REPRODUCTIVE BIOLOGY OF *NYMPHAEA PUBESCENS* WILLD. (NYMPHAEACEAE)

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Received: 18.06.2021 / Accepted: 10.09.2021

Abstract: *Nymphaea pubescens* Willd. is a notorious invasive species of aquatic ecosystem. Understanding the reproductive biology of this one is essential towards prevention and management of its invasive potential. However, little is known about the sexual and asexual mechanisms of reproduction of this species. Thus, exploration on the reproductive biology of *N. pubescens* Willd. has been conducted with emphasis on the documentation of the morphology of its reproductive parts and monitoring of its rate of germination. The reproductive biology of *N. pubescens* Willd. influences its invasive potential. The striking flowers of this species attract numerous pollinators such as insects and humans. Each fruit of this species harbors 11-14 seeds that could germinate at the rate of 100%. The morphology of the seeds aids in its ability to successfully establish population since it is more resilient to biotic and abiotic disturbances. The rhizome of this plant has great contribution to its invasiveness. It is considered as the main challenge in the control of this species as it is embedded underground below the water surface which is not reached by any control protocols. Thus, it is highly recommended for the control managers not to just focus its control methods on the superficial surfaces, but should also consider including soil under water bodies to ensure complete removal of the rhizome and other vegetative parts to avoid regrowth and re-establishment of the population of this invasive species.

Keywords: asexual reproduction, *Nymphaea pubescens*, Nymphaeaceae, rhizome, seeds, sexual

Introduction:

Nymphaea pubescens Willd. is a member of aquatic lilies, distinguished from other species through its hairy petiole and pinkish flowers. It is a perennial herb distributed commonly throughout the shallow lakes and ponds of temperate and tropical Asia (Guruge et al. 2016). This macrophyte is cultivated for horticultural purpose used as an offering in temples, traditional medicine for diabetes (Karthiyayini et al. 2011; Angadi et al. 2013) and its seeds were used as an alternative to rice and a potential source of raw material for food industry (Nizam and Arampath 2016; Aliyu et al. 2017). However, this species is reported as an invasive one of aquatic ecosystems and seasonal tanks in countries like Sri Lanka and United Kingdom (Munasinghe et al. 2010).

The excessive growth of aquatic species led to serious water quality problems

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particularly in the usability and management of water resources (Chapman et al. 1974). Aquatic plants have caused increase siltation in water bodies thereby reducing its clarity. Its invasiveness triggered deleterious impacts causing the alteration of the natural function and services of an ecosystem resulting in economic and ecological instability (Chandrasena and Rao 2017). Thus, calls for a need to understand the factors that promote the invasion of these species to prevent and manage such environmental dilemma.

Invasive species is only capable of excessive proliferation if they can manage successful reproduction (Blackburn et al. 2011; Mazzolari et al. 2017). Most of these plants display a wide array of reproductive mechanism either through vegetative or sexual reproduction (Albert et al. 2015); and these mechanisms influence the success of invasion as it determines the number of propagules that are needed in population establishment. Thus, knowledge in plant reproduction is a necessity to understand biological invasions (Ward et al. 2012; Redmond and Stout 2018). The quality and quantity of seeds and the rate of its germination, the ability to attract pollinating vectors and seed dispersers, the ability to self-fertilize (Redmond and Stout 2018) and to asexually reproduce are among the information that needs to be established to effectively understand the invasiveness of a species. All of these factors related to strategies of reproduction could provide insights into the invasion mechanisms of alien species and prove essential in formulating and implementing prevention and control strategies (Sun et al. 2018). However, regardless of the eminent need for this knowledge, little information is available on the reproductive mechanisms and pollination requirements of most invasive species (Traveset and Richardson 2014; Mazzolari et al. 2017) and even explorations on the contribution of asexual reproduction to the invasion process are scarce (Budde et al. 2011; Mazzolari et al. 2017). Thus, this paper intended to explore the reproductive biology of *N. pubescens* Willd., to document the morphology of its reproductive parts and record its germination rate.

