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# A Morphological Identification in Fish of the Genus *Puntius* Hamilton 1822 (Cypriniformes: Cyprinidae) of Some Wetlands in Northeast Thailand

[1]T. Champasri, [2]R. Rapley, [3]M. Duangjinda and [4]A. Suksri
[1]Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Department of Biosciences, School of Life Sciences, University of Hertfordshire, College Lane, Hatfield, Herts AL10 9AB, UK
[3]Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

**Abstract:** This investigation was carried out at the Department of Fisheries, Khon Kaen University, Khon Kaen, Thailand in collaboration with the Department of Biosciences, University of Hertfordshire, Hatfield, United Kingdom. The work was carried out in the 2003 to 2006 to taxonomically identify fish of the genus *Puntius* Hamilton 1822 harvested from 15 wetlands in Northeast Thailand. Some considerable amounts of fish samples were collected and used for morphological characteristics identifications both measuring and counting. The fish samples were kept in a 10% formalin solution for two weeks and then transferred to a 75% ethanol alcohol for laboratory determinations. Thirty fish samples from each location were used. The results showed that there were 9 fish species of the genus *Puntius* Hamilton 1822 harvested from 15 locations (provinces). They include: *Puntius altus*, *Puntius aurotaeniatus*, *Puntius binotatus*, *Puntius gonionotus*, *Puntius leiacanthus*, *Puntius orphoides*, *Puntius partipentazona*, *Puntius schwanenfeldi* and *Puntius wetmorei*. The fish samples of the 9 species were morphologically identified where the information include 45 characteristics on morphological measurements and 18 characteristics for counting. However, with this work only 20 important characteristics are included. The obtained results were calculated through the computer programmes commencing from the use of an Excel programme for raw data inputs followed by Statistica (PCA values), Multivariate by SAS and then NTSYSpc2.10 for UPGMA dendrogram figure. The results showed that the 20 important characteristics did not provide any multivariate values of significant differences found among the 9 fish species. The values on Euclidean distances of the 9 species ranged from 0.31-0.61 where there were no significant differences found among the 9 fish species of the genus *Puntius* Hamilton 1822, thus the fish of the 9 species could not be shifted or split into to other genera apart from the genus *Puntius* Hamilton 1822.

**Key words:** Fish species, morphological identification, *Puntius* Hamilton 1822, wetlands, similarity correlation coefficients

## INTRODUCTION

Amongst different kinds of fresh water fish, the fish of the genus *Puntius* Hamilton 1822 has been considered as an important source of protein being used for man daily diets apart from other uses such as animal feed and etc. For Thailand in particular, an annual harvest of this type of fish alone increased from 46,276 tons in the 2000 to 82,363 tons in the 2004. Whilst in northeastern region alone the harvest reaches a figure of 2,956 tons in the 2000 and then an increase of 6,889 tons in the 2004 (Anonymous, 2005). The large increases in the amounts of harvested fish in Thailand may signify economic impact of the fish of the genus *Puntius* Hamilton 1822. Northeastern region of Thailand has both natural and artificial wetlands of 21,914 units where all of them retain some large amounts of annual rainwater throughout the year. Many of the wetlands occupy land area of many hectares they range from 6.25 to 27387.375 ha for Huay Suaten of Khon Kaen and Ubonratana dam of Khon kaen province, respectively with a total regional area of 168,854.36 km$^2$. Most of the natural wetlands had been developed from back swamps and low terraces where the building up of sediments has been collectively added underneath from year to year since many of them located on different types of soil series but most of them belong to Oxic Paleustults, a great soil group. Most of them possess a similar ecological environment, e.g., it has

**Corresponding Author:** Thongchai Champasri, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

several plant species grown under water and some floating throughout the area where several kinds of fish, birds and other animals thrive on throughout the year, particularly the fish of Cyprinidae family, which the fish of this family could easily be found in all of the wetland areas (Anonymous, 1997).

