei Aspekte, die zunächst separat in einzelnen Kapiteln bearbeitet werden und anschließend in einen funktionellen und evolutiven Zusammenhang gesetzt werden. Nach einer allgemeinen, themenübergreifenden Einleitung (Kapitel 1) beschäftigen sich die folgenden drei Kapitel (Kapitel 2-4) mit der α-Taxonomie der Gattung. Es werden zwei Artgruppen revidiert, deren Umfang dank eigener Aufsammlungen von mir und meinen Kollegen in den letzten Jahren deutlich wurde. Desweiteren werden zusätzlich zwei neue Arten beschrieben, die die morphologische Variabilität von *Nasa* zusätzlich erweitern und damit den Umfang der Diversität der Gattung abschließend definieren. Der zweite Teil der Arbeit besteht aus mehreren Studien zur Blütenbiologie von *Nasa* und anderen Gattungen der Loasaceae subfam. Loasoideae. Die Unterfamilie grenzt sich von den übrigen Vertretern der Loasaceae durch das Vorhandensein einer komplexen Blütenmorphologie ab, die einige funktionelle Besonderheiten aufweist. Unter Anderem zeigen die Pflanzen eine Bewegung der Staubblätter aus den kahnförmigen Petalen ins Blütenzentrum. Vor etwa 20 Jahren wurde entdeckt, dass diese Bewegung bei einigen Vertretern durch das Verhalten der Bestäuber ausgelöst werden kann während diese zur Nektaraufnahme bestimmte Blütenorgane bewegen (sog. Nektarschuppen). Die allgemeine Blütenmorphologie ließ vermuten, dass es sich dabei um ein eher weitverbreitetes Phänomen innerhalb der Loasoideae handeln könnte. Der Artenreichtum einiger Gattungen, allen voran *Nasa*, deutet wiederum an, dass die komplexe, funktionelle Blütenbiologie Teil einer erfolgreichen Reproduktionsstrategie ist und eine zentrale Rolle bei der Radiation einiger Gattungen in den südamerikanischen Anden gespielt haben könnte.

Die beiden Themen werden zunächst separat bearbeitet, wobei die einzelnen Kapitel Beiträgen entsprechen, die bereits in Fachzeitschriften veröffentlicht wurden, bei diesen eingereicht sind oder in Kürze eingereicht werden. Dies ist den jeweiligen Fußnoten zu Beginn eines Kapitels zu entnehmen. Jedes Kapitel enthält dementsprechend eigene Abschnitte zur Einleitung, zu den Materialien und Methoden, Ergebnissen und eine Diskussion. Kapitel 9 diskutiert zusammenfassend die Ergebnisse der Kapitel 2-8 und stellt sie in einen gemeinsamen Zusammenhang. Diese Synopsis der Erkenntnisse aus der Systematik und Ökologie bildet den Abschluss der vorliegenden Arbeit, der folgende wissenschaftliche Fragestellungen zu Grunde lagen:

1. Ist die Interaktion mit spezialisierten Bestäubern so eng wie frühere Studien implizieren?
2. Welche regulatorischen Mechanismen liegen der autonomen und thigmonastischen Staubblattbewegung zu Grunde?

3. Kann anhand bestehender Phylogenien und den Ergebnissen aus der vergleichenden Analyse der Staubblattbewegung erkannt werden, wann diese innerhalb der Loasaceae subfam. Loasoideae entstanden ist?
4. Lassen sich unterschiedliche Merkmalsausprägungen der thigmonastischen Staubblattbewegung feststellen und gibt es eine graduelle Zunahme der Komplexität, die sich phylogenetisch nachvollziehen lässt?
5. Steht die komplexe Blütenbiologie und Interaktion mit den Bestäubern für die Pflanzen in einem günstigen Kosten-Nutzen Verhältnis und ist sie in jedem Fall lohnenswert?
6. Kann ein sekundärer Verlust der Fähigkeit zur thigmonastischen Staubblattbewegung bestätigt werden, wie er bereits für zwei Arten von *Caiophora* beschrieben wurde, die von Kolibris bzw. Kleinsäugern bestäubt werden?
7. Ist die thigmonastische Staubblattbewegung eine "key-invention" und hat sie zur großen Diversität und weiten Verbreitung der Loasoideae beigetragen?

Um diese Fragen zu beantworten wurden sowohl klassisch-botanische Methoden zur Klärung der α-Taxonomie, als auch zahlreiche experimentelle Ansätze zur Untersuchung der mannigfaltigen Aspekte der Blütenbiologie genutzt.
Kapitel 1 stellt einleitend die hier behandelte Pflanzengruppe vor, beleuchtet grundlegende Aspekte ihrer Ökologie, formuliert eine wissenschaftliche Hypothese und listet die Ziele der Arbeit auf. Es wird desweiteren erläutert, welche Ansätze zur Bearbeitung der Themenkomplexe gewählt wurden und eine Übersicht über die Gliederung der Arbeit gegeben.
Die Kapitel 2 bis 4 widmen sich der Systematik von *Nasa*. Zwei infragenerische Gruppen konnten erkannt werden und ihr taxonomischer Umfang wird nach aktuellem Kenntnisstand in ausführlichen Revisionen dargestellt. Diese Arbeiten erfolgten mit Hilfe der umfassenden Bearbeitung von Herbarmaterial, eigenen Feldstudien und durch Kultivierung zahlreicher Sippen in den institutseigenen Versuchsgewächshäusern.
In Kapitel 2 wird die morphologisch schwer zu definierende *Nasa poissoniana* Gruppe revidiert, deren Monophylie zunächst in molekularen Studien erkannt wurde. Die Gruppe zeichnet sich durch eine hohe Variabilität in zahlreichen morphologischen Merkmalen aus, die innerhalb von *Nasa* ihrerseits plesiomorphen Charakter haben. Entsprechend den molekularen Erkenntnissen wurde eine Art, *N. raimondii* (ehem. *N. stuebeliana* Gruppe), nun der *N. poissoniana* Gruppe zugeordnet. Die namensgebende *N. poissoniana* zerfällt nun in zwei disjunkte Unterarten [subsp. *poissoniana* (Urb. & Gilg) Weigend und subsp. *glandulifera* T.Henning & Weigend], die an dieser Stelle neu beschrieben werden.
Kapitel 3 ist teilweise ein Nachtrag zu Kapitel 2, da hier zwei Arten von *Nasa* neu beschrieben werden, von denen eine eindeutig der zu diesem Zeitpunkt kürzlich revidierten *N. poissoniana* Gruppe zugerechnet werden muss. Zwei Aufsammlungen dieser neuen Art erreichten mich kurz nach dem Erscheinen der Revision und konnten nicht mehr in diese einfließen. Die zweite in diesem Kapitel neu beschriebene Art ist *N. sanchezii*, eine isolierte, gut dokumentierte Art aus NordPeru. Sie konnte bisher weder morphologisch noch molekular zufriedenstellend positioniert werden und wird hier zunächst formal beschrieben.
Kapitel 4 ist eine Revision einer der größten und interessantesten Artengruppen in *Nasa*. Die *Nasa ranunculifolia* Gruppe konnte erst erarbeitet werden nachdem während zahlreicher Feldreisen genügend, und vor allem qualitativ hochwertiges Material zu Ihrer Evaluation zur Verfügung stand. Die Mitglieder dieser Gruppe sind Teil der hochandinen Klade *Nasa* ser. *Grandiflorae* und sind vor allem in den Puna- und Paramohabitaten Perus beheimatet. Neben

zwei neuen Arten (*N. basilica* und *N. tulipadiaboli*) wurde für zwei Arten (*N. ranunculifolia* und *N. rugosa*) ein Unterartkonzept angewendet, um der graduellen morphologischen Variabilität und den vorgefundenen Verbreitungsmustern gerecht zu werden. Dabei wurden mehrere bereits beschriebene Arten neu bewertet (*N. ranunculifolia*, *N. macrantha*, *N. cymbopetala* und *N. macrorrhiza*) und auf Unterartrang in *N. ranunculifolia* eingezogen, die zusätzlich um weitere fünf Unterarten ergänzt wurde (subspp. *pamparomasii*, *guzmangoensis*, *bolivarensis*, *patazensis* und *huanucoensis*). Die nah verwandte, vor allem ostandine *N. rugosa* enthält nach der nun vorliegenden Bearbeitung ebenfalls vier Unterarten, die morphologisch und geographisch unterschieden werden können. Zusammen mit Kapitel 2 und 3 stellt die Revision dieser Gruppe den Abschluss der α-taxonomischen Bearbeitung von *Nasa* dar.