Materials and methods:

Flower and fruit of *N. pubescens* Willd. were collected in the Lake Pinamaloy, Don Carlos, Bukidnon (Fig. 1, Annexes). Morphological characteristics of the reproductive parts were described. Twelve mature seeds of the species were collected and were grown in 3 plastic containers representing the replicates with 4 seeds each. The seeds were grown inside a container with water as the growing medium. Observations of the germination rate lasted for 7 day.

Results and discussion:

Morphological descriptions of the *N. pubescens* Willd. flowers and fruits are shown in Table 1. The species is noted for its solitary terminal flower with pink admirable petals and sepals. Numerous numbers of stamen and pistil were also observed (Fig. 2, Annexes). The flower is bisexual, which made this species capable of self-fertilization wherein the aid of pollinators is no longer or less of a necessity for this plant to reproduce. The ability to self-fertilize enhances its reproductive success, hence diminishing the problems encountered in cross pollination, where pollinators and the uncertainty of fertilization during long-distance pollination are being considered (Busch and Delph 2012; Shivanna 2014; Razanajatovo et al. 2016; Van Etten et al. 2017).

The orientation of its ovary is superior with 11-14 locules harboring the same number of ovules which would develop to its fruit after fertilization. The striking color of the flower of *N. pubescens* Willd. does not just attract insects and birds but also humans, able to become a viable pollinator and disperser of the pollen and seeds of this plant.

The fruit of *N. pubescens* Willd. is known to be a berry of 15-20 mm long seeds embedded in it (Fig. 3, Annexes). Each

individual fruit of *N. pubescens* Willd. harbors 11-14 seeds with 100% rate of germination that starts around the 3rd day of inoculation (Tab. 2, Fig. 4-5, Annexes).

Table no. 1 Morphological description of the Reproductive parts of *N. pubescens* Willd.

Characters	Description
Flower	
No. of petals	10-15
Petal color	Pink
No. of sepals	4
Sepal color	Pink
Stamen	Present (Numerous)
Pistil	Present (11-14)
Ovary orientation	Superior
No. of locule	11-14
No. of ovule	11-14
Fruit	
Type of fruit	berry
Fruit size	40-50 mm
No. of seeds	11-14
Size of seeds	15-20 mm

Table no. 2 Rate of Germination of the *N. pubescens* Willd. seeds

	Rate of Germination (4 seeds/ replicate)	Day of germination first observed in each replicates
Replicate 1	100%	3rd day
Replicate 2	100%	3rd day
Replicate 3	100%	3rd day
Average	100%	

This rate of germination is high enabling the growth of a new propagule; however comparing the number of seeds produced by *N. pubescens* Willd. with other common invasive species such as Poaceae and Cyperaceae, the seed quantity of this species is quite low as other invasive species may harbor a much higher number of seeds in a single branch. The quantity of seeds produced by a plant greatly affects the number of its population in an area and thereby influences the invasiveness of the species (Mazzolari et al. 2017). Nonetheless, ecologist and area managers should not be complacent in monitoring the invasiveness of *N. pubescens* Willd. as the seeds of this plant were more resilient to environmental disturbance compared to other invasive species as it is noted to be heavier and features a hardened pericarp. This characteristic is useful in protecting the delicate embryo inside the seed against physical biotic and abiotic pressures/ disturbance, thus increasing the potential of higher germination rate and propagule establishment in natural environment. According to Leishman (2001) and Moles and Westoby (2006), heavier seeds and fruits are better in terms of quality as largely provisioned seeds are capable of sustaining themselves in situations of limited resources where the seedlings are inclined to rely on its stored reserves.

Aside from this, *N. pubescens* Willd. is also capable of reproducing asexually and

expanding its population to a larger area through its stolon and rhizomes. These modified stems persist through the ground under the waterbodies such as lakes and ponds which are the common habitat of this species, enabling it to increase in number and area of its proliferation. According to Peng et al. (2017) asexual reproduction has great contribution on the invasiveness of the species as it does not rely on pollinators and dispersers to reproduce.