According to the published information, the fish of the genus *Puntius* Hamilton 1822 belongs to the family Cyprinidae and it is commonly known as Silver barb. It is one of the most important commercial fish for human food consumption. Smith (1945) reported that in Thailand the fish of this genus comprises of 36 nominal species and most of them thrive on well in most natural fresh water resources such as lakes and rivers. Nevertheless, Rainboth (1996a, b) did identify the fish of this genus into another three genera based on morphological characteristics. The three genera include *Systomus*, *Barbodes* and *Hypsibarbus*. From this information, it seems more likely that the author did provide somewhat of an inadequate explanation in differentiating the fish of the three established genera where one may find that the differences found among them could be relatively small, e.g. numbers of: lateral line scales, circumpeduncular scales, gill rakers, predorsal scales and horizontal scale rows between lateral line and mid-dorsal scale rows at dorsal-fin origin and etc. Roberts (1989) with fish of the genus *Puntius* Hamilton 1822 reported that there were some small differences found among the fish species of this genus where the impact on environmental conditions could have been influenced the changes on morphological characteristics of the fish. Thus the small changes found should be used to classify the fish of the genus *Puntius* Hamilton 1822 into species rather than genera. The findings of the three genera of the fish of the genus *Puntius* Hamilton 1822 had perhaps created an academic controversial issue among fish taxonomists. Other published data concerning fish identification were carried out by Bush and Adams (2007) where they measured landmarks on head of fish of Arctic charr and they claimed that their method gave an ultimate accuracy since it is freed from the effect of body size as stated by Adams *et al.* (2003). This current investigation aims to justify the fish of the genus *Puntius* Hamilton 1822 if this genus of the fish could split into other genera due to their different identities. Therefore, it may be of tangible value to carry out laboratory works with the use of the fish of the genus *Puntius* Hamilton 1822 collected from some wetlands in northeastern region of Thailand again in order to identify more clearly if this genus of the fish could provide evidences whether or not the genetic variation of the genus could have been changed beyond a similarity range of genetic correlation coefficients where the fish of the genus *Puntius* Hamilton 1822 have been identified into other three genera as stated by Rainboth (1996a, b). The same morphological measurements method by Rainboth (1996a, b) were used again for this research apart from a small modification made where the added information could possibly offer a clearer image of the fish.

## MATERIALS AND METHODS

This study was carried out at the Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Northeast Thailand to investigate the variability of fish of the genus *Puntius* Hamilton 1822 harvested from fifteen different locations of wetlands in Northeast Thailand. The 15 locations include the following provinces, they are: Nong Kai; Nakhon Phanom; Mukdaharn; Mahasarakham; Roi-Et; Sisaket; Kalasin; Chaiya Phume; Udon Thani; Nakhon Ratchasima; Sakon Nakhon; Nong Bua Lampoo; Yasothon; Khon Kaen and Loei. An amount of 1,500 individual fish samples was collected from week to week started from August 2005 to February 2006. The fish samples were collected from the villagers right after each harvest. Each individual fish was immediately preserved in a 10% formalin solution where the samples were kept not more than two weeks and later stored in an ethanol alcohol (75%) and then the fish samples were used for morphometric identification. The measurement methods being used for this investigation were the same as those reported by Hubbs and Lagler (1947) and Rainboth (1996a, b) but with some small modifications where a Vernier Caliper was used to measure distances of each landmark of each specimen of individual fish. A scale in millimeter was used for the measurement in length and then the readings were recorded in the data-sheets for computations and calculations. All of the measured characteristics such as numbers of measurement points and the counting of different numbers of fish organs, e.g., numbers of lateral line scales, circumpeduncular scales and others are shown in Fig. 1. There were 9 species of fish of the genus *Puntius* Hamilton 1822 collected from 15 wetlands in Northeast Thailand. The different sizes of the fish samples of the 9 species were randomly chosen where each location represents by 30 fish samples. The average values of each specific morphological measurement (being measured from No. 1 up to 14 of Fig. 1) together with numerical mean values (being counted from 15-20) were recorded. The measurements were carried out commencing from a specific point to the other ended point of the fish body, whilst the different counts were carried out with the use of different fish