Die Kapitel 5-8 beschäftigen sich mit der Blütenbiologie der Loasaceae subfam. Loasoideae. Kapitel 5 wurde im Sinne einer Fallstudie durchgeführt. Anhand von Daten aus Feld- und Gewächshausexperimenten wurde die komplexe Interaktion der Pflanzen und ihrer Bestäuber untersucht. Exemplarisch wird der Ablauf der Bestäubung und die funktionelle Blütenbiologie am Beispiel von *Nasa macrothyrsa* und ihrer Blütenbesucher (v.a. Holzbienen der Art *Neoxylocopa lachnea*) dargestellt und in einen größeren theoretischen Zusammenhang (pollen presentation theory) gesetzt und diskutiert.

Kapitel 6 befasst sich darauf aufbauend mit dem Kosten-Nutzen Verhältnis der funktionellen Blütenbiologie der Pflanzen. Um eine allgemeingültige Aussage treffen zu können, wurden mehrere Arten zweier Gattungen (*Nasa* und *Caiophora*) untersucht. Die Arten wurden so ausgewählt, dass jeweils fakultativ autogame annuelle und fakultativ xenogame perennierende Arten beider Gattungen vertreten waren, die zusätzlich eine andere Bestäubergilde nutzen (Kurz- und Langzungenbienen und Kolibris). So konnten Unterschiede und Anpassungen an die jeweiligen Bestäubungssysteme und etwaige Überlagerungen dieser Anpassungen durch phylogenetische Zwänge erkannt und diskutiert werden.

Der generelle Ablauf der Staubblattbewegung und die Möglichkeiten der Pflanzen diesen zu steuern sind Gegenstand von Kapitel 7. Mehrere abiotische Faktoren konnten als passive Steuerungsmechanismen experimentell nachgewiesen werden. Desweiteren wurde der Einfluss des Blütenalters, die räumlichen Details der Reizabgabe durch den Bestäuber und die Möglichkeit der Anpassung der Bewegungsgeschwindigkeit an die aktuelle Besuchshäufigkeit der Bestäuber untersucht.

Kapitel 8 schließlich gibt einen Überblick über das Vorhandensein und den Ablauf der Staubblattbewegung innerhalb der Loasoideae. Zahlreiche Arten wurden mittels eines Standartexperiments untersucht und miteinander verglichen. Diese Studie zielt darauf ab, unabhängig von den in Kapitel 5-7 untersuchten Details die Fähigkeit zur Thigmonastie und individuelle Muster des zeitlichen Bewegungsablaufes über die Gattungsgrenzen hinweg zu erkennen. Dabei konnten einige Besonderheiten herausgearbeitet werden die in einen Zusammenhang zum jeweiligen Bestäuber, zu besonderen reproduktiven Merkmalen der jeweiligen Arten oder zu deren phylogenetischer Position stehen.

Die Kernaussagen, sowohl der einzelnen Kapitel als auch der beiden thematischen Teile der Arbeit, werden in Kapitel 9 abschließend zunächst getrennt zusammengefasst und diskutiert. Im letzten Teil wird der hier beobachtete Zusammenhang zwischen Systematik und Ökologie besprochen. Die gegenseitige Bedingung und die notwendige gemeinsame Bearbeitung beider Themenkomplexe für das Verständnis der Evolution von *Nasa*, der Loasoideae und anderer Pflanzengruppen wird verdeutlicht und Ansätze für die nachfolgende Bearbeitung werden aufgezeigt.

Contribution to Chapters

Chapter 2: Henning T, Weigend M. 2009. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical Journal of the Linnean Society* 161: 278-301.
Own contributions: Designed work (together with M. Weigend), collected material (in part), revised material, prepared figures (in part), and wrote the manuscript (together with M.Weigend).

Chapter 3: Henning T, Weigend M. 2011. Two new species of *Nasa* (Loasaceae) from Andean South America. *Phytotaxa* 26: 1-8.
Own contributions: Designed work (together with M. Weigend), collected material (in part), revised material, prepared figures, and wrote the manuscript (together with M. Weigend).

Chapter 4: Henning T, Rodríguez E, Weigend M. 2011. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae pro parte*, Loasaceae). *Botanical Journal of the Linnean Society* 167: 47-93.
Own contributions: Designed work (together with M. Weigend), collected material (in part), revised material, prepared figures (most), and wrote the manuscript (together with M.Weigend and E. Rodríguez).

Chapter 5: Weigend M, Ackermann M, Henning T. 2010. Reloading the revolver - male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales). *Biological Journal of the Linnean Society* 100. 124-131.
Own contributions: Designed field and glasshouse experiments (together with M. Weigend & M. Ackermann), collected data in the field and glasshouse (together with M. Ackermann), performed statistical analyses, prepared figures, and wrote the manuscript (together with M. Weigend).

Chapter 6: Henning T, Ackermann M, Weigend M. 2012. Complex, but not cheap - floral rewards and floral behaviour in six species of Loasaceae. submitted to *American Journal of Botany,* manuscript ID: AJB-D-12-00023.
Own contributions: Designed study (together with M. Weigend), designed and performed experiments (together with M. Weigend & M. Ackermann), performed statistical analyses, prepared figures, wrote the manuscript (together with M. Weigend).

Chapter 7: Henning T, Weigend M. in prep. Total control - pollen presentation and floral longevity are modulated by light, temperature and visitation rates. To be submitted to *Plos One*
Own contribution: Designed study (together with M. Weigend), designed and performed experiments, performed statistical analyses, prepared figures, and wrote the manuscript.

Chapter 8: Henning T, Weigend M. in prep. Stamen movement and pollen packaging in Loasaceae subfam. Loasoideae. To be submitted to *BMC Evolutionary Biology*
Own contribution: Designed study (together with M. Weigend), designed and performed experiments, performed statistical analyses, prepared figures, and wrote the manuscript.

List of Publications

Weigend M, Henning T, Schneider C. 2003. A revision of *Nasa* Ser. *Carunculatae* (Loasaceae Subfam. Loasoideae). *Systematic Botany* 28(4): 765-781.

Weigend M, Dostert N, Henning T, Schneider C, Rodríguez E. 2006. Valid publication for 101 species and subspecies names of the genera *Nasa* and *Aosa* (Loasaceae: Cornales). *Revista Peruana de Biología* 13(1): 71-84.

Henning T, Weigend M. 2009. Two novel and critically endangered subspecies of *Nasa humboldtiana* (Loasaceae) from Peru. *Botanische Jahrbücher für Systematik* 127: 473-488.

Henning T, Weigend M. 2009. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical Journal of the Linnean Society* 161: 278-301.

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Weigend M, Ackermann M, Henning T. 2010: Reloading the revolver - male fitness as simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Cornales, Loasaceae). *Biological Journal of the Linnean Society* 100: 124-131.

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Henning T, Weigend M. 2011. Two new subspecies of *Nasa* (Loasaceae) from Andean South America. *Phytotaxa* 26: 1-8.