Controlling the proliferation of *N. pubescens* Willd. is quite challenging since it is limited to uprooting the plant. However, such method has rendered ineffective due to the rhizome that are steadily and sturdily embedded underground. During physical uprooting of the plants, these modified stems are left below the ground thus still enabling such species to regrow and establish new propagule. Chemical control of this invasive species was not considered as it may contaminate the water bodies and harm other existing terrestrial and aquatic organisms and also the community which may be relying its water source on aquatic ecosystem. This leaves area managers and ecologists to depend more on physical or mechanical protocols of control; albeit there is a need to develop control methods and not just focus on superficial uprooting of the plants, but should formulate a protocol that would be effective in including the eradication of stolon and rhizomes of *N. pubescens* Willd.

Conclusions:

The present paper revealed the invasive potential of *N. pubescens* Willd. and the big role of reproductive biology to its invasiveness. The morphology of the species specially of its sexual and asexual parts promotes the success of its reproduction and its ability to establish population at a wider range. The modified stems (stolon and rhizome) are considered as a great contributor to its invasiveness which allows *N. pubescens* Willd. to proliferate profusely in aquatic ecosystem. These stolon and underground rhizomes rendered unreachable by control measures as they are situated below the water bodies. Thus, underwater methods of mechanical control are also recommended to ensure elimination of rhizome, stolon and other vegetative parts which may aid in the regrowth of this species. Studies on monitoring the viability of seeds and further exploration on the asexual reproduction of *N. pubescens* Willd. are recommended to acquire more fundamental and substantial information on its contribution to the invasiveness of this species. These researches would be an essential addition to the existing knowledge on reproductive and invasive biology.

Rezumat:

BIOLOGIA REPRODUCERII LA *NYMPHAEA PUBESCENS* WILLD. (NYMPHAEACEAE)

Nymphaea pubescens Willd. este o specie invazivă notorie a ecosistemului acvatic. Înțelegerea biologiei reproductive a acesteia este esențială pentru prevenirea și gestionarea potențialului său invaziv. Cu toate acestea, se cunosc prea puține lucruri despre mecanismele sexuale și asexuale de reproducere ale acestei specii. Astfel, explorarea biologiei reproductive la *N. pubescens* Willd. a fost realizată cu accent pe documentarea morfologiei părților sale de reproducere și monitorizarea ratei sale de germinare. Biologia reproductivă a *N. pubescens* Willd. influențează potențialul său invaziv. Florile acestei specii atrag numeroși polenizatori precum insectele și oamenii. Fiecare fruct al acestei specii adăpostește 11-14 semințe care ar putea germina cu o rată de 100%. Morfologia semințelor ajută la capacitatea sa de a stabili cu succes populația, deoarece este mai rezistentă la tulburările biotice și abiotice. Rizomul acestei plante are o mare contribuție la invazivitatea sa. Este considerat principala provocare în controlul acestei specii, deoarece este încorporat în pământ sub suprafața apei, care nu este atinsă

de niciun protocol de control. Astfel, este foarte recomandat managerilor să nu-și concentreze metodele de control doar pe suprafețele superficiale, ci ar trebui să ia în considerare și includerea solului de sub corpurile de apă pentru a asigura îndepărtarea completă a rizomilor și a altor părți vegetative, pentru a evita creșterea și restabilirea populației acestei specii invazive.