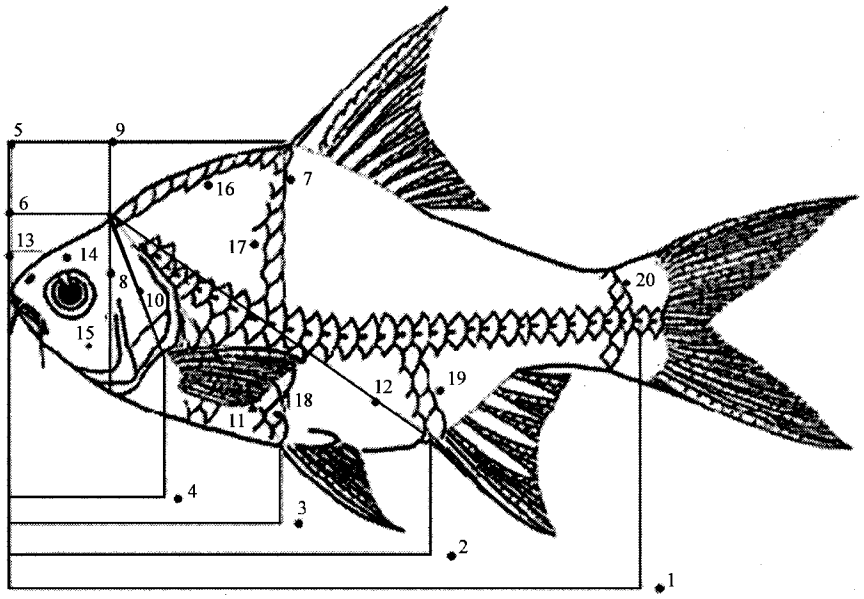


Fig. 1: Point numbers on morphological measurements and counts of different organs of fish of the genus *Puntius* Hamilton 1822. Numbers (1 up to 20) represent both sizes of specific fish organs

organs where appropriate. The measurements were carried out with the following characteristics, i.e., (1) snout to urocentrum, (2) preanal length, (3) prepelvic length, (4) prepectoral length, (5) predorsal length, (6) preoccipal length, (7) dorsal origin to pelvic insertion, (8) head depth at occiput, (9) occiput to dorsal origin, (10) occiput to pectoral insertion, (11) occiput to pelvic insertion, (12) occiput to anal insertion, (13) snout length and (14) interorbital width. The numbers of counting were carried out with (15) total gill rakers, (16) predorsal scale rows, (17) upper transverse, (18) scale from Lateral line to pelvic fin origin, (19) lower transverse scale rows and (20) circumpeduncular scale rows.

The modified truss system on measurement is the one found on item No. 12, which is only a modified landmark being used and the rest are those of Rainboth (1996b). The collected results were analysed with the use of computer programmes where appropriate and the 20 important characteristics were used for a dendrogram illustration of clusters where many steps in calculating the data commenced from the use of an Excel programme where sets of raw data were fed into the computer by hand and after the completion of each data set then they were ready for further computations, i.e., with the use of STATISTICA Computer Programme (StatSoft, 1995) where it gave information derived from canonical variate analysis (CVA) and then followed by principal component analysis (PCA) and the programme continued to calculate further to attain values on trait correlation matrix and the attained component values were used for group correlations with the use of NTSYSpc2.10 programme (Rohlf, 2000). Finally they were further computed through with the use of a SAS Computer Programme for multivariate analysis of variance (MANOVA) where several statistical values were used for the assessment on the discriminatory effectiveness of the analysis where the use of Wilks' Lambda ($\lambda$) of Wilks (1932) was applied.

## RESULTS

**The nine collected species of fish of the genus *Puntius* Hamilton 1822:** The results showed that the fish collected from 15 different locations in Northeast Thailand consisted of 9 nominal species. These 9 nominal species were identified according to their respective species along with names of the authors of the published works and their respective numbers of the harvested locations (Fig. 2a-i), i.e., *Puntius altus*; (Fig. a) *P. aurotaeniatus*; (Fig. 2b) *P. binotatus*; (Fig. 2c) *P. gonionotus*; (Fig. 2d) *P. leiacanthus*; (Fig. 2e) *P. orphoides*; (Fig. 2f) *P. partipentazona*; (Fig. 2g) *P. schwanefeldi*; (Fig. 2h) *P. wetmorei* and (Fig. 2i). These nine nominal fish species possess a similar body structure with respect to morphological definitions although the sizes may not be the same.