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Congress contributions

Weigend M, Henning T, Gottschling M. 2003. Growth habit and shoot morphology in *Nasa* and other Loasaceae. Abstract and poster. 16th International symposium „Biodiversität und Evolutionsbiologie“ der Deutschen Botanischen Gesellschaft. Frankfurt, September 21st-27th, 2003.

Henning T, Weigend M, Schneider C. 2004. Flower longevity, self pollination and stamen movement in Loasaceae subfam Loasoideae. Abstract and poster. 7th annual meeting of the Gesellschaft für biologische Systematik (GfBS). Stuttgart, September 14th-17th, 2004. 1st Price for best Poster.

Henning T, Weigend M, Schneider C. 2005. Triggered pollen presentation, timing and pollination in Loasaceae subfam. Loasoideae. Abstract and talk. 18th annual meeting of the Gesellschaft für Tropenökologie (GTÖ). Berlin, February 22nd-25th, 2005.

For reasons of data protection, the curriculum vitae is not included in the online version

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Appendices to the Chapters

Appendix A to Chapter 2:

A1: *Nasa chenopodiifolia* (Desr.) Weigend - additional specimens examined: PERU. Department Ancash, Prov. Bolognesi: Chiquián, 3100–3200 m, Ferreyra 7298 (MO); Chiquián, 3150–3400 m, Weigend & Dostert 97/176 (M, USM, F); Prov. Corongo: road from Yanac to Sihuas, 2800 m, Weigend et al. 5038 (HUT, B, M, USM); Prov. Huaylas: Huaylas, Bosque Huamanripe, Albán 8958 (USM); road from Pamparomas to Karka, 2808 m, 27.ix.2006, Weigend et al. 8467 (USM, B); ditto, 2850 m, 05.v.2000,Weigend 2000/620 (HUSA, HUT, NY, WU); ditto, 2900 m, 04.v.2000, Weigend 2000/606 (HUSA, HUT, NY, WU); Pamparomas, Quebrada Muschomarca, 2826 m, 14.v.2003, Weigend et al. 7685 (USM, B); from Moro to Pamparomas, 1904 m, 26.iv.2006, Weigend et al. 8433 (USM, B); Prov. Santa: Lomas de Lupín, Ferreyra 8650 (MO); Lomas de La Chay, 40 km N of Barranca, 475 m, Stork et al. 9211 (G, UC); Department Arequipa, Prov. Condesuyos, Chuquiribamba, km 224, 2700 m, 23.iv.1967, Vargas 19395 (CUZ); Prov. Caylloma: Cabanaconde, Mirador de Achachihua, 3320 m, 18.iii.2006, Cáceres et al. 3430 (HUSA); Department Junin. Prov. Tarma: Tarma to Acobamba, 3050 m, Stork 10950 (F, G, UC); Department Lima. Prov. Huarochiri: Cerro Jurco, 2400 m, Cerrate 5756 (MO, USM); Chosica to Surco, km 70 on Central Highway, 1700–1800 m, Ferreyra 6947 (MO); Sta Eulalia, NE of Huinca, 2345–2450 m, Gentry & Smith 36113 (MO); San Mateo, Gutte & Lopez 162 (LP); above Barba Blanca, 15 km NE of Chosica on Río Santa Eulalia, 1700 m, Horton 10997 (G, UC); San Mateo, Martinet 43 (P); Distr. Matucana, Central highway km 94, 1800 m, Saunders 269 (BM); Llaucha, next to Bosque de Zaraté, 3000 m, Valencia 864 (USM); Matucana, 2400 m, Weigend et al. 97/461 (F, M, USM); ditto, cultivated at Munich (K, US); Tambo de Viso, 2900 m, Weigend et al. 8261 (USM, B); Prov. Huaral. Shucuy Huanca, road to Huascoy, 2500–2800 m, Acleto 3 (USM); Prov. Cajatambo: Banos de Churin, 2500 m, Sandeman 5385 (OXF); Ambar, 2010 m, 16.iv.1939, Stork 11445 (UC); Prov. Lima: valley S of Matucana, 2400 m, Goodspeed 33075 (G, MO, UC, US); Matucana, Macbride & Featherstone 179 (F, S, US); – Prov. Oyón: road from Oyón to Churín, 2900 m, Weigend et al. 5534 (HUT, B, M, USM); Department Moquegua. Prov. General Sánchez Cerro: from Omate to Carabaya [16°138´07.5´S, 070°58´´01´W], 2678 m, 25.iii.2009, Weigend & Ackermann 9258 (BSB, HUSA, USM); ditto, above Carabaya [16°37´´51.1´S, 070°57´´59.3´W], 2799 m, 26.iii.2009,Weigend & Ackermann 9268 (BSB, HUSA, USM); Prov. Torata: Torata, 2200–2300 m, Weberbauer 7394 (BM, F, US).

A2: *Nasa urens* (Jacq.) Weigend - additional specimens examined: PERU. Department Ancash, Prov. Casma: Lomas de Lupín, 31–32 km N of Paramonga, 330 m, 1.xi.1986, Dillon & Santisteban 4698 (NY, USM); – Prov. Santa, Lomas de Lupin, 350– 500 m, 7.viii.1948, Ferreyra 3913 (MO, USM); Lomas de Lupín, 350–500 m, 23.ix.1952, Ferreyra 8653 (USM); ditto, Lomas de Casma, 400–500m, Ferreyra 8055 (MO USM); Lomas de Mongon, near San Rafael, between Casma and Huarmey, 450 m, 17.ix.1938, Stork et al. 9189 (UC); Department Arequipa, Prov. Arequipa: Cobija, S. Peru, Cuming 937 (E); [probably Islay, Arequipa] D'Orbigny s.n. (P); without precise locality: Gaudichaud s.n. (MO); 500–600 m, 20.xii.1959, Ferreyra 13993 (MO, USM); Lomas de Okopa, 400–600 m, 11.x.1955, Ferreyra 11464 (MO, USM); Prov. Camaná: Lomas de Camaná, 32 km S of Camaná, 1000 m, 5.xi.1983, Dillon & Dillon 3863 (USM); between Camaná and Arequipa, km 161–162, 400–500 m, 10.xi.1947, Ferreyra 2568 (USM); Camaná, Lomas de Camaná, 600 m, 2.xii.1955, Ferreyra 11689 (USM); Lomas de Camaná, 20.xi.1961,