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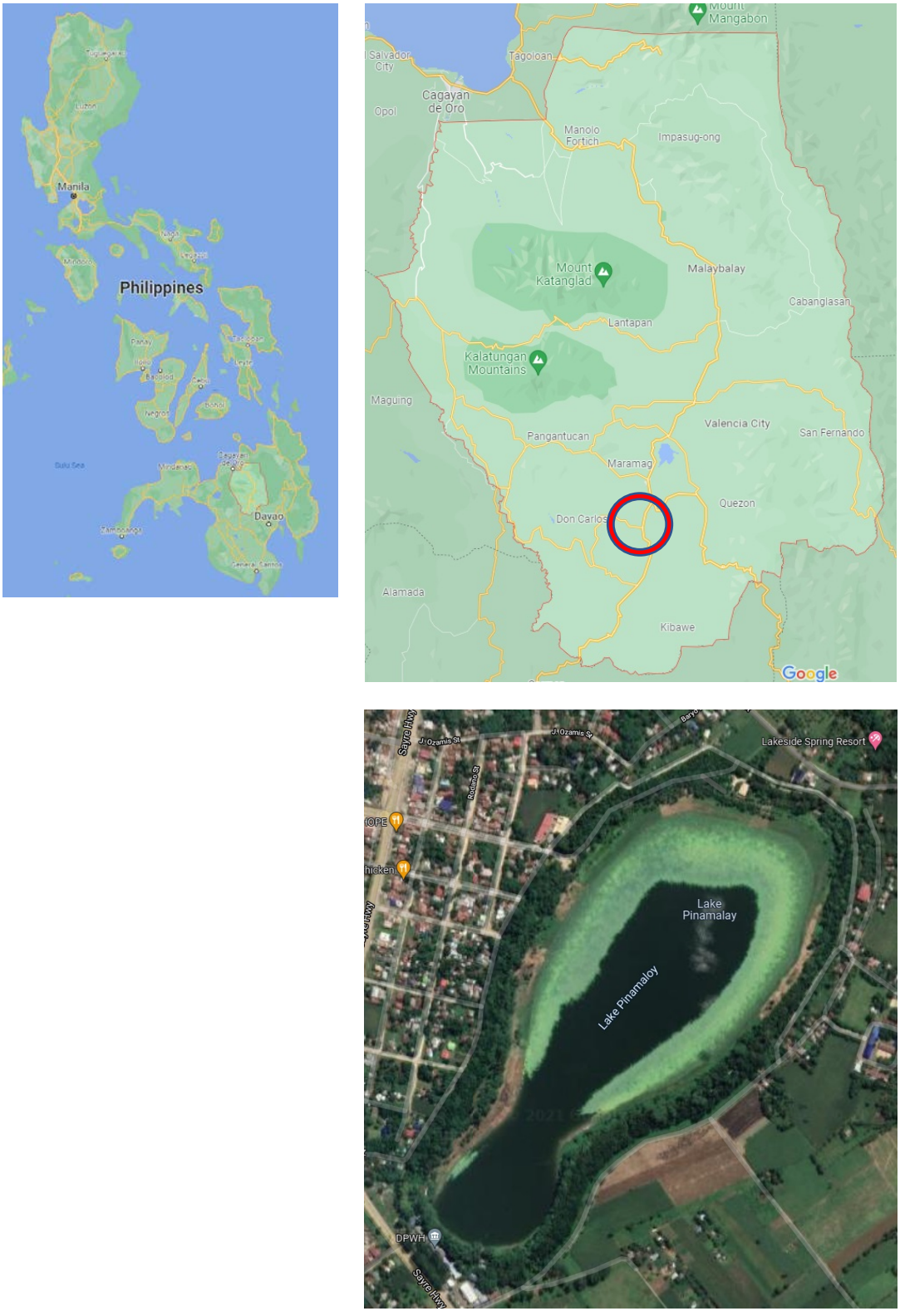
Annexes:

Figure no. 1 Lake Pinamaloy, Don Carlos, Bukidnon. a. Map and Satellite view of the sampling site; b. sampling site infested by *N. pubescens* Willd

a.



b.

Istros – Museum of Braila "Carol I"

Figure no. 2 Flower of *N. pubescens* Willd. a - fully bloomed flower; b - sepals; c - ovary, stamen and pistil

Figure no. 3 Fruit of *N. pubescens* Willd. a - dorsal side of the fruit; b - ventral side of the fruit with the locules and seeds; c - seed

a.

b.

c.

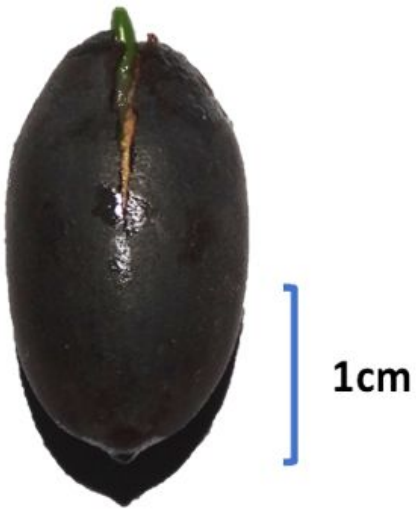


Figure no. 4 Germinated seeds of *N. pubescens* Willd.

Figure no. 5 Growth of the seeds of *N. pubescens* Willd.