**Morphological characteristics identifications:** The results showed that Wilks' Lambda values of the 19

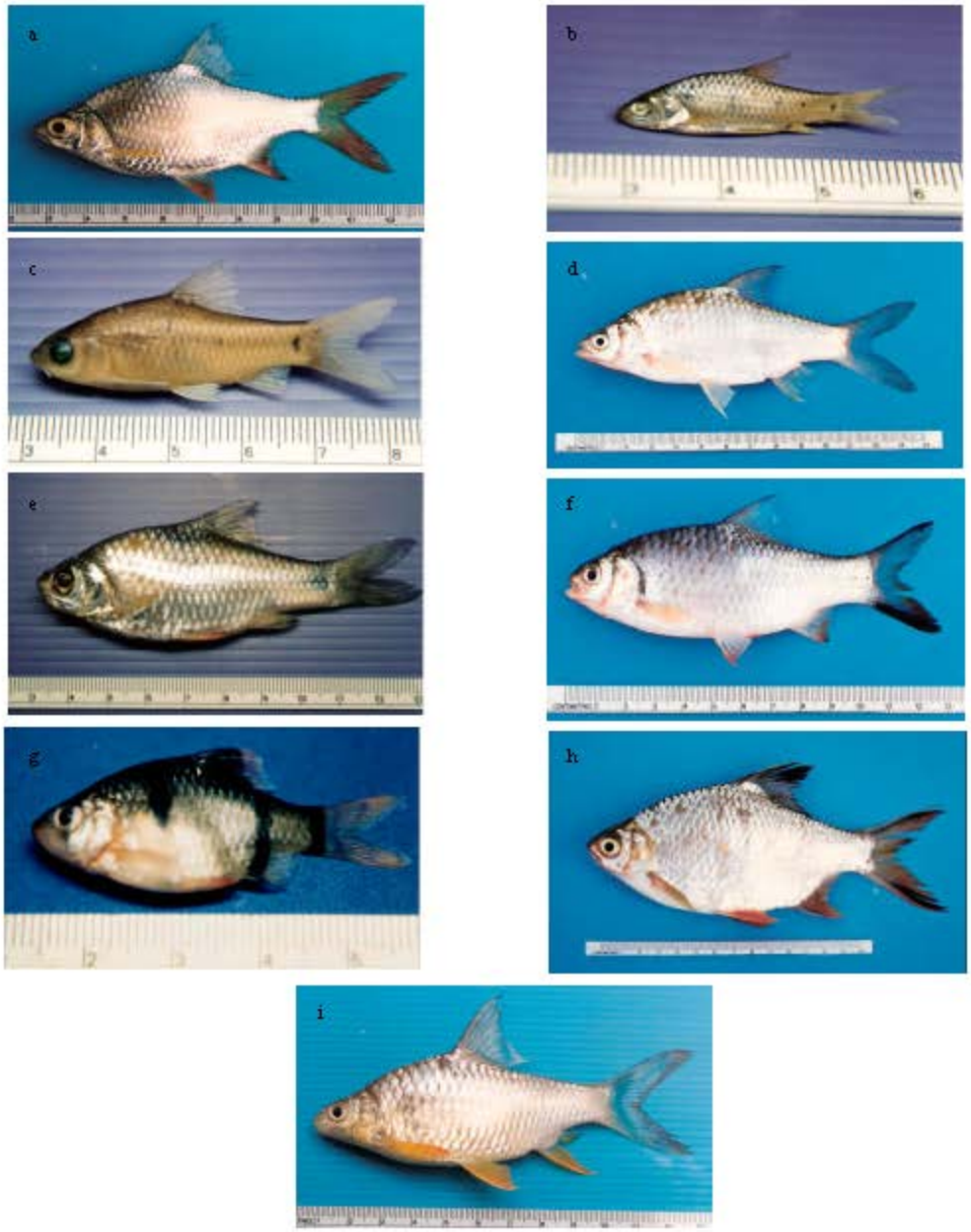


Fig. 2a-i: Photographs of the nine species of fish with their respective numbers of the collected locations of the genus *Puntius* Hamilton 1822 being collected from 15 wetlands of the 15 provinces (locations) in Northeast Thailand where 1 = Kalasin; 2 = Yasothorn; 3 = Udonthani; 4 = Chaiyaphum; 5 = Srisaket; 6 = Mahasarakham; 7 = Sakonnakhon; 8 = Roi Et; 9 = Nong Khai; 10 = Nakhonphanom; 11 = Mukdahan; 12 = Nakhonratchasima; 13 = Khon Kaen; 14 = Nongbualumphu and 15 = Loei (a) *Puntius altus* (Gunther, 1868) Harvested from locations 3, 9, 10, 11 and 13, (b) *Puntius aurotaeniatus* (Tirant, 1885) Harvested from locations 1, 3, 6, 11 and 15, (c) *Puntius binotatus* (Valenciennes, 1842) Harvested from locations 3, 7, 9, 11 and 14, (d) *Puntius gonionotus* (Bleeker, 1850) Harvested from locations 1, 3, 6, 9 and 13, (e) *Puntius leiacanthus* (Bleeker, 1860) Harvested from locations 2, 4, 5, 7 and 9, (f) *Puntius orphoides* (Valenciennes, 1842) Harvested from locations 6, 8, 12, 14 and 15, (g) *Puntius partipentazona* (Fowler, 1934) Harvested from locations 1, 3, 4, 8 and 13, (h) *Puntius schwanenfeldi* (Bleeker, 1853) Harvested from locations 3, 9, 10, 11 and 13 and (i) *Puntius etmorei* (Smith, 1931) Harvested from locations 1, 9, 10, 11 and 13