Tovar 3454 (USM); 5 km N of Atiquipa, 20.ix.1938, Worth &. Morrison 15652 (UC); Camana, 5 km N of Atiquipa, 20.ix.1938, Worth & Morrison 15652 (UC); Prov. Caravelí, Atiquipa, near Chala, 250–300 m, 20.x.1946, Ferreyra 1512 (USM); Lomas de Cháparra, 400–500 m, 14.x.1956, Ferreyra 11973 (USM, MO); Lomas de Atiquipa, 400–500 m, 15.xi.1986, Ferreyra 20793 (USM); Caraveli, 5 km S of Chala, Lomas de Capac, 225 m, 14.ix.1957, Hutchison 1299 (NY, USM); Lomas de Atiquipa, 600 m, 09.xi.1953, Vargas 10910 (CUZ) Panamericana Sur km 621.5, 1.5 km N of Cháparra turnoff, 4.x.1997, Weigend & Förther 97/732 (USM); Prov. Islay: Quebrada del Guerreso, without precise location, 650 m, 08.xi.2002, Caceres et al. 2871 (HUSA); Lomas de Mejia, 8 km of Mejia 500–600 m, 25.10.1983, Dillon & Dillon 3706 (USM); Lomas de Mollendo, 4 km E of Matarani on Arequipa road, 500 m, 17.xi.1986, Dillon et al. 4825 (NY,USM); road downwards from Ljoya to Mollendo, 500 m, 12.i.1949, Ferreyra 6385 (USM); Islay, above Mollendo, 500 m, 13.xi.1949, Ferreyra 6420 (USM); Lomas de Mollendo, x.1972, Herrera-Rivera & Chavez 20 (MO, USM); Department Lima, Prov. Chancay: Lomas de Atocongo, 80 m, 04.viii.1974, Antay-V. s.n. (USM); Lomas de Chancay, Araña 443 (M, USM); Lomas de Atocongo, 19.x.1986, Baldeón-M. 131 (USM); Lomas de Lachay, Bernardi 16398 (UC); Lomas de Lachay, 300–700 m, 7.ix.1996, Cano-E et al. 7595 (USM); Lomas de Lachay, 15.xii.1960, 460 m, Cerrate 3620 (USM); Iguanil, near Huaral, 200–400 m, 4.x.1987, Cerrate 9162 (USM); Lomas de Iguanil, 4.x.1987, Del Carpio et al. 455 (USM); Lomas de Atocongo, 16.viii.1953, 300–400 m, Ferreyra 9519 (USM); Lomas de Atocongo, 28 km S of Lima, 28.ix.1947, Ferreyra 2432 (USM); Lomas de Atocongo, 200–300 m, 26.vii.1956, Ferreyra 11821 (USM); Lomas de Atocongo, 5.x.1945, Ferreyra 159 (USM); Lomas de Atocongo, 13.x.1956, Ferreyra 5536 (USM); Lomas de Lachay, 4.xi.1952, 350 m, Ferreyra 8778 (USM); Lomas de Chancay 160–200 m, 24.ix.1952, Ferreyra 8685 (MO, USM); Lomas de Lachay, 14.vii.1948, 300 m, Ferreyra 3851 (USM); Pativilca, Lomas de San Jerónimo, 250–300 m, 1.xi.1965, Ferreyra & Cerrate 16546 (USM); Lomas de Lachay, Gutte et al. 3410 (LP); E of Pachacamac, Cerro de Manzano, 300–600 m, Knapp et al. 8323 (MO); Lomas de Atocongo, 350 m, viii.1974, Miloslavich s.n. (USM); Lomas deAtocongo, Müller 942 (LP); Atocongo, near Pachacamac, 300 m, Müller 3706 (LP); Lomas de Chancay, Müller & Kigan 3518 (LP); Lomas de Lachay, Müller et al. 739 (LP); Lomas de Lachay, 12.ix.1943, Ridoutt s.n. (USM no. 12904); Lomas de Lachay, 15.xi.1942, 1200 m, Ridoutt s.n. (USM no. 12490); Lima, Cerro Jeronimo, 5.xi.1939, Soukup 1306 (USM); Lomas de Pasomayo, S of Chancay, 0–400 m, 4.x.1938, Stork & Vargas 9345 (UC); Lomas de Lachay, 300 m, 27.x.1957, Tóvar 2603 (USM); Atocongo, 2.xi.1957, Tóvar 2633 (USM); Lomas de Lachay, 200–600 m Vargas 4702 (MO); Lomas de Lachay 100–500 m, 26.ix.1997, Weigend & Förther 97/542 (USM); Quebrada Verde N of Pachacamac, 250–350 m, 27.ix.1997, Weigend & Förther 97/552 (USM); Prov. Lima: Lomas de Amancaes, Asplund 13761 (NY); Barranca, Cuming 1072 (E, K); Lomas de Lurín, Yato Sisa, San Fernando, 200–350 m, 1.x.1987, Del Carpio & Chávez 445 (USM); Quebrada Verde, 325 m, Ferreyra 3917 (MO); Ferreyra 3913 (US); Ferreyra 3960 (USM); Lomas de Amancaes, 7.x.1948, Ferreyra 4053 (MO, USM); Lima, Lomas del Manzano, near Lurín, 150–180 m, 10.ix.1989, Ferreyra 20977 (USM); Barranca, Isern 2529 (F); Barranca, Ochoa 588 (F); Barranca, Mathews 729 (E, W); Barranca, Mexía 4011 (MO); Barranca, Ridoutt s.n. (USM no. 12146); Barranca, Wawra 103 (W); Barranca, Wawra 434 (W); Barranca, 250 m, 12.vii.1927, Weberbauer s.n. (USM); Panamericana north of Lima, Serpentin de Pacasmayo, 872 m, 24.ix.2001, Weigend & Skrabal 5889 (HUT, B, M, USM); Prov. Yauyos: road from Quilmana to Panamericana, km 122 on Panamericana, Lomas de Quilmaná, 320 m, 08.x.2002, Weigend et al. 7326 & 7327 (B, USM); Department Moquegua, Lomas de Ilo, 79 km SW of Moquegua, 260–460 m, 18.x.1983, Dillon & Dillon 3653 (USM); Department Tacna, Prov. Tacna, Lomas de Sama Grande, 700–900 m, 10.x.1997, Weigend & Förther 97/810 (USM).

A3: *Nasa poissoniana* subsp. *poissoniana* (Urb. & Gilg) Weigend - additional specimens examined: PERU: Department Cuzco, Prov. Anta: El Chaccan, 3695 m, Brunel 864 (MO); Chequerce, Anta to Urubamba, Hawkes et al. 5098 (NY); – Prov. Calca: Calca, Cano et al. 7889 (M, USM); between Calca, Huaman & Choqque, Hammarlund 619 (S); Pisac on Río Urubamba, 3000 m, Solomon 3033 (MO); Calca, 13 km NW of Pisac, 24.ii.1963, Ugent & Ugent 3944 (UC, WISC); Calca, road from Pisac to Calca, 3000 m, Weigend & Weigend 2000/287 (NY, M); Calca, road from Yucay to Calca, 2900–3000 m, Weigend & Weigend 2000/167 (NY); – Prov. Cuzco: Cuzco, Balfour-Gourlay 105 (CBGE, E); Sacsayhuaman, 3600 m, Herrera 2363 (F, G, S); valley of Urubamba, Herrera 3380 (G); Cuzco, 3000–3600 m, Herrera s.n. anno 1923 (US); Sacsayhuaman, Pennell 13571 (F, NY); Sacsayhuaman, Sandeman s.n. (BM); Cuzco, Soukup 41 (F); Sacsayhuaman, Soukup 10602 (USM); 4 km from Cuzco on road to Pisac, Ugent 4085 (US); Prov. Urubamba: village Ollantaytambo, hillsides [13°15´´33´S, 072°15´´31´W], 01.iv.2009, Ackermann 756 (BSB, HUSA, USM); Chinchero, 3800 m, Davis et al. 1673 (F, USM); Urubamba Valley, 3000 m, Herrera 3380 (F); Urubamba, Pumahuanca, 72 km N of Cuzco, 3100 m, 11.iii.1987, Nunez 7453 (USM); ditto, Nunez 7426 (USM); Pumahuanca, 2900 m, 18.i.1949, Vargas 7592 (CUZ); Yahuarmaqui, 2820 m, 14.iii.1950, Vargas 9296 (CUZ); Urubamba, road from Urubamba to Ollantaytambo, 290–3000 m, Weigend & Weigend 2000/208 (NY); Prov. Quispicanchis: Pikillacta, 3250 m, 28.iv.1946, Vargas 6038 (CUZ).

A4: *Nasa weigendii* E. Rodr. - additional specimens examined: PERU: Department La Libertad, Prov. Sanchez Carrion, road from Huamachuco to Chagual-Pataz, after Chugay and between Molino Viejo and Aricapampa, 2389 m, 20.iv.2004, Weigend & Schwarzer 7913 (B, F, HUSA, USM, HUT) – cultivated at Berlin from this collection, Weigend & Schwarzer 7913-C (AAU, CP, HUSA, HUT, UPS, B, FR, K, NY, M, MO, P, TEX, UC, USM, WISC). Prov. Pataz, Pueblo Nuevo Los Alisos, 2630 m, 06.v.2003, Sagástegui et al. 17255 (HAO).