Table 1: Items being used for statistical analysis (Multivariate analysis, MANOVA) of twenty morphological characteristics of fish of the genus *Puntius* Hamilton 1822. The fish samples of the nine species were harvested from fifteen wetlands in Northeast Thailand, (items No. 1-14 were carried out by measuring and No. 15-20 by counting)

| Measuring and counting characteristics | Wilks' Lambda | F-test | p-level (p > F) |
|---|---|---|---|
| Snout to urocentrum | 0.9000 | 1.00 | 0.3434 |
| Preanal length | 0.8820 | 0.27 | 0.8468 |
| Prepelvic length | 0.5282 | 1.79 | 0.2497 |
| Prepectoral length | 0.3629 | 2.93 | 0.1390 |
| Predorsal length | 0.0156 | 42.15 | 0.0233 |
| Preoccipal length | 0.0456 | 13.96 | 0.0676 |
| Dorsal origin to pelvic insertion | 0.0034 | 19.16 | 0.0052 |
| Head depth at occiput | 0.0021 | 14.31 | 0.0032 |
| Occiput to dorsal origin | 0.0015 | 56.53 | 0.0022 |
| Occiput to pectoral insertion | 0.0008 | 86.00 | 0.0013 |
| Occiput to pelvic insertion | 0.0001 | 35.72 | 0.0002 |
| Occiput to anal insertion | 0.0018 | 64.04 | 0.0027 |
| Snout length | 0.0013 | 48.19 | 0.0021 |
| Interorbital width | 0.0025 | 25.92 | 0.0039 |
| Total gill rakers | 0.0008 | 30.66 | 0.0012 |
| Predorsal scale rows | 0.0024 | 80.58 | 0.0036 |
| Upper transverse | 0.0590 | 10.63 | 0.0872 |
| Scale from Lateral line to pelvic fin origin | 0.0244 | 26.67 | 0.0364 |
| Lower transverse scale rows | 0.0727 | 8.50 | 0.1071 |
| Circumpeduncular scale rows | 0.0017 | 76.00 | 0.0027 |

All values of p-level are non significant (NS)

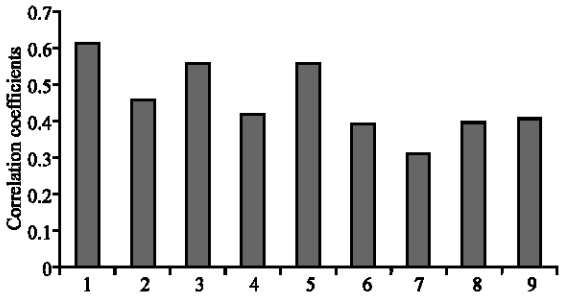


Fig. 3: The distribution of correlation coefficients on morphological similarity of the nine fish species of the genus *Puntius* Hamilton 1822 harvested from the fifteen wetlands in Northeast Thailand where 1 = *P. wetmorei*; 2 = *P. schwanenfeldi*; 3 = *P. altus*; 4 = *P. orphoides*; 5 = *P. leiacanthus*; 6 = *P. gonionotus*; 7 = *P. aurotaeniatus*; 8 = *P. binotatus* and 9 = *P. partipentazona*

characteristics of the 9 species of fish of the genus *Puntius* Hamilton 1822 derived from Multivariate analysis ranged from 0.0001 to 0.900 for occiput pelvic insertion and snout to urocentrum, respectively (Table 1). There were no significant differences found among the 9 fish species tested where p-level (p > F) values ranged from 0.0012 to 0.8468 for total gill rakers and preanal length, respectively.