A5: *Nasa ferruginea* (Urb. & Gilg) Weigend - additional specimens examined: BOLIVIA. Department La Paz, Prov. Bautista Saavedra: Charazani, E of Chullina, 3400 m, Gutte & Herzog. 450 (LP); Charazani, near Kapna, 3500 m, Herzog 755 (LP). – PERU. Department Cuzco, Prov. Urubamba, Urubamba, km 88–95, 16.iii.1943, Vargas 3391 (CUZ); Habaspapa Piri Peñas, 2800–3400 m, 23.iv.1946, Vargas 5959 (CUZ); road from Ollantaytambo to Quillabamba, 3400 m, 23.ii.2000, Weigend & Weigend 2000/407 (HUSA, NY); Prov. Calca, Manto, Amparaes, Lares, 3399 m, 21.i.2003, Valenzuela et al. 1120 (MO); road from Calca to Amparaes, 3500 m, 06.ii.2000, Weigend & Weigend 2000/199 (HUSA, NY); Prov. Paucartambo, Had. Cachupata, 19.v.1942, Vargas 2835 (CUZ, UC); Puyupata, 3200 m, 16.iii.1953, Woytkowski 587 (USM); Prov. Quispicanchis, Marcapata, 3400 m, 14.vii.1945, Vargas 5180 (CUZ); ditto, near Huayllay, 3360 m, 11.xii.1938, Vargas 9709 (UC); Department Puno, Prov. Sandia: road from Cuyocuyo passing Baños de Cuyocuyo, 3550 m, 26.ix.02, Ackermann 400 (USM, B); 2–6 km S of Limbani, 3550–3650 m, Metcalf 30461 (G, UC, US); Prov. Sandia, 2200 m, Weberbauer 562 (G); Prov. Carabaya: Puente San Fernando, 3190 m, 31.xii.1947, Vargas 6983 (CUZ); Prov. Macusani: road from Ollachea to Macusani, 3200 m, 01.ii.2000, Weigend & Weigend 2000/115 (HUSA, NY); Prov. San Antonio de Putina: Sino, 3200 m, ii.1993, Rossel 106 (LPB);

A6: *Nasa raimondii* (Standl. & F.A.Barkley) Weigend - additional specimens examined: PERU: Department Apurimac, Prov. Abancay: road from Abancay to Curahuasi, km 20, 3000–3400 m 21.ii.2000, M. & K. Weigend 2000/397 (HUSA, M, NY, USM); road from Abancay to Curahuasi, S of

the pass height, 3400–3500 m [13°32´´S, 072°45´´W], 21.ii.2000, M. & K. Weigend 2000/289 (HUSA, M, NY, USM); Prov. Andahuaylas: Laguna Pachuca, near Paccha, 3000 m, 10.vii.2004, Schwarzer 15 (B); Department Cuzco, Prov. La Convención: Santa Teresa, Choqékiraw, 2826–3128 m, 19.v.2004, Valenzuela et al. 3565 (MO); Prov. Paucartambo: above Paucartambo, c. 3600 m, 06.v.1939, Balls B6791 (UC); Prov. Urubamba: Cechobamba in Urubamba valley, 3300 m, Herrera 1550 (US); Hacienda Tuncapata Stihit, Vargas 2672 (F); Department Huancavelica, Prov. Tayacaja: between Colcabamba and Paucartambo, 3800–3900 m, 20.iv.1954, Tovar 2027 (USM).

A7: *Nasa vargasii* (J.F.Macbr.) Weigend - additional specimens examined: PERU: Department Apurimac, Prov. Abancay: Border to Cuzco, bridge over Río Apurimac, 2400 m, Skog 1120 (NY); road from Abancay to Andahuaylas, at the banks of Río Pachachaca/ Apurimac, near old bridge [13°39´´S, 072°56´´W], Weigend & Weigend 2000/313 (HUSA, NY); Prov. Andahuyalas: Cunyacc, 2020 m, 26.3.1948, Vargas 7112 (CUZ); Quebrada Honda, 1800–2200 m, 23.ii.1950, Vargas 9173 (CUZ); Chincheros to Pampa, 2300 m, 26.iv.1959, Vargas 12814 (CUZ); Prov. Chincheros: road from Andahuaylas to Ayacucho, km 108, 1700–2000 m, 17.ii.2000, Weigend & Weigend 2000/338 (HUSA, NY); Prov. Grau, Kainancka, 2300 m, 9.iii.1946, Vargas 5823 (CUZ); Department Cuzco, Prov. Anta, Anta, iii.1937, Vargas 197 (UC); Sisal to Limatambo, 2300 m, 15.iii.1963, Vargas 14337 (CUZ); Anta to Wallpachaca, 2300 m, 25.iii.1968, Vargas 20577 (CUZ); road from Curahuasi to Cuzco, before bridge over Apurimac, 2000 m [13°33´´S, 072°35´´W], 21.ii.2000, Weigend & Weigend 2000/396 (HUSA, NY); Prov. Urubamba: Macchu Picchu, Colpani Grande, 1705 m, 19.iv.2003, Valenzuela et al. 1874 (MO); Prov. La Convención: Distr. Santa Teresa, Yantile [13°08´´S, 072°33´´W], 1800 m, Huamantupa et al. 4075 (MO); Department Huánuco, Prov. Pachitea: Distrito Chaglla, Piedra Grande, station near Rio Santo Domingo, 1700 m, Macbride 3897 (G, F, S, US); Prov. Ambo: Ambo, Macbride 3154 (US, F); between Ambo & Huancabamba, 2380 m, Ochoa 14553 (F); road from Huánuco to Cerro de Pasco, 27.3 km from Ambo, 2300 m, 03.iv.2001, Weigend et al. 5463 (HUT, B, M, USM).

Appendix B to Chapter 4:

B1: *Nasa magnifica* (Urb. & Gilg) Weigend - additional specimens examined: PERU. Department Ancash. Prov. Bolognesi: near Aquia, 3200–3300 m, Ferreyra 7550 (US); Rio Grande/Rio Chacchan, above Pariacoto, 3530 m, Weigend et al. 7669 (B, USM); Prov. Huaylas: Cordillera Negra, Pamparomas, Llamahuilca, 2800–3200 m, Weigend & Dostert 97/132 (F, M, USM); ditto, Albán 9047 (M, USM); ditto, road from Pamparomas to Caraz, 3240 m, Henning & Schulz 08/06 (B, USM); ditto, 3153 m, Weigend et al. 8476 (B, USM); Prov. Huaraz: Huaraz, 10 km from Cochabamba, 2870 m, Smith & Buddensiek 10947 (NY); Department La Libertad. Prov. Otuzco: El Granero, Llaguen, Otuzco, 2900 m, 1.vi.1951, Lopez 1513 (HUT); Department Lima. Prov. Huarochiri: above Infernillo, between San Mateo and Casapalca, 3300–3350 m, Ferreyra 7707 (US); ditto, Weigend et al. 97/468 (F, M, USM); ditto, cultivated at Munich (K, NY, US, W); ditto, Ferreyra 6980 (MOL, US); ditto, Goodspeed et al. 11514 (K); ditto, Müller et al. 61 (LP); San Mateo, Sandeman s.n. (K, OXF); above Matucana, 3300–3500 m, Saunders 293 (BM); 4 km E of San Mateo, 3600 m, Dillon 2525 (F); Rio Blanco, 3000–3500 m, Killip & Smith 30608 (US); ditto, Killip & Smith 21625 (US); Matucana, Macbride & Featherstone 369 (US); Bosque de Zaraté, Gigantón, 2850 m, Valencia 329 (M, USM); Bosque de Zárate, Gatero, 2800 m, Müller & Gutte 9529 (LP); Bosque de Zárate, above San Bartolo, 2700–2800 m, Weigend & Dostert 97/11 (M,

USM); Prov. Yauyos: road from Huancayo to San Vicente de Cañete, c. 2 km below Huancachi, near San Tomás, 3965 m, Weigend & Skrabal 5881 (B, M, USM); Huancracha, above Tupe, 3300 m, Cerrate 1176 (B, USM); Department Huancavelica. Prov. Castrovirreina: Near Córdoba, 3050–3300 m, Metcalf 30282 (G, US); Prov. Huaytará: below Ranranayo on road to Pisco, 3320 m, Weigend & Förther 97/617 (M); below Ranranayo on road to Pisco, 3320 m, Weigend & Förther 97/617-C (M); Province unknown [probably Lima]: Tambo de Vibo (?), Martinet 173 (P); ditto, Martinet 942 (P); Department Moquegua. Prov. General Sánchez Cerro: between Puquina and Omate, 3200 m, Weigend et al. 7759 (USM, B).

B2: *Nasa profundilobata* (Werderm.) Weigend - additional specimens examined: ECUADOR: Prov. Azuay: road to Quinoas from Laguna Surocucho, Weigend & Horn s.n. (cult at Munich, K, M); ditto, Sparre 18839 (S); Bosque Protector Llaviuco, path to laguna Surocucho, 1–5 km from entrance, 3150 m, Alvarez et al. 2752 (BSB, NY); road from Cuenca to Molleturo, 2500 m, Huttel 1023 (QCA, QCNE); El Chorro, 6 km above Molleturo on road to Cuenca, 2800–2900 m, Harling & Andersson 22879 (GB).

B3: *Nasa ranunculifolia* (Kunth) Weigend subsp. *ranunculifolia* - additional specimens examined: PERU. Department Cajamarca. Prov. Cajamarca: Cajamarca–Bambamarca road, 3500–3800 m, Smith & Vazquez 3458 (NY, MO); road from Celendin to Chachapoyas, 3700 m, Henning & Schneider 208 (B, HUT, USM); Jalca, 3450–3500 m, Sánchez & Tejada 3426 (F); Hueco Grande above Quiruvilca, 4150 m, Leiva & Leiva 1064 (F, M); Cumbemayo, Reichlen 128 (P); ditto, 3500 m, Dillon et al. 3550 (MO); ditto, Weigend et al. 97/355 (F, MSB, USM); ditto, Gutte & Gutte 3954 (LP); ditto, Henning & Schneider 167 (B, HUT, USM); Sánchez & Cabanillas 10437 (B, CPUN); Huacarumi, Ochoa & Salas 16044 (US); Prov. Celendin: 53 km E of Cajamarca on road to Celendin, 3750–3850 m, Weigend et al. 97/367 (F, MSB, USM); ditto, 3345 m, Henning & Brokamp 5 (B, USM); road Cajamarca to Hualgayoc, km 25–30, 3200–3500 m, Dostert 98/127 (CPUN, F, MSB, USM); road Cajamarca to Bambamarca, 3500–3800 m, Smith & Vasquez 3458 (NY). Prov. San Miguel: Cerro Ponga la Mesa, 3600 m, Henning & Schneider 180 (B, HUT, USM); Prov. Hualgayoc: 3600 m, Weigend et al. 7514 (B, HUT, USM); Department La Libertad. Prov. Santiago de Chuco: road from Quiruvilca to Huamachuco, 20 km before Huamachuco, 4200 m, Weigend et al. 97/238 (F, MSB, USM); road from Santiago de Chuco to Huamachuco, 4111 m, Weigend & Schwarzer 7887 (B, HUSA, HUT, USM); Cruz de Shilte, Hda Llaguén, 3200 m, López 4724 (HUT); Huevo Grande, above Quiruvilca, 4120 m, Leiva & Leiva 1064 (HAO†, MO, MSB); Sauca, Santiago de Chuco, Sagástegui et al. 11949 (HUT, M, MO); Quesquenda, Jalca de Quiruvilca, 4000 m, López & Sagástegui 2882 (US); Jalca de Quesquenda, 4050 m, Straw 2534 (M, USM, US); ditto, 4000 m, Sagastegui et al. 17185 (B, HAO†, HUT); ditto, 4050 m, Sagastegui & Zapata 16440 (B, HAO†); Prov. Sánchez Carrion: Pampas de Julia, 3600 m, Sagástegui 11139 (HUT); Laguna El Toro, Quiruvilca, 4100 m, Sagástegui & Bernal 3027 (HUT); Trujillo–Huamachuco road, near El Toro & Santa Cruz Chiquita lakes, 3800–3900 m, Smith & Vazquez 3303 (MO, NY); Santiago to Shorey road, 11 km from Santiago, 3620 m, Smith 2316 (MO); Pampa de Julia, Sagástegui et al. 11139 (MO, NY); road from Otuzco to Huamachuco, c. 10 km before Shorey on road from Otuzco, Weigend et al. 97/239 (F, MSB, USM); Prov. Otuzco: Cerro Sango between Motil and Shorey, 3300–3400 m, López 967 (US); Department Ancash. Prov. Pallasca: road to Pastobueno from Huamachuco–Trujillo road, 36 km from Huamachuco, 6 km from Pastobueno, 4300 m, Duncan et al. 2639 (F, MO).

B4: *Nasa ranunculifolia* (Kunth) Weigend subsp. *cymbopetala* (Urb. & Gilg) Weigend - additional specimens examined: PERU. Department La Libertad. Prov. Huamachuco: Callacuyan, Shulcahuanga and surrounding area, 3900–4050 m, Cano et al. 12784 (B, USM); Department Ancash. Prov. Pallasca: Consuzo, 3850 m, López 2386 (HUT); Prov.
Huaylas: Huasacarán National Park, near Auquispuquío, 4000 m, Smith et al. 12093 (HUT, MO, F); Huasacarán National Park, Quebrada Los Cedros, 4020–4700 m, Smith et al. 9907 (F, HUT); 5 km below Cahuish tunnel, 4250–4350 m, Smith & Buddensiek 11084 (MO); Prov. Huari: 39 km from Catac E to tunnel of Cavish on road to Chavin, 6 km after the tunnel, 3700 m, Aronson 982 (M, MO); road from Chavín de Huántar to Catac, 4000 m, Weigend et al. 5164 (B, HUT, M, USM); Quebrada Ayash, 3800– 3850 m, Cano et al. 13223 (B, USM); San Marcos, Carhuayoc, margins of the Quebrada de Callapo, 3500–3700 m, Cano et al. 13499 (B, USM); Huari, 2 km to Abra, 4200–4400 m, Cano et al. 14436 (B, USM); Prov. Huaraz: Cerro San Cristobal, Huaraz, 3800 m, 8.vii.1977, Evangelista s.n. (MO, NY); Prov. Bolognesi: Huancar, near Chiquián, 3800–3880 m, Cerrate 224 (USM); above Chiquián, 3800–3880 m, Ferreyra 5773 (MO, MOL, US); from Conococha west to Panamericana, c. 4000 m, Weigend & Dostert 97/196 (F, M, USM); road Rio Grande/Rio Chachan, above Pariacoto, 4042 m, Weigend et al. 7701 (B, USM); Prov. Yungay: Huascarán, Lake Llanganuco to Portachuelo, 3860–4100 m, Smith 8226 (MO, NY); road from Yungay to Yauya, near Laguna Llanganuco, 3500–3800 m, Gentry et al. 37392 (MO); Prov. Recuay: Laguna de Querococha, 4050 m, 25.v.1970, López et al. 7518 (F, NY, US); Prov. Carhuaz: Cordillera Blanca, road from Carhuaz to Chacas, Quebrada Ulta, 3900–4200 m, Weigend & Dostert 97/146 (F, M, NY, USM, W); ditto, 4050 m, Frimer & Nielsen 112 (AAU); Tamboraque, 4300 m, Duncan et al. 2639 (F, MO); Prov. San Luis: road from San Luis to Huari, before Laguna Huachococha, 4000–4500 m, Weigend et al. 5136 (B, HUT, M, USM); Department Junín. Prov. Yauli: Distr. Marcapomacocha, near Minas Venturosa, 4200 m, Ochoa & Orrillo 14529 (F, US); Department Lima. Prov. Huarochiri: Distr. San Mateo, Río Blanco, 3000–3500 m, 15–17.iv.1929, Killip & Smith 21710 (F, US); ditto, Killip & Smith 21639 (US); ditto, Macbride 2877 (US); Saunders 326 & 326 A (BM); Chicla, from San Mateo to Casapalca, 3700 m, Ferreyra 6515 (US); ditto, Ferreyra 6514 (M, US, USM); 2 km N of Chicla on road to Oroya, 3700–3800 m, Poston 221 (MO, US); Chicla, 3700 m, Asplund 11313 (S); Bellavista, 3600 m, Weigend et al. 97/466 (F, M, USM); Rio Blanco, Macbride & Featherstone 2977 (F, S, US); Prov. Canta: Huamantanga, Matthews 515 (E, BR, OXF); above Culluhuay, Gutte 3177 (LP); Prov. Oyón: road from Yanahuanca to Oyón, 4330 m, Weigend et al. 5526 (B, HUT, M, USM); Province unknown: Pavón 1864 (G); Abadía 1833 (G).