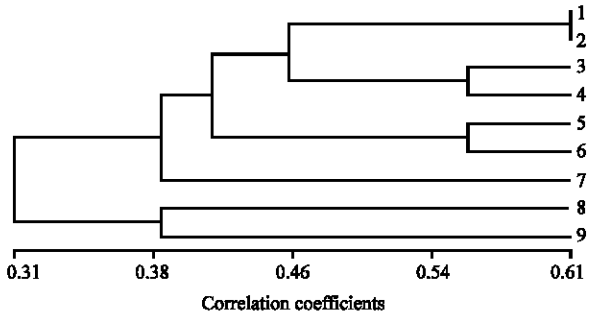


Fig. 4: A dendrogram structure of clusters on similarity correlation coefficients of the nine fish species of the genus *Puntius* Hamilton 1822 based on cluster analysis of UPGMA (Rohlf, 2001), where 1 = *P. wetmorei*; 2 = *P. orphoides*; 3 = *P. leiacanthus*; 4 = *P. gonionotus*; 5 = *P. aurotaeniatus*; 6 = *P. binotatus*; 7 = *P. partipentazona*; 8 = *P. schwanenfeldi* and 9 = *P. altus*

**Morphological similarity correlation coefficients:** For the distribution of individual fish species, the results revealed that the 9 fish species of the genus *Puntious* Hamilton 1822 gave similarity coefficient values ranged from 0.31 to 0.61 for *Puntius aurotaeniatus* and *Puntius wetmorei*, respectively. There was no significant difference found among the 9 fish species tested (Fig. 3).

**A dendrogram structure on similarity correlation coefficients:** With the results derived from a Dendrogram structure of clusters, the results revealed that the 9 fish species could possibly be grouping into two main groups, i.e. the first main group includes *Puntius altus* and *Puntius schwanenfeldi* and the second group include *Puntius wetmorei*, *Puntius orphoides*, *Puntius leiacanthus*, *Puntius gonionotus*, *Puntius aurotaeniatus*, *Puntius binotatus* and *Puntius partipentazona*. However, of the 9 fish species, it was found that similarity correlation coefficient values were within a range of 0.31 to 0.61 only (Fig. 4). There was no significant difference found among the 9 fish species tested.

## DISCUSSION

Of the 15 wetlands in Northeast Thailand, in terms of ecological environments, the wetland areas occupied with fresh water throughout the year. The villagers in each wetland partly carried out fishing activities for fishes for their alternative occupation. There had been a variety of plant species grown within the littoral wetland areas of the reservoirs where birds and other creatures are inhibited.

All of the reservoirs have been occurring with, more or less, clear or transparent water where within a depth of a few meters, some certain amount of radiant energy from the sun could pass through to the ground area for some water plants that submerged under water to synthesize their food. Most of the wetland areas may be presumably recognized as natural water resources of fresh water reservoirs where annual rainwater derives from the monsoon filled them up from year to year and aided more living creatures to survive, particularly the fishes of numerous species apart from the fish of the genus *Puntius* Hamilton 1822.