B5: *Nasa ranunculifolia* (Kunth) Weigend subsp. *macrantha* (Urb. & Gilg) Weigend - additional specimens examined: PERU. Department Junín. Prov. Junín: above Capillacocha, near Carhuamayo, 4300 m, Tovar 2410 (USM); Prov. Yauli: Marcapomacocha, near Minas Venturosa, 4200 m, Ochoa & Orrilla 14529 (F); road to Illic, Palca, 2750 m, Díaz. & Baldeón 2213 (M, MO, USM); Prov. Huancayo: road from Huancayo to Huancavelica, 3916 m, Weigend et al. 5801 (B, HUT, M, USM); La Juntay, 4700 m, Killip & Smith 22047 (US); Prov. Jauja: from Jauja, 4000 m, bought on the market in Tarma, Weigend &
Dostert 97/113 (M, USM); road from Palca to Jauja, 8.5 km from Ricrán towards Tambillo, 4170 m, Weigend et al. 5705 (B, HUT, M, USM); Huancayo, road towards Cerro Huamancarpa, near Colca, 3700 m, 8.iii.1990, Yarupaitán 96 (USM); Prov. Tarma: road from Palca to Maraynioc, 3925

m, Weigend et al. 5660 (B, HUT, M, USM); Prov. Concepción: Distr. Comas or Andamarca, km 103 left of road Concepción–Satipo, 14 000 ft, Saunders 1091 (UC); Department Huancavelica. Prov. Tayacaja: Hacienda Alalay, between Mejorada and Pampas, 3550 m, Tovar 1307 (USM); Hacienda Huari, Asnaj Puquio, 4100 m, Lourteig 3142 (P, USM); Department Cuzco. Prov. Calca: Kelcani to Lares, 3000–4100 m, Vargas 11946 (CUZ); road from Manto to Calca, after Lares, 4600 m, Weigend & Weigend 2000/204 (HUSA, M, NY, USM); road from Calca to Lares, 4000 m, Ackermann et al. 275 (B, USM). Prov. Urubamba, Cuncani, 3600 m, Tupayachi 1504 (CUZ); ditto, Ollantaytambo, Garrapata, Pajonal, 3382 m, Valenzuela et al. 6393 (MO); Department Apurímac: Prov. Abancay; Ampay, 4250 m, Vargas 1022 (CUZ). Mountains NE of the road from Curahuasi to Abancay, before pass, 4000–4100 m, Weigend & Weigend 2000/ 276 (HUSA, M, NY, USM); Department Pasco: Prov. Pasco: Cerro de Pasco, road from Cerro de Pasco to Oxapampa, km 152 after Carhuamayo, 4350 m, Weigend et al. 5475 (B, HUT, M, USM).

B6: *Nasa ranunculifolia* (Kunth) Weigend subsp. *macrorrhiza* (Urb. & Gilg) Weigend - additional specimens examined: PERU. Department Ancash. Prov. Huaylas: Cordillera Negra, path from Karka (Moro, Pamparomas) to the Laguna Negra Huacanan, 4100–4300 m, Weigend & Dostert 97/142 (F, M, USM); ditto, 4300 m, Weigend & Salas 2000/618 (HUT, M, NY, USM, WU); ditto, 4373 m, Henning & Schulz 08/08 (B, USM).

Appendix C to Chapter 6:

C1: Tab. 6.3 Statistical comparison of anthers and pollen grains presented thigmonastically between the species examined

	VS.	*Caiophora*			*Nasa*			
		stenocarpa	*arechavaletae*	*carduifolia*	*urens*	*macrothyrsa*	*ranunculifolia*	
Caiophora	*stenocarpa*	X	***0.000***	*0.589*	***0.000***	***0.000***	***0.000***	*anthers presented thigmonastically*
	arechavaletae	***0.000***	X	***0.000***	***0.000***	*0.124*	*0.853*	
	carduifolia	*0.403*	***0.000***	X	***0.000***	***0.000***	***0.000***	
Nasa	*urens*	***0.000***	*0.180*	***0.000***	X	***0.000***	***0.000***	
	macrothyrsa	***0.000***	*0.142*	***0.000***	***0.000***	X	*0.228*	
	ranunculifolia	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	X	

pollen grains presented thigmonastically

C2: Tab. 6.4 Statistical comparison of ovules per flower and pollen grains per anther between the species examined.

	VS.	*Caiophora*			*Nasa*			
		stenocarpa	*arechavaletae*	*carduifolia*	*urens*	*macrothyrsa*	*ranunculifolia*	
Caiophora	*stenocarpa*	X	***0.000***	***0.000***	***0.000***	***0.000***	***0.001***	*no. of ovules per flower*
	arechavaletae	*0.214*	X	***0.000***	***0.000***	***0.000***	***0.002***	
	carduifolia	*0.436*	***0.000***	X	***0.000***	***0.000***	***0.001***	
Nasa	*urens*	*0.089*	***0.000***	*0.247*	X	*0.244*	***0.001***	
	macrothyrsa	*0.135*	***0.000***	*0.796*	*0.436*	X	***0.001***	
	ranunculifolia	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	X	

no. of pollen grains per anther

Appendix D to Chapter 8:

D1: Voucher information for the plant material used in Chapter 8:

Plakothira parviflora J. FLORENCE: Weigend s.n. (MSB, F)
Xylopodia klaprothioides WEIGEND: Weigend 97/450 (M, USM)
Huidobria fruticosa PHIL.: Kern 6 (BSB)
Presliophytum heucheraefolium (KILIP) WEIGEND: Weigend 7691 (BSB, USM)
Presliophytum incanum (GRAHAM) WEIGEND: Weigend 97/12 (M, USM)
Aosa rupestris (HOOK.) WEIGEND: Weigend 7138 (B, M, BM)
Nasa chenopodiifolia (JUSS.) WEIGEND: Weigend et al. 8433 (USM, B)
Nasa dillonii WEIGEND: Weigend 7556 (B, USM)
Nasa dyeri (URB. & GILG) Weigend ssp. *australis* Dostert & Weigend: Dostert 98/80 (M, USM)
Nasa macrothyrsa (URB. & GILG) WEIGEND: Weigend et al. 7471 (B, M, USM, HUT)
Nasa moroensis WEIGEND: Weigend 8424 (BSB, HUT, USM); Weigend 7694 (BSB, HUT, M, USM)
Nasa olmosiana (GILG EX J.F.MACBR.) WEIGEND: Dostert 98/163 (BSB, M); Weigend 8541 (B, USM)
Nasa picta (HOOK.F.) WEIGEND: Weigend & Dostert 98/158 (M, USM)
Nasa poissoniana (URB. & GILG) WEIGEND: Weigend 8007 (B, USM, HUT, HUSA, NY, MO, UC, K, P, BM)
Nasa ranunculifolia (KUNTH) WEIGEND subsp. *ranunculifolia* HENNING & WEIGEND: Henning 06/05 (B, USM),
Nasa triphylla (JUSS.) WEIGEND subsp. *flavipes* WEIGEND & DOSTERT: Weigend & Dostert 98/203 (M, USM)
Nasa triphylla (Juss.) WEIGEND subsp. *triphylla* WEIGEND & DOSTERT: Ackermann 602 (BSB)
Nasa triphylla (JUSS.) WEIGEND subsp. *triphylla* WEIGEND & DOSTERT: Weigend 9031 (BSB)
Nasa urens: (JACQ.) WEIGEND: Weigend 7327 (B, USM)
Nasa vargasii (J.F. MACBR.) WEIGEND: Weigend 5463 (B, HUT, M, USM)
Nasa weigendii E. RODR.: Weigend & Schwarzer 7913 (B, F, HUSA, USM, HUT)
Blumenbachia hieronymi URB.: Ackermann 601 (BSB)
Blumenbachia insignis SCHRAD.: Weigend 7475 (BSB)
Blumenbachia latifolia CAMBESS.: Weigend 9135 (BSB)
Loasa acerifolia Weigend 9142 (BSB)
Loasa gayana URB. & GILG: Weigend 7057 (B, M, NY)
Loasa insons POEPP.: Weigend 8724 (BSB),
Loasa nitida LAM.: Weigend 7346 (B, USM)
Loasa sclareifolia JUSS. : Weigend 8183 (BSB, M)
Loasa tricolor LINDL.: Weigend 9010 (BSB)
Loasa triloba JUSS.: Weigend 9008 (BSB)
Scyphanthus cf. *elegans* D.DON.: Gardner & Knees 8351C (cultivated in Berlin from Gardner & Knees 8351 (BSB)
Scyphanthus elegans D.DON.: Weigend 9032C (BSB, F, NY, MO, UC, WIS)
Caiophora arechavaletae (URB.) URB. & GILG: Weigend 9330 (B, F, M, MO)
Caiophora canarinoides (LENNÉ & K. KOCH) URB. & GILG: Ackermann 395 (BSB, HUSA, M, USM)
Caiophora cf. *andina* URB. & GILG: Schlumpberger 663 (BSB, LPB)
Caiophora coronata (GILLIES EX ARN.) HOOK. & ARN.: Weigend 9152 (BSB)
Caiophora carduifolia C. PRESL.: Ackermann 762 (BSB, HUSA, USM)
Caiophora cirsiifolia C. PRESL.: Weigend 7559 (BSB)
Caiophora cirsiifolia C. PRESL.: Weigend 7697 (BSB, USM)
Caiophora cirsiifolia C. PRESL..: Weigend 9043 (BSB)
Caiophora cirsiifolia C. PRESL.: Henning 08/30 (USM)
Caiophora stenocarpa URB. & GILG: Ackermann & Kollehn 288 (BSB, HUSA, M, USM, NY, F)

D2 : Tab. 8.3 Average stamen numbers per flower of all species of Loasaceae subfam. Loasoideae examined in Chapter 8, if available (in alphabetical order) .

Tribe	Species	voucher	Stamens	n
			mean	flowers
Loaseae	*Aosa rupestris*	Weigend 7138	79	7
Loaseae	*Blumenbachia hieronymi*	Ackermann 601	81	7
Loaseae	*Blumenbachia insignis*	Weigend 7475	85	17
Loaseae	*Blumenbachia latifolia*	Weigend 9135	45	12
Loaseae	*Caiophora arechavaletae*	Weigend 9330	105	10
Loaseae	*Caiophora canarinoides*	Ackermann 395	n.a.	
Loaseae	*Caiophora carduifolia*	Ackermann & Kollehn 288	n.a.	
Loaseae	*Caiophora* cf. *andina*	Schlumpberger 663	110	8
Loaseae	*Caiophora coronata*	Weigend 9152	n.a.	
Loaseae	*Caiophora cirsiifolia*	Henning 08/30	92	24
Loaseae	*Caiophora cirsiifolia*	Weigend 7559	n.a.	
Loaseae	*Caiophora cirsiifolia*	Weigend 7697	n.a.	
Loaseae	*Caiophora cirsiifolia*	Weigend 9043	96	12
Loaseae	*Caiophora stenocarpa*	Ackermann 758	116	10
Loaseae	*Huidobria fruticosa*	T. Kern 6	n.a.	
Loaseae	*Loasa gayana*	Weigend 7057	65	7
Loaseae	*Loasa nitida*	Weigend 7346	n.a.	
Loaseae	*Loasa acerifolia*	Weigend 9142	60	13
Loaseae	*Loasa sclareifolia*	Weigend 8183	n.a.	
Loaseae	*Loasa insons*	Weigend 8724	n.a.	
Loaseae	*Loasa tricolor*	Weigend 9010	50	12
Loaseae	*Loasa triloba*	Weigend 9008	36	12
Loaseae	*Nasa chenopodiifolia*	Weigend 8433	51	10
Loaseae	*Nasa dillonii*	Weigend 7556	n.a.	
Loaseae	*Nasa dyeri* ssp. *australis*	Dostert 98/80	71	10
Loaseae	*Nasa macrothyrsa*	Weigend 7471	105	30
Loaseae	*Nasa moroensis*	Weigend 7694	102	10
Loaseae	*Nasa moroensis*	Weigend 8424	n.a.	
Loaseae	*Nasa olmosiana*	Weigend 8541	71	19
Loaseae	*Nasa olmosiana*	Dostert 98/163	n.a.	
Loaseae	*Nasa picta*	Dostert 98/158	n.a.	
Loaseae	*Nasa poissoniana*	Weigend 8007	67	10
Loaseae	*Nasa ranunculifolia* ssp. *ranunculifolia*	Henning 06/05	109	8
Loaseae	*Nasa triphylla* ssp. *flavipes*	Dostert 98/203	83	10
Loaseae	*Nasa triphylla* ssp. *triphylla*	Ackermann 602	88	10
Loaseae	*Nasa triphylla* ssp. *triphylla*	Weigend 9031	85	10
Loaseae	*Nasa urens*	Weigend 7327	71	40
Loaseae	*Nasa vargasii*	Weigend 5463	111	31
Loaseae	*Nasa weigendii*	Weigend 7913	84	10
Klaprothieae	*Plakothira parviflora*	Weigend s.n.	34	7
Loaseae	*Presliophytum heucheraefolium*	Weigend 7691	120	7
Loaseae	*Presliophytum incanum*	Weigend 97/12	65	9
Loaseae	*Scyphanthus elegans*	Weigend 9032	134	26
Loaseae	*Scyphanthus* cf. *elegans*	Gardner & Knees 8351	242	10
Klaprothieae	*Xylopodia klaprothioides*	Weigend 97/450	60	10