There has been some published information being accumulated during the past decade on morphological identification of fish of the genus *Puntius* Hamilton 1822, e.g., with the work of Rainboth (1996a, b), this worker claimed that the fish of the genus *Puntius* Hamilton 1822 could be split into three genera due to its morphological characteristics and he gave out three names of the genera as *Barbodes*, *Systomus* and *Hypsibarbus*. It seems more likely that the three additional genera of the fish establish some controversial viewpoints among taxonomists and scientists to perceive and accountable. This could possibly be attributable to perhaps some inadequate and inaccurate amounts of available explanations where it led to an ambiguous explanation, perhaps the failure in using computer programmes to establish appropriate evidences in differentiating the fish species since there were no intensive and conceivable evidences produced on morphological characteristics of the new genera distributed, particularly with the facts presented evidences on similarity correlation coefficients including clusters of a dendrogram structure of similarity coefficients and it is noticeable that the fish of the three new genera found within his text possessed exactly the same number or nearly the same numbers of many characteristics such as the pelvic fin and dorsal fin, predorsal scales and scales above lateral lines, whilst other organs possessed a very small difference where the small differences could have been influenced by natural habitations (Roberts, 1989). The use of morphological characteristics on phenotypic variation of the fish of Archtic charr by Bush and Adams (2007) emphasized the high accuracy of morphological measurements of the fish, thus published information on morphological characteristics of the fish of the genus *Puntius* Hamilton 1822 within the past decades must be used and taken into account as a scope of identification, Smith (1945), Jayaram (1981), Mohsin and Ambak (1983), Roberts (1989) and Talwar and Jhingran (1991). These workers did taxonomically explain a wide range of many characteristics of the fish of the genus *Puntius* Hamilton 1822. It seems more likely that the differences due to morphological characteristics among the three genera of the fish, which Rainboth (1996a, b) identified could actually be the fish of a single genus of the *Puntius* Hamilton 1822 where he failed to provide vividly the distinctive characteristics of the fish as given by many previously stated authors and his given definitions among the three new genera were not relatively cleared. Thus the fish of the genus *Puntius* Hamilton 1822 could not be split into another three genera as such. For example, the explanation found by Rainboth (1996a) on his three new genera, he was not able to provide more definite identities of the fish and the differences described between the two genera of *Barbodes* and *Hypsibarbus* on dorsal-fin spine were more or less the same where the phrases of the explanation repeatedly explained and unable to point out the reasons why the genus of *Puntius* Hamilton 1822 must be changed or shifted to other genera. Furthermore, the explanations on the differentiation of the fish on pages 102-103 on maxillary barbels where the fish gave, more or less, a similar amount of barbels were inadequately presented. Thus the fish of the genus *Puntius* Hamilton 1822 could not be classified into different genera as he deliberately described in his FAO guidebook for fishery workers.

The fifteen wetland areas in Northeast Thailand occupied with only 9 fish species of the genus *Puntius* Hamilton 1822. This minority number of the fish species found may be attributable to the changes in habitation of fish or perhaps the distribution of other fish species of this genus may be found in some other rivers or other wetland areas instead of inhibited under wetland conditions of these fifteen locations, since it has been reported by Smith (1945) that there were approximately 36 nominal species of the fish of the genus *Puntius* Hamilton 1822 available in Thailand. With 20 morphological characteristics being measured and counted on the fish samples collected from 15 wetland areas in Northeast Thailand, the raw data were computed through different computer programmes commenced from Excel followed by STATISTICA and Multivariate Analysis where the final results had been attained and illustrated. The numbers of measurements and counts being used may be considered to be an adequate number for morphological measurements and counts of this type of fish when compared with some other works such as the works of Klingenberg (1996), Elvira and Almodovar (2000), Iguchi *et al.* (2003), Poulet *et al.* (2004) and Chen *et al.* (2005) where many of them had been using less than 12 characteristics for their taxonomic identifications. The results revealed that there has been no significant

differences found within 20 important morphological characteristics being measured and counted. However, the nine fish species could be classified into two main groups based on cluster analysis on the values of the Euclidean matrix distances with the use of the Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) being computed through Sahn programme of NTSYS of Rohlf (2000), i.e., the first group consisted of *Puntius altus* and *Puntius schwanenfeldi* and the second group include another seven species, i.e., *Puntius partipentazona*, *P. binotatus*, *P. aurotaeniatus*, *P. gonionotus*, *P. leiacanthus*, *P. orphoides* and *P. wetmorei*. In spite of the evidences of the two cluster groups, it was found that the results on similarity correlation coefficients ranged only from 0.31 up to 0.61. Thus the results signified no distinguish differences found among the nine species of the fish where all of the attained values with respect to correlation coefficients were not exceeded a value of 0.78. Salini *et al.* (2004) showed that correlation coefficient values being calculated for the search in differentiating fish not exceeded a value of 0.78 could be considered insignificant thus the fish of the *Puntius* Hamilton 1822 could not be classified into other genera due to their close relationships among similarity correlation coefficients. They further stated that morphological variation among the fish of Hilsa (*Tenualosa ilisha*) from different sites within the Bangladesh is more likely to be a consequence of environmental variation than a strong genetic selection pressure as reported by Campana *et al.* (2000). Therefore, the results found with this work could possibly be of tangible value in testifying the fish of the genus *Puntius* Hamilton 1822 whether or not this genus of the fish could be classified or shifted into other genera. In addition, the results on laboratory investigations with the use of random amplified polymorphic DNA (RAPD) may be used to confirm this finding.